

OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

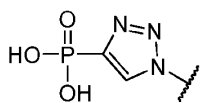
[0001] This application claims priority to United States Provisional Application No. 63/620,768, filed January 12, 2024, the entirety of which is incorporated herein by reference.

BACKGROUND

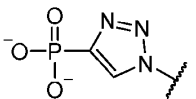
[0002] Oligonucleotides are useful for many purposes. Various technologies for preparing oligonucleotides have been reported.

SUMMARY

[0003] Various chemical modifications have been reported to improve properties and activities of oligonucleotides. For example, 5'-end $-P(O)(OH)_2$ group which may exist in a salt form as $-P(O)(O^-)_2$, in some cases together with another chemical moiety, has been incorporated at 5'-end of various oligonucleotides to improve properties and/or activities of the oligonucleotides, e.g., as RNAi agents. In some embodiments, an oligonucleotide comprises the following group attached to its 5'-end sugar carbon atom, in which a phosphonate is bonded to a triazole ring:



In some embodiments, it exists in salt form as an anion:



Various technologies have been reported to incorporate a phosphonate group. Typically, during oligonucleotide synthesis, $-P(O)(OH)_2$ groups are incorporated as protected groups (e.g., as $-P(O)(OMe)_2$, $-P(O)(OEt)_2$, etc.) which are later deprotected. Among other things, the present disclosure encompasses the recognition that such protected groups can be challenging to deprotect under typical oligonucleotide synthesis conditions. In some embodiments, extra steps, harsher conditions and/or longer reaction time are required. In some embodiments, such protected groups lead to lower oligonucleotide yield and/or purity. In some embodiments, such protected phosphate groups increase operation complexity. In some embodiments, such protected phosphate groups lower manufacturing efficiency. In some embodiments, such protected phosphate groups increase manufacturing cost. In some embodiments, the present disclosure provides to address these challenges.

[0004] In some embodiments, the present disclosure provides technologies for incorporating $-P(O)(OH)_2$ into oligonucleotides with higher yield, higher purity, higher efficiency, lower operation complexity, milder reaction condition, shorter preparation time, and/or lower cost, etc. when compared to using, e.g., $-P(O)(OMe)_2$, or $-P(O)(OEt)_2$. In some embodiments, the present disclosure provides protected $-P(O)(OH)_2$ groups which can be deprotected under oligonucleotide synthesis conditions, e.g., during cleavage and/or deprotection, utilized for oligonucleotides without $-P(O)(OH)_2$ groups.

[0005] In some embodiments, the present disclosure provides technologies comprising $-P(O)(OR^{PG})_2$ wherein each R^{PG} is a group that is labile to oligonucleotide synthesis conditions, e.g., during cleavage and/or deprotection, utilized for oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, each R^{PG} is a group that can be removed under oligonucleotide synthesis conditions, e.g., during cleavage and/or deprotection, utilized for oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, $-P(O)(OR^{PG})_2$ is a protected $-P(O)(OH)_2$ group that is converted to $-P(O)(OH)_2$ which may exist in a salt form, e.g., under oligonucleotide synthesis conditions, e.g., during cleavage and/or deprotection, utilized for oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, the P atom is bonded to a carbon atom.

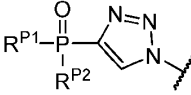
[0006] For example, in some embodiments, R^{PG} comprises 1) an alpha carbon atom bonded to the oxygen to which it is attached, wherein the alpha carbon atom is bonded to a hydrogen, and 2) a beta carbon atom, wherein the beta carbon atom is bonded to a group R^{PG11} that can facilitate removal of R^{PG} (e.g., compared to ethyl), such as an electron-withdrawing group. In some embodiments, R^{PG11} is an electron-withdrawing group. In some embodiments, R^{PG11} is R^s as described herein. In some embodiments, R^{PG11} is $-CN$. In some embodiments, R^{PG11} is $-L^s-R^{s11}$ wherein each L^s and R^{s11} is independently as described herein. In some embodiments, R^{PG} is $-CH_2-R^s$, wherein the $-CH_2-$ is independently optionally substituted and R^s is as described herein. In some embodiments, the $-CH_2-$ is optionally mono-substituted. In some embodiments, R^{PG} is R^{P1} as described herein. In some embodiments, R^{PG} is R^{P2} as described herein. In some embodiments, R^{PG} is $-O-CH_2CH_2CN$.

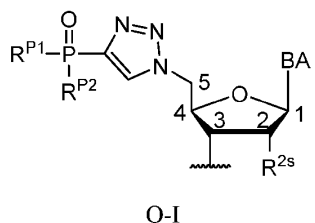
[0007] In some embodiments, R^{PG} is a group labile to an acid. In some embodiments, R^{PG} is $-CH_2-O(CO)R^{PG12}$, wherein R^{PG12} is trisubstituted methyl and the $-CH_2-$ is optionally substituted. In some embodiments, R^{PG} is $-CH_2-O(CO)R^{PG12}$, wherein R^{PG12} is $-C(R^{s11})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, R^{PG} is R^{PG12} , wherein R^{PG12} is trisubstituted methyl. In some embodiments, R^{PG12} is $-C(R^{s11})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, the carbon of the trisubstituted methyl or $-C(R^{s11})_3$ is bonded to three carbon atoms. In some embodiments, R^{PG12} is optionally substituted t-butyl. In some embodiments, R^{PG12} is t-butyl. In some embodiments, R^{PG} is R^{P1} or R^{P2} as described herein. In some embodiments, R^{PG} is $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl. In some embodiments, R^{PG} is

–O–CH₂–O–C(O)–tBu.

[0008] In some embodiments, –P(O)(OR^{PG})₂ has the structure of –P(O)(R^{P1})(R^{P2}) as described herein (e.g., in formula I or formula I'), wherein each of R^{P1} and R^{P2} is independently as described herein.

[0009] In some embodiments, the present disclosure provides oligonucleotides comprising –P(O)(OR^{PG})₂ as described herein. In some embodiments, such oligonucleotides are useful for preparing or delivering oligonucleotides comprising –P(O)(OH)₂ which may exist in various salt forms. In some embodiments, oligonucleotides comprising –P(O)(OR^{PG})₂ as described herein are administered to a subject for preventing or treating conditions, disorders or diseases. In some embodiments, oligonucleotides comprising –P(O)(OR^{PG})₂ as described herein are utilized for preparing oligonucleotides comprising –P(O)(OH)₂ which may exist in various salt forms. For example, in some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising

, wherein each of R^{P1} and R^{P2} is –O–CH₂CH₂CN or –O–CH₂–O–C(O)–R^P, wherein R^P is optionally substituted tert-butyl. In some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is –O–CH₂CH₂CN or –O–CH₂–O–C(O)–R^P, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is –F, –Cl, –Br, –I, –CN, –N₃, –NO, –NO₂, –L^{2s}–R^{2a}, –L^{2s}–OR^{2a}, –L^{2s}–SR^{2a}, –L^{2s}–N(R^{2a})₂, –O–L^{2s}–OR^{2a}, –O–L^{2s}–SR^{2a}, or –O–L^{2s}–N(R^{2a})₂, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R';

each L is independently a bivalent, optionally substituted group selected from C₁₋₁₀ aliphatic and C₁₋₁₀ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen,

phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{SC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$;

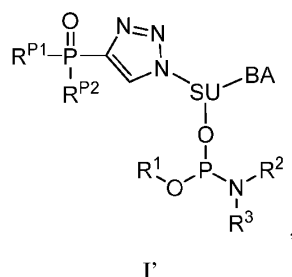
$-\text{Cy}-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{CO}_2\text{R}$, or $-\text{S}(\text{O})_2\text{R}$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

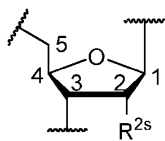
[0010] In some embodiments, the present disclosure provides nucleoside phosphoramidites comprising $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$. In some embodiments, $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ is bonded to the sugar of a nucleoside (e.g., 5' carbon). In some embodiments, it is bonded to another moiety, e.g., an optionally substituted triazole ring (e.g., as in formula I or formula I') which is bonded to the sugar of a nucleoside (e.g., 5' carbon). In some embodiments, a phosphoramidite comprises a chiral auxiliary.

[0011] In some embodiments, the provided technology provides a compound of formula I' or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl;



SU is a sugar (e.g., R^{2s} , wherein R^{2s} is as described herein);

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $\text{---}(\text{A})\text{---}(\text{R}^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently ---F , ---Cl , ---Br , ---I , ---CN , ---N_3 , ---NO , ---NO_2 , $\text{---L}^s\text{---R}^{s11}$, $\text{---L}^s\text{---OR}^{s11}$, $\text{---L}^s\text{---SR}^{s11}$, $\text{---L}^s\text{---N}(\text{R}^{s11})_2$, $\text{---O---L}^s\text{---OR}^{s11}$, $\text{---O---L}^s\text{---SR}^{s11}$, $\text{---O---L}^s\text{---N}(\text{R}^{s11})_2$, $\text{---C}(\text{R}^{s11})_3$ or $\text{---L}^s\text{---Si}(\text{R}^{s11})_3$;

each R^{s11} is independently R' ;

each L^s is independently L;

each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by ---Cy--- , ---O--- , ---S--- , $\text{---N}(\text{R}')\text{---}$, $\text{---C}(\text{O})\text{---}$, $\text{---C}(\text{S})\text{---}$, $\text{---C}(\text{NR}')\text{---}$, $\text{---C}(\text{O})\text{N}(\text{R}')\text{---}$, $\text{---N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')\text{---}$, $\text{---N}(\text{R}')\text{C}(\text{O})\text{O---}$, $\text{---S}(\text{O})\text{---}$, $\text{---S}(\text{O})_2\text{---}$, $\text{---S}(\text{O})_2\text{N}(\text{R}')\text{---}$, $\text{---SC}(\text{O})\text{---}$, or $\text{---C}(\text{O})\text{O---}$;

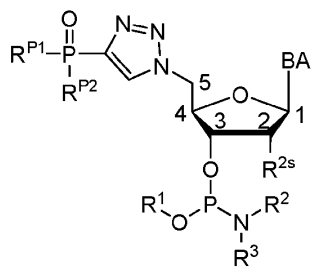
---Cy--- is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently ---R , $\text{---C}(\text{O})\text{R}$, $\text{---CO}_2\text{R}$, or $\text{---S}(\text{O})_2\text{R}$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0012] In some embodiments, the provided technology provides a compound of formula I or a salt

thereof:



I

wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $\text{---}(\text{A})-(R^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; t is 0-5;

each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R^{s11}$, $-L^s-OR^{s11}$, $-L^s-SR^{s11}$, $-L^s-N(R^{s11})_2$, $-O-L^s-OR^{s11}$, $-O-L^s-SR^{s11}$, $-O-L^s-N(R^{s11})_2$, $-C(R^{s11})_3$ or $-L^s-Si(R^{s11})_3$;

each R^{s11} is independently R' ;

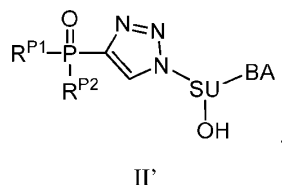
each L^s is independently L;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

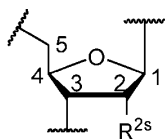
[0013] In some embodiments, a compound of formula I or a salt thereof is useful as a nucleoside phosphoramidite for oligonucleotide synthesis. In some embodiments, a compound of formula I' or a salt thereof is useful as a nucleoside phosphoramidite for oligonucleotide synthesis.

[0014] In some embodiments, the present disclosure provides nucleosides comprising $-P(O)(OR^{PG})_2$. In some embodiments, $-P(O)(OR^{PG})_2$ is bonded to the sugar of a nucleoside (e.g., 5' carbon). In some embodiments, it is bonded to another moiety, e.g., an optionally substituted triazole ring (e.g., as in formula I or formula I') which is bonded to the sugar of a nucleoside (e.g., 5' carbon). In some embodiments, the present disclosure provides a compound of formula II' or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

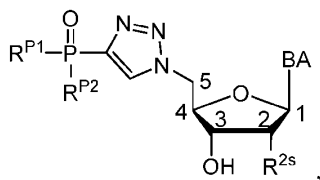


SU is a sugar (e.g., R^{2s}), wherein R^{2s} is as described herein); and

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase.

[0015] In some embodiments, the present disclosure provides a compound of formula II or a salt

thereof:



II

wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

[0016] each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, the present disclosure provides technologies for preparing

oligonucleotides and compositions thereof. In some embodiments, oligonucleotides comprise $-P(O)(OH)_2$ which may exist in various salt forms. In some embodiments, oligonucleotides comprise $-P(O)(OR^{PG})_2$ as described herein. In some embodiments, the present disclosure provides technologies for preparing phosphoramidites. In some embodiments, the present disclosure provides technologies for preparing nucleosides.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0017] Technologies of the present disclosure may be understood more readily by reference to the following detailed description of certain embodiments.

Definitions

[0018] As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001.

[0019] As used herein in the present disclosure, unless otherwise clear from context, (i) the term "a" or "an" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; (iii) the terms "comprising", "comprise", "including" (whether used with "not limited to" or not), and "include" (whether used with "not limited to" or not) may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; (iv) the term "another" may be understood to mean at least an additional/second one or more; (v) the terms "about" and "approximately" may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (vi) where ranges are provided, endpoints are included.

[0020] Unless otherwise specified, description of oligonucleotides and elements thereof (e.g., base sequence, sugar modifications, internucleotidic linkages, linkage phosphorus stereochemistry, patterns thereof, etc.) is from 5' to 3'. As those skilled in the art will appreciate, in some embodiments, oligonucleotides may be provided and/or utilized as salt forms, particularly pharmaceutically acceptable salt forms, e.g., sodium salts. As those skilled in the art will also appreciate, in some embodiments, individual oligonucleotides within a composition may be considered to be of the same constitution and/or structure even though, within such composition (e.g., a liquid composition), particular such oligonucleotides might be in different salt form(s) (and may be dissolved and the oligonucleotide chain

may exist as an anion form when, e.g., in a liquid composition) at a particular moment in time. For example, those skilled in the art will appreciate that, at a given pH, individual internucleotidic linkages along an oligonucleotide chain may be in an acid (H) form, or in one of a plurality of possible salt forms (e.g., a sodium salt, or a salt of a different cation, depending on which ions might be present in the preparation or composition), and will understand that, so long as their acid forms (e.g., replacing all cations, if any, with H⁺) are of the same constitution and/or structure, such individual oligonucleotides may properly be considered to be of the same constitution and/or structure.

[0021] Aliphatic: As used herein, “aliphatic” means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or a substituted or unsubstituted monocyclic, bicyclic, or polycyclic hydrocarbon ring that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or combinations thereof. In some embodiments, aliphatic groups contain 1-50 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-20 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-9 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-7 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1, 2, 3, or 4 aliphatic carbon atoms. Suitable aliphatic groups include linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0022] Alkenyl: As used herein, the term “alkenyl” refers to an aliphatic group, as defined herein, having one or more double bonds.

[0023] Alkyl: As used herein, the term “alkyl” is given its ordinary meaning in the art and may include saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In some embodiments, alkyl has 1-100 carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 carbon atoms in its backbone (e.g., C₁-C₂₀ for straight chain, C₂-C₂₀ for branched chain), and alternatively, about 1-10. In some embodiments, cycloalkyl rings have from about 3-10 carbon atoms in their ring structure where such rings are monocyclic, bicyclic, or polycyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl group may be a lower alkyl group, wherein a lower alkyl group comprises 1-4 carbon atoms (e.g., C₁-C₄ for straight chain lower alkyls).

[0024] Alkynyl: As used herein, the term “alkynyl” refers to an aliphatic group, as defined herein,

having one or more triple bonds.

[0025] Aryl: The term “aryl”, as used herein, used alone or as part of a larger moiety as in “aralkyl,” “aralkoxy,” or “aryloxyalkyl,” refers to monocyclic, bicyclic or polycyclic ring systems having a total of five to thirty ring members, wherein at least one ring in the system is aromatic. In some embodiments, an aryl group is a monocyclic, bicyclic or polycyclic ring system having a total of five to fourteen ring members, wherein at least one ring in the system is aromatic, and wherein each ring in the system contains 3 to 7 ring members. In some embodiments, each monocyclic ring unit is aromatic. In some embodiments, an aryl group is a biaryl group. The term “aryl” may be used interchangeably with the term “aryl ring.” In certain embodiments of the present disclosure, “aryl” refers to an aromatic ring system which includes, but is not limited to, phenyl, biphenyl, naphthyl, binaphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term “aryl,” as it is used herein, is a group in which an aromatic ring is fused to one or more non-aromatic rings, such as indanyl, phthalimidyl, naphthimidyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[0026] Chiral control: As used herein, “chiral control” refers to control of the stereochemical designation of the chiral linkage phosphorus in a chiral internucleotidic linkage within an oligonucleotide. As used herein, a chiral internucleotidic linkage is an internucleotidic linkage whose linkage phosphorus is chiral. In some embodiments, a control is achieved through a chiral element that is absent from the sugar and base moieties of an oligonucleotide, for example, in some embodiments, a control is achieved through use of one or more chiral auxiliaries during oligonucleotide preparation, which chiral auxiliaries often are part of chiral phosphoramidites used during oligonucleotide preparation. In contrast to chiral control, a person having ordinary skill in the art will appreciate that conventional oligonucleotide synthesis which does not use chiral auxiliaries cannot control stereochemistry at a chiral internucleotidic linkage if such conventional oligonucleotide synthesis is used to form the chiral internucleotidic linkage. In some embodiments, the stereochemical designation of each chiral linkage phosphorus in each chiral internucleotidic linkage within an oligonucleotide is controlled.

[0027] Chirally controlled oligonucleotide composition: The terms “chirally controlled oligonucleotide composition”, “chirally controlled nucleic acid composition”, and the like, as used herein, refers to a composition that comprises a plurality of oligonucleotides (or nucleic acids) which share a common base sequence, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is R_p or S_p in the composition (“stereodefined”), not a random R_p and S_p mixture as non-chirally controlled internucleotidic linkages). In some embodiments, a chirally controlled oligonucleotide composition comprises a plurality of oligonucleotides (or nucleic acids) that share: 1) a common base sequence, 2) a common pattern of

backbone linkages, and 3) a common pattern of backbone phosphorus modifications, wherein the plurality of oligonucleotides (or nucleic acids) share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled or stereodefined internucleotidic linkages, whose chiral linkage phosphorus is Rp or Sp in the composition (“stereodefined”), not a random Rp and Sp mixture as non-chirally controlled internucleotidic linkages). Level of the plurality of oligonucleotides (or nucleic acids) in a chirally controlled oligonucleotide composition is pre-determined/controlled or enriched (e.g., through chirally controlled oligonucleotide preparation to stereoselectively form one or more chiral internucleotidic linkages) compared to a random level in a non-chirally controlled oligonucleotide composition. In some embodiments, about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition are oligonucleotides of the plurality. In some embodiments, about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a chirally controlled oligonucleotide composition that share the common base sequence, the common pattern of backbone linkages, and the common pattern of backbone phosphorus modifications are oligonucleotides of the plurality. In some embodiments, a level is about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides in a composition, or of all oligonucleotides in a composition that share a common base sequence (e.g., of a plurality of oligonucleotide or an oligonucleotide type), or of all oligonucleotides in a composition that share a common base sequence, a common pattern of backbone linkages, and a common pattern of backbone phosphorus modifications, or of all oligonucleotides in a composition that share a common base sequence, a common pattern of base modifications, a common pattern of sugar modifications, a common pattern of internucleotidic linkage types, and/or a common pattern of internucleotidic linkage modifications. In some embodiments, the plurality of oligonucleotides share the same stereochemistry at about 1-50 (e.g., about 1-10, 1-20, 5-10, 5-20, 10-15, 10-20, 10-25, 10-30, or about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or

20, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) chiral internucleotidic linkages. In some embodiments, the plurality of oligonucleotides share the same stereochemistry at about 1%-100% (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, or at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%) of chiral internucleotidic linkages. In some embodiments, oligonucleotides (or nucleic acids) of a plurality share the same pattern of sugar and/or nucleobase modifications, in any. In some embodiments, oligonucleotides (or nucleic acids) of a plurality are various forms of the same oligonucleotide (e.g., acid and/or various salts of the same oligonucleotide). In some embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same constitution. In some embodiments, level of the oligonucleotides (or nucleic acids) of the plurality is about 1%-100%, (e.g., about 5%-100%, 10%-100%, 20%-100%, 30%-100%, 40%-100%, 50%-100%, 60%-100%, 70%-100%, 80%-100%, 90%-100%, 95%-100%, 50%-90%, or about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) of all oligonucleotides (or nucleic acids) in a composition that share the same constitution as the oligonucleotides (or nucleic acids) of the plurality. In some embodiments, each chiral internucleotidic linkage is a chiral controlled internucleotidic linkage, and the composition is a completely chirally controlled oligonucleotide composition. In some embodiments, oligonucleotides (or nucleic acids) of a plurality are structurally identical. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%, typically at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 95%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 96%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 97%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 98%. In some embodiments, a chirally controlled internucleotidic linkage has a diastereopurity of at least 99%. In some embodiments, a percentage of a level is or is at least (DS)^{nc}, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more) and nc is the number of chiral linkage phosphorus as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more). In some embodiments, a percentage of a level is or is at least (DS)^{nc}, wherein DS is a diastereopurity as described in the present disclosure (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or

more) and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 5-50, 5-40, 5-30, 5-25, 5-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more). In some embodiments, a percentage of a level is or is at least $(DS)^{nc}$, wherein DS is 95%-100%. For example, when DS is 99% and nc is 10, the percentage is or is at least 90% $((99\%)^{10} \approx 0.90 = 90\%)$. In some embodiments, level of a plurality of oligonucleotides in a composition is represented as the product of the diastereopurity of each chiral linkage phosphorus. In some embodiments, level of a plurality of oligonucleotides in a composition is represented as the product of the diastereopurity of each chirally controlled internucleotidic linkage in the oligonucleotides. In some embodiments, diastereopurity of an internucleotidic linkage connecting two nucleosides in an oligonucleotide (or nucleic acid) is represented by the diastereopurity of an internucleotidic linkage of a dimer connecting the same two nucleosides, wherein the dimer is prepared using comparable conditions, in some instances, identical synthetic cycle conditions (e.g., for the linkage between N_x and N_y in an oligonucleotide $\dots N_x N_y \dots$, the dimer is $N_x N_y$). In some embodiments, not all chiral internucleotidic linkages are chirally controlled internucleotidic linkages, and the composition is a partially chirally controlled oligonucleotide composition. In some embodiments, a non-chirally controlled internucleotidic linkage has a diastereopurity of less than about 80%, 75%, 70%, 65%, 60%, 55%, or of about 50%, as typically observed in stereorandom oligonucleotide compositions (e.g., as appreciated by those skilled in the art, from traditional oligonucleotide synthesis, e.g., the phosphoramidite method). In some embodiments, oligonucleotides (or nucleic acids) of a plurality are of the same type. In some embodiments, a chirally controlled oligonucleotide composition comprises non-random or controlled levels of individual oligonucleotide or nucleic acids types. For instance, in some embodiments a chirally controlled oligonucleotide composition comprises one and no more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises more than one oligonucleotide type. In some embodiments, a chirally controlled oligonucleotide composition comprises multiple oligonucleotide types. In some embodiments, a chirally controlled oligonucleotide composition is a composition of oligonucleotides of an oligonucleotide type, which composition comprises a non-random or controlled level of a plurality of oligonucleotides of the oligonucleotide type.

[0028] Comparable: The term “comparable” is used herein to describe two (or more) sets of conditions or circumstances that are sufficiently similar to one another to permit comparison of results obtained or phenomena observed. In some embodiments, comparable sets of conditions or circumstances are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will appreciate that sets of conditions are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a

reasonable conclusion that differences in results obtained or phenomena observed under the different sets of conditions or circumstances are caused by or indicative of the variation in those features that are varied.

[0029] Cycloaliphatic: The term “cycloaliphatic,” “carbocycle,” “carbocyclyl,” “carbocyclic radical,” and “carbocyclic ring,” are used interchangeably, and as used herein, refer to saturated or partially unsaturated, but non-aromatic, cyclic aliphatic monocyclic, bicyclic, or polycyclic ring systems, as described herein, having, unless otherwise specified, from 3 to 30 ring members. Cycloaliphatic groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cycloheptyl, cycloheptenyl, cyclooctyl, cyclooctenyl, norbornyl, adamantyl, and cyclooctadienyl. In some embodiments, a cycloaliphatic group has 3–6 carbons. In some embodiments, a cycloaliphatic group is saturated and is cycloalkyl. The term “cycloaliphatic” may also include aliphatic rings that are fused to one or more aromatic or nonaromatic rings, such as decahydronaphthyl or tetrahydronaphthyl. In some embodiments, a cycloaliphatic group is bicyclic. In some embodiments, a cycloaliphatic group is tricyclic. In some embodiments, a cycloaliphatic group is polycyclic. In some embodiments, “cycloaliphatic” refers to C₃–C₆ monocyclic hydrocarbon, or C₈–C₁₀ bicyclic or polycyclic hydrocarbon, that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule, or a C₉–C₁₆ polycyclic hydrocarbon that is completely saturated or that contains one or more units of unsaturation, but which is not aromatic, that has a single point of attachment to the rest of the molecule.

[0030] Heteroaliphatic: The term “heteroaliphatic”, as used herein, is given its ordinary meaning in the art and refers to aliphatic groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). In some embodiments, one or more units selected from C, CH, CH₂, and CH₃ are independently replaced by one or more heteroatoms (including oxidized and/or substituted forms thereof). In some embodiments, a heteroaliphatic group is heteroalkyl. In some embodiments, a heteroaliphatic group is heteroalkenyl.

[0031] Heteroalkyl: The term “heteroalkyl”, as used herein, is given its ordinary meaning in the art and refers to alkyl groups as described herein in which one or more carbon atoms are independently replaced with one or more heteroatoms (e.g., oxygen, nitrogen, sulfur, silicon, phosphorus, and the like). Examples of heteroalkyl groups include, but are not limited to, alkoxy, poly(ethylene glycol)-, alkyl-substituted amino, tetrahydrofuranyl, piperidinyl, morpholinyl, etc.

[0032] Heteroaryl: The terms “heteroaryl” and “heteroar-”, as used herein, used alone or as part of a larger moiety, e.g., “heteroaralkyl,” or “heteroaralkoxy,” refer to monocyclic, bicyclic or polycyclic ring systems having a total of five to thirty ring members, wherein at least one ring in the system is aromatic

and at least one aromatic ring atom is a heteroatom. In some embodiments, a heteroaryl group is a group having 5 to 10 ring atoms (i.e., monocyclic, bicyclic or polycyclic), in some embodiments 5, 6, 9, or 10 ring atoms. In some embodiments, each monocyclic ring unit is aromatic. In some embodiments, a heteroaryl group has 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indoliziny, purinyl, naphthyridinyl, and pteridinyl. In some embodiments, a heteroaryl is a heterobiaryl group, such as bipyridyl and the like. The terms "heteroaryl" and "heteroar-", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Non-limiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H-quinoliziny, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolyl, tetrahydroisoquinolyl, and pyrido[2,3-b]-1,4-oxazin-3(4H)-one. A heteroaryl group may be monocyclic, bicyclic or polycyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl group, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0033] Heteroatom: The term "heteroatom", as used herein, means an atom that is not carbon or hydrogen. In some embodiments, a heteroatom is boron, oxygen, sulfur, nitrogen, phosphorus, or silicon (including oxidized forms of nitrogen, sulfur, phosphorus, or silicon; charged forms of nitrogen (e.g., quaternized forms, forms as in iminium groups, etc.), phosphorus, sulfur, oxygen; etc.). In some embodiments, a heteroatom is silicon, phosphorus, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is silicon, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is oxygen, sulfur or nitrogen.

[0034] Heterocycle: As used herein, the terms "heterocycle," "heterocyclyl," "heterocyclic radical," and "heterocyclic ring", as used herein, are used interchangeably and refer to a monocyclic, bicyclic or polycyclic ring moiety (e.g., 3-30 membered) that is saturated or partially unsaturated and has one or more heteroatom ring atoms. In some embodiments, a heterocyclyl group is a stable 5- to 7-membered monocyclic or 7- to 10-membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0-3 heteroatoms

selected from oxygen, sulfur and nitrogen, the nitrogen may be N (as in 3,4-dihydro-2H-pyrrolyl), NH (as in pyrrolidinyl), or ⁺NR (as in N-substituted pyrrolidinyl). A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms “heterocycle,” “heterocyclyl,” “heterocyclyl ring,” “heterocyclic group,” “heterocyclic moiety,” and “heterocyclic radical,” are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3H-indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl. A heterocyclyl group may be monocyclic, bicyclic or polycyclic. The term “heterocyclylalkyl” refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[0035] Internucleotidic linkage: As used herein, the phrase “internucleotidic linkage” refers generally to a linkage linking nucleoside units of an oligonucleotide or a nucleic acid. In some embodiments, an internucleotidic linkage is a phosphodiester linkage, as extensively found in naturally occurring DNA and RNA molecules (natural phosphate linkage (–OP(=O)(OH)O–), which as appreciated by those skilled in the art may exist as a salt form). In some embodiments, an internucleotidic linkage is a modified internucleotidic linkage (not a natural phosphate linkage). In some embodiments, an internucleotidic linkage is a “modified internucleotidic linkage” wherein at least one oxygen atom or –OH of a phosphodiester linkage is replaced by a different organic or inorganic moiety. In some embodiments, such an organic or inorganic moiety is selected from =S, =Se, =NR’, –SR’, –SeR’, –N(R’)₂, B(R’)₃, –S–, –Se–, and –N(R’)–, wherein each R’ is independently as defined and described in the present disclosure. In some embodiments, an internucleotidic linkage is a phosphotriester linkage, phosphorothioate linkage (or phosphorothioate diester linkage, –OP(=O)(SH)O–, which as appreciated by those skilled in the art may exist as a salt form), or phosphorothioate triester linkage. In some embodiments, a modified internucleotidic linkage is a phosphorothioate linkage. In some embodiments, an internucleotidic linkage is one of, e.g., PNA (peptide nucleic acid) or PMO (phosphorodiamidate Morpholino oligomer) linkage. In some embodiments, a modified internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a modified internucleotidic linkage is a neutral internucleotidic linkage (e.g., n001 in certain provided oligonucleotides). It is understood by a person of ordinary skill in the art that an internucleotidic linkage may exist as an anion or cation at a given pH due to the existence of acid or base moieties in the linkage.

[0036] Linkage phosphorus: as defined herein, the phrase “linkage phosphorus” is used to indicate

that the particular phosphorus atom being referred to is the phosphorus atom present in the internucleotidic linkage, which phosphorus atom corresponds to the phosphorus atom of a phosphodiester internucleotidic linkage as occurs in naturally occurring DNA and RNA. In some embodiments, a linkage phosphorus atom is in a modified internucleotidic linkage, wherein each oxygen atom of a phosphodiester linkage is optionally and independently replaced by an organic or inorganic moiety. In some embodiments, a linkage phosphorus atom is chiral (e.g., as in phosphorothioate internucleotidic linkages). In some embodiments, a linkage phosphorus atom is achiral (e.g., as in natural phosphate linkages).

[0037] Modified nucleobase: The terms "modified nucleobase", "modified base" and the like refer to a chemical moiety which is chemically distinct from a nucleobase, but which is capable of performing at least one function of a nucleobase. In some embodiments, a modified nucleobase is a nucleobase which comprises a modification. In some embodiments, a modified nucleobase is capable of at least one function of a nucleobase, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases. In some embodiments, a modified nucleobase is substituted A, T, C, G, or U, or a substituted tautomer of A, T, C, G, or U. In some embodiments, a modified nucleobase in the context of oligonucleotides refer to a nucleobase that is not A, T, C, G or U.

[0038] Modified nucleoside: The term "modified nucleoside" refers to a moiety derived from or chemically similar to a natural nucleoside, but which comprises a chemical modification which differentiates it from a natural nucleoside. Non-limiting examples of modified nucleosides include those which comprise a modification at the base and/or the sugar. Non-limiting examples of modified nucleosides include those with a 2' modification at a sugar. Non-limiting examples of modified nucleosides also include abasic nucleosides (which lack a nucleobase). In some embodiments, a modified nucleoside is capable of at least one function of a nucleoside, e.g., forming a moiety in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases.

[0039] Modified nucleotide: The term "modified nucleotide" includes any chemical moiety which differs structurally from a natural nucleotide but is capable of performing at least one function of a natural nucleotide. In some embodiments, a modified nucleotide comprises a modification at a sugar, base and/or internucleotidic linkage. In some embodiments, a modified nucleotide comprises a modified sugar, modified nucleobase and/or modified internucleotidic linkage. In some embodiments, a modified nucleotide is capable of at least one function of a nucleotide, e.g., forming a subunit in a polymer capable of base-pairing to a nucleic acid comprising an at least complementary sequence of bases.

[0040] Modified sugar: The term "modified sugar" refers to a moiety that can replace a sugar. A modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar. In some embodiments, as described in the present disclosure, a modified sugar is substituted ribose or deoxyribose. In some embodiments, a modified sugar comprises a 2'-modification.

Examples of useful 2'-modification are widely utilized in the art and described herein. In some embodiments, a 2'-modification is 2'-F. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C₁₋₁₀ aliphatic. In some embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a modified sugar is a bicyclic sugar (e.g., a sugar used in LNA, BNA, etc.). In some embodiments, in the context of oligonucleotides, a modified sugar is a sugar that is not ribose or deoxyribose as typically found in natural RNA or DNA.

[0041] Nucleic acid: The term "nucleic acid", as used herein, includes any nucleotides and polymers thereof. The term "polynucleotide", as used herein, refers to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA) or a combination thereof. These terms refer to the primary structure of the molecules and, thus, include double- and single-stranded DNA, and double- and single-stranded RNA. These terms include, as equivalents, analogs of either RNA or DNA comprising modified nucleotides and/or modified polynucleotides, such as, though not limited to, methylated, protected and/or capped nucleotides or polynucleotides. The terms encompass poly- or oligo-ribonucleotides (RNA) and poly- or oligo-deoxyribonucleotides (DNA); RNA or DNA derived from N-glycosides or C-glycosides of nucleobases and/or modified nucleobases; nucleic acids derived from sugars and/or modified sugars; and nucleic acids derived from phosphate bridges and/or modified internucleotidic linkages. The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified internucleotidic linkages. Examples include, and are not limited to, nucleic acids containing ribose moieties, nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. Unless otherwise specified, the prefix poly- refers to a nucleic acid containing 2 to about 10,000 nucleotide monomer units and wherein the prefix oligo- refers to a nucleic acid containing 2 to about 200 nucleotide monomer units.

[0042] Nucleobase: The term "nucleobase" refers to the parts of nucleic acids that are involved in the hydrogen-bonding that binds one nucleic acid strand to another complementary strand in a sequence specific manner. The most common naturally-occurring nucleobases are adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, a naturally-occurring nucleobases are modified adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a naturally-occurring nucleobases are methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a nucleobase comprises a heteroaryl ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In some embodiments, a nucleobase comprises a heterocyclic ring wherein a ring atom is nitrogen, and when in a nucleoside, the nitrogen is bonded to a sugar moiety. In some embodiments, a nucleobase is a "modified nucleobase," a nucleobase other than adenine (A), guanine (G), uracil (U), cytosine (C), and thymine (T). In some embodiments, a modified nucleobase is substituted A,

T, C, G or U. In some embodiments, a modified nucleobase is a substituted tautomer of A, T, C, G, or U. In some embodiments, a modified nucleobases is methylated adenine, guanine, uracil, cytosine, or thymine. In some embodiments, a modified nucleobase mimics the spatial arrangement, electronic properties, or some other physicochemical property of the nucleobase and retains the property of hydrogen-bonding that binds one nucleic acid strand to another in a sequence specific manner. In some embodiments, a modified nucleobase can pair with all of the five naturally occurring bases (uracil, thymine, adenine, cytosine, or guanine) without substantially affecting the melting behavior, recognition by intracellular enzymes or activity of the oligonucleotide duplex. As used herein, the term “nucleobase” also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleobases and nucleobase analogs. In some embodiments, a nucleobase is optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G, or U. In some embodiments, a “nucleobase” refers to a nucleobase unit in an oligonucleotide or a nucleic acid (e.g., A, T, C, G or U as in an oligonucleotide or a nucleic acid).

[0043] Nucleoside: The term “nucleoside” refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or a modified sugar. In some embodiments, a nucleoside is a natural nucleoside, e.g., adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, or deoxycytidine. In some embodiments, a nucleoside is a modified nucleoside, e.g., a substituted natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In some embodiments, a nucleoside is a modified nucleoside, e.g., a substituted tautomer of a natural nucleoside selected from adenosine, deoxyadenosine, guanosine, deoxyguanosine, thymidine, uridine, cytidine, and deoxycytidine. In some embodiments, a “nucleoside” refers to a nucleoside unit in an oligonucleotide or a nucleic acid.

[0044] Nucleotide: The term “nucleotide” as used herein refers to a monomeric unit of a polynucleotide that consists of a nucleobase, a sugar, and one or more internucleotidic linkages (e.g., phosphate linkages in natural DNA and RNA). The naturally occurring bases [guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)] are derivatives of purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included. Nucleotides are linked via internucleotidic linkages to form nucleic acids, or polynucleotides. Many internucleotidic linkages are known in the art (such as, though not limited to, phosphate, phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphotriesters, phosphorothionates, H-phosphonates, phosphoramidates, boranophosphates, methylphosphonates, phosphonoacetates, thiophosphonoacetates and other variants of the phosphate

backbone of native nucleic acids, such as those described herein. In some embodiments, a natural nucleotide comprises a naturally occurring base, sugar and internucleotidic linkage. As used herein, the term "nucleotide" also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified nucleotides and nucleotide analogs. In some embodiments, a "nucleotide" refers to a nucleotide unit in an oligonucleotide or a nucleic acid.

[0045] Oligonucleotide: The term "oligonucleotide" refers to a polymer or oligomer of nucleotides, and may contain any combination of natural and non-natural nucleobases, sugars, and internucleotidic linkages.

[0046] Oligonucleotides can be single-stranded or double-stranded. A single-stranded oligonucleotide can have double-stranded regions (formed by two portions of the single-stranded oligonucleotide) and a double-stranded oligonucleotide, which comprises two oligonucleotide chains, can have single-stranded regions for example, at regions where the two oligonucleotide chains are not complementary to each other. Example oligonucleotides include, but are not limited to structural genes, genes including control and termination regions, self-replicating systems such as viral or plasmid DNA, single-stranded and double-stranded RNAi agents and other RNA interference reagents (RNAi agents or iRNA agents), shRNA, antisense oligonucleotides, ribozymes, microRNAs, microRNA mimics, supermirs, aptamers, antimirs, antagomirs, Ul adaptors, triplex-forming oligonucleotides, G-quadruplex oligonucleotides, RNA activators, immuno-stimulatory oligonucleotides, and decoy oligonucleotides.

[0047] Oligonucleotides of the present disclosure can be of various lengths. In particular embodiments, oligonucleotides can range from about 2 to about 200 nucleosides in length. In various related embodiments, oligonucleotides, single-stranded, double-stranded, or triple-stranded, can range in length from about 4 to about 10 nucleosides, from about 10 to about 50 nucleosides, from about 20 to about 50 nucleosides, from about 15 to about 30 nucleosides, from about 20 to about 30 nucleosides in length. In some embodiments, an oligonucleotide is from about 9 to about 39 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 26 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 27 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 28 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 29 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 30 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 31 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 32 to about 70 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 60 nucleosides in length. In some embodiments, an oligonucleotide is from about 25 to about 50 nucleosides in length. In some embodiments, an

oligonucleotide is from about 25 to about 40 nucleosides in length. In some embodiments, an oligonucleotide is from about 30 to about 40 nucleosides in length. In some embodiments, the oligonucleotide is at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides in length. In some embodiments, an oligonucleotide is at least 4 nucleosides in length. In some embodiments, an oligonucleotide is at least 5 nucleosides in length. In some embodiments, an oligonucleotide is at least 6 nucleosides in length. In some embodiments, an oligonucleotide is at least 7 nucleosides in length. In some embodiments, an oligonucleotide is at least 8 nucleosides in length. In some embodiments, an oligonucleotide is at least 9 nucleosides in length. In some embodiments, an oligonucleotide is at least 10 nucleosides in length. In some embodiments, an oligonucleotide is at least 11 nucleosides in length. In some embodiments, an oligonucleotide is at least 12 nucleosides in length. In some embodiments, an oligonucleotide is at least 15 nucleosides in length. In some embodiments, an oligonucleotide is at least 15 nucleosides in length. In some embodiments, an oligonucleotide is at least 16 nucleosides in length. In some embodiments, an oligonucleotide is at least 17 nucleosides in length. In some embodiments, an oligonucleotide is at least 18 nucleosides in length. In some embodiments, an oligonucleotide is at least 19 nucleosides in length. In some embodiments, an oligonucleotide is at least 20 nucleosides in length. In some embodiments, an oligonucleotide is at least 25 nucleosides in length. In some embodiments, an oligonucleotide is at least 26 nucleosides in length. In some embodiments, an oligonucleotide is at least 27 nucleosides in length. In some embodiments, an oligonucleotide is at least 28 nucleosides in length. In some embodiments, an oligonucleotide is at least 29 nucleosides in length. In some embodiments, an oligonucleotide is at least 30 nucleosides in length. In some embodiments, an oligonucleotide is at least 31 nucleosides in length. In some embodiments, an oligonucleotide is at least 32 nucleosides in length. In some embodiments, an oligonucleotide is at least 33 nucleosides in length. In some embodiments, an oligonucleotide is at least 34 nucleosides in length. In some embodiments, an oligonucleotide is at least 35 nucleosides in length. In some embodiments, an oligonucleotide is at least 36 nucleosides in length. In some embodiments, an oligonucleotide is at least 37 nucleosides in length. In some embodiments, an oligonucleotide is at least 38 nucleosides in length. In some embodiments, an oligonucleotide is at least 39 nucleosides in length. In some embodiments, an oligonucleotide is at least 40 nucleosides in length. In some embodiments, an oligonucleotide is 25 nucleosides in length. In some embodiments, an oligonucleotide is 26 nucleosides in length. In some embodiments, an oligonucleotide is 27 nucleosides in length. In some embodiments, an oligonucleotide is 28 nucleosides in length. In some embodiments, an oligonucleotide is 29 nucleosides in length. In some embodiments, an oligonucleotide is 30 nucleosides in length. In some embodiments, an oligonucleotide is 31 nucleosides in length. In some embodiments, an oligonucleotide is 32 nucleosides in length. In some embodiments, an oligonucleotide is 33 nucleosides in length. In some embodiments, an oligonucleotide is 34 nucleosides in length. In some

embodiments, an oligonucleotide is 35 nucleosides in length. In some embodiments, an oligonucleotide is 36 nucleosides in length. In some embodiments, an oligonucleotide is 37 nucleosides in length. In some embodiments, an oligonucleotide is 38 nucleosides in length. In some embodiments, an oligonucleotide is 39 nucleosides in length. In some embodiments, an oligonucleotide is 40 nucleosides in length. In some embodiments, each nucleoside counted in an oligonucleotide length independently comprises a nucleobase comprising a ring having at least one nitrogen ring atom. In some embodiments, each nucleoside counted in an oligonucleotide length independently comprises A, T, C, G, or U, or optionally substituted A, T, C, G, or U, or an optionally substituted tautomer of A, T, C, G or U.

[0048] Optionally Substituted: As described herein, compounds of the disclosure may contain optionally substituted, substituted and/or unsubstituted moieties. In general, the term “substituted,” means that one or more hydrogens of the designated moiety are independently replaced with a substituent. Unless otherwise indicated, an “optionally substituted” group may independently have a substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with two or more substituents, the substituents may be either the same or different at every position. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Various substituents are described below.

[0049] Monovalent substituents are independently halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$; $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}Ph$, which may be substituted with R° ; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R° ; $-CH=CHPh$, which may be substituted with R° ; $-(CH_2)_{0-4}O(CH_2)_{0-1}$ -pyridyl which may be substituted with R° ; $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)N(R^\circ)_2$; $-N(R^\circ)C(S)N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)N(R^\circ)_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSi(R^\circ)_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)N(R^\circ)_2$; $-C(S)N(R^\circ)_2$; $-C(S)SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)N(R^\circ)_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2N(R^\circ)_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2N(R^\circ)_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NH)N(R^\circ)_2$; $-Si(R^\circ)_3$; $-OSi(R^\circ)_3$; $-P(R^\circ)_2$; $-P(OR^\circ)_2$; $-OP(R^\circ)_2$; $-OP(OR^\circ)_2$; $-N(R^\circ)P(R^\circ)_2$; $-B(R^\circ)_2$; $-OB(R^\circ)_2$; $-P(O)(R^\circ)_2$; $-OP(O)(R^\circ)_2$; $-N(R^\circ)P(O)(R^\circ)_2$; $-(C_{1-4}$ straight or branched alkylene) $O-N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene) $C(O)O-N(R^\circ)_2$; wherein each R° may be independently substituted as defined below and is independently hydrogen, C_{1-10} (e.g., C_{1-6} , C_{1-4} , etc.) aliphatic, C_{1-10} (e.g., C_{1-6} , C_{1-4} , etc.) heteroaliphatic having 1-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, C_{6-10} (e.g., C_6 , C_{10} , etc.) aryl, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having

1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur, $-\text{CH}_2-(\text{C}_{6-10}$ (e.g., C_6 , C_{10} , etc.) aryl), $-\text{O}(\text{CH}_2)_{0-1}(\text{C}_{6-10}$ (e.g., C_6 , C_{10} , etc.) aryl), $-\text{CH}_2-(5-10$ (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur), $-\text{O}(\text{CH}_2)_{0-1}(5-10$ (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur), a 3-10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, or partially unsaturated ring having 0-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, or, notwithstanding the definition above, two independent occurrences of R° , taken together with their intervening atom(s), form a 3-10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aromatic ring (for aromatic ring, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered) having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below.

[0050] Monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, $-(\text{CH}_2)_{0-2}\text{R}^\bullet$, $-(\text{haloR}^\bullet)$, $-(\text{CH}_2)_{0-2}\text{OH}$, $-(\text{CH}_2)_{0-2}\text{OR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{CH}(\text{OR}^\bullet)_2$, $-\text{O}(\text{haloR}^\bullet)$, $-\text{CN}$, $-\text{N}_3$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{R}^\bullet$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OH}$, $-(\text{CH}_2)_{0-2}\text{C}(\text{O})\text{OR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{SR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{SH}$, $-(\text{CH}_2)_{0-2}\text{NH}_2$, $-(\text{CH}_2)_{0-2}\text{NHR}^\bullet$, $-(\text{CH}_2)_{0-2}\text{NR}^\bullet_2$, $-\text{NO}_2$, $-\text{SiR}^\bullet_3$, $-\text{OSiR}^\bullet_3$, $-\text{C}(\text{O})\text{SR}^\bullet$, $-(\text{C}_{1-4}$ straight or branched alkylene) $\text{C}(\text{O})\text{OR}^\bullet$, or $-\text{SSR}^\bullet$ wherein each $\text{R}^\bullet$ is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-\text{CH}_2\text{Ph}$, $-\text{O}(\text{CH}_2)_{0-1}\text{Ph}$, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents on a saturated carbon atom of R° are independently $=\text{O}$ or $=\text{S}$.

[0051] Divalent substituents are independently the following: $=\text{O}$, $=\text{S}$, $=\text{NNR}^*_{2-3}$, $=\text{NNHC}(\text{O})\text{R}^*$, $=\text{NNHC}(\text{O})\text{OR}^*$, $=\text{NNHS}(\text{O})_2\text{R}^*$, $=\text{NR}^*$, $=\text{NOR}^*$, $-\text{O}(\text{C}(\text{R}^*)_{2-3})_{2-3}\text{O}-$, or $-\text{S}(\text{C}(\text{R}^*)_{2-3})_{2-3}\text{S}-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents that are bound to vicinal substitutable carbons of an “optionally substituted” group are independently $-\text{O}(\text{CR}^*_{2-3})_{2-3}\text{O}-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0052] Substituents on the aliphatic group of R^* are independently halogen, $-\text{R}^\bullet$, $-(\text{haloR}^\bullet)$, $-\text{OH}$, $-\text{OR}^\bullet$, $-\text{O}(\text{haloR}^\bullet)$, $-\text{CN}$, $-\text{C}(\text{O})\text{OH}$, $-\text{C}(\text{O})\text{OR}^\bullet$, $-\text{NH}_2$, $-\text{NHR}^\bullet$, $-\text{NR}^\bullet_2$, or $-\text{NO}_2$, wherein each $\text{R}^\bullet$ is

unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently C₁₋₄ aliphatic, -CH₂Ph, -O(CH₂)₀₋₁Ph, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0053] Substituents on a substitutable nitrogen are independently -R[†], -NR[†]₂, -C(O)R[†], -C(O)OR[†], -C(O)C(O)R[†], -C(O)CH₂C(O)R[†], -S(O)₂R[†], -S(O)₂NR[†]₂, -C(S)NR[†]₂, -C(NH)NR[†]₂, or -N(R[†])S(O)₂R[†]; wherein each R[†] is independently hydrogen, C₁₋₆ aliphatic which may be substituted as defined below, unsubstituted -OPh, or an unsubstituted 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur, or, notwithstanding the definition above, two independent occurrences of R[†], taken together with their intervening atom(s) form an unsubstituted 3-12 (e.g., 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0054] Substituents on the aliphatic group of R[†] are independently halogen, -R[•], -(haloR[•]), -OH, -OR[•], -O(haloR[•]), -CN, -C(O)OH, -C(O)OR[•], -NH₂, -NHR[•], -NR[•]₂, or -NO₂, wherein each R[•] is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently C₁₋₄ aliphatic, -CH₂Ph, -O(CH₂)₀₋₁Ph, or a 3-6 (e.g., 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0055] Partially unsaturated: As used herein, the term “partially unsaturated” refers to a ring moiety that includes at least one double or triple bond. The term “partially unsaturated” is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[0056] Pharmaceutical composition: As used herein, the term “pharmaceutical composition” refers to an active agent, formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, an active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray

applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0057] Pharmaceutically acceptable: As used herein, the phrase “pharmaceutically acceptable” refers to those compounds, materials, compositions and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0058] Pharmaceutically acceptable carrier: As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0059] Pharmaceutically acceptable salt: The term “pharmaceutically acceptable salt”, as used herein, refers to salts of such compounds that are appropriate for use in pharmaceutical contexts, i.e., salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66: 1-19 (1977). In some embodiments, pharmaceutically acceptable salt include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate,

bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, a provided compound comprises one or more acidic groups, e.g., an oligonucleotide, and a pharmaceutically acceptable salt is an alkali, alkaline earth metal, or ammonium (e.g., an ammonium salt of $N(R)_3$, wherein each R is independently defined and described in the present disclosure) salt. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, a pharmaceutically acceptable salt is a sodium salt. In some embodiments, a pharmaceutically acceptable salt is a potassium salt. In some embodiments, a pharmaceutically acceptable salt is a calcium salt. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate and aryl sulfonate. In some embodiments, a provided compound comprises more than one acid groups, for example, an oligonucleotide may comprise two or more acidic groups (e.g., in natural phosphate linkages and/or modified internucleotidic linkages). In some embodiments, a pharmaceutically acceptable salt, or generally a salt, of such a compound comprises two or more cations, which can be the same or different. In some embodiments, in a pharmaceutically acceptable salt (or generally, a salt), all ionizable hydrogen (e.g., in an aqueous solution with a pKa no more than about 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2; in some embodiments, no more than about 7; in some embodiments, no more than about 6; in some embodiments, no more than about 5; in some embodiments, no more than about 4; in some embodiments, no more than about 3) in the acidic groups are replaced with cations. In some embodiments, each phosphorothioate and phosphate group independently exists in its salt form (e.g., if sodium salt, $-O-P(O)(SNa)-O-$ and $-O-P(O)(ONa)-O-$, respectively). In some embodiments, each phosphorothioate and phosphate internucleotidic linkage independently exists in its salt form (e.g., if sodium salt, $-O-P(O)(SNa)-O-$ and $-O-P(O)(ONa)-O-$, respectively). In some embodiments, a pharmaceutically acceptable salt is a sodium salt of an oligonucleotide. In some embodiments, a pharmaceutically acceptable salt is a sodium salt of an oligonucleotide, wherein each acidic phosphate and modified phosphate group (e.g., phosphorothioate, phosphate, etc.), if any, exists as a salt form (all sodium salt).

[0060] Predetermined: By predetermined (or pre-determined) is meant deliberately selected or non-random or controlled, for example as opposed to randomly occurring, random, or achieved without control.

Those of ordinary skill in the art, reading the present specification, will appreciate that the present disclosure provides technologies that permit selection of particular chemistry and/or stereochemistry features to be incorporated into oligonucleotide compositions, and further permits controlled preparation of oligonucleotide compositions having such chemistry and/or stereochemistry features. Such provided compositions are “predetermined” as described herein. Compositions that may contain certain oligonucleotides because they happen to have been generated through a process that are not controlled to intentionally generate the particular chemistry and/or stereochemistry features are not “predetermined” compositions. In some embodiments, a predetermined composition is one that can be intentionally reproduced (e.g., through repetition of a controlled process). In some embodiments, a predetermined level of a plurality of oligonucleotides in a composition means that the absolute amount, and/or the relative amount (ratio, percentage, etc.) of the plurality of oligonucleotides in the composition is controlled. In some embodiments, a predetermined level of a plurality of oligonucleotides in a composition is achieved through chirally controlled oligonucleotide preparation.

[0061] Protecting group: The term “protecting group,” as used herein, is well known in the art and includes those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, the entirety of which is incorporated herein by reference. Also included are those protecting groups specially adapted for nucleoside and nucleotide chemistry described in *Current Protocols in Nucleic Acid Chemistry*, edited by Serge L. Beaucage et al. 06/2012, the entirety of Chapter 2 is incorporated herein by reference. Suitable amino-protecting groups include methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-*t*-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenyl)ethyl carbamate (Bpoc), 1-(3,5-di-*t*-butylphenyl)-1-methylethyl carbamate (*t*-Bumeoc), 2-(2'- and 4'-pyridyl)ethyl carbamate (Pyoc), 2-(*N,N*-dicyclohexylcarboxamido)ethyl carbamate, *t*-butyl carbamate (BOC), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, *N*-hydroxypiperidinyll carbamate, alkylidithio carbamate, benzyl carbamate (Cbz), *p*-methoxybenzyl carbamate (Moz), *p*-nitrobenzyl carbamate, *p*-bromobenzyl carbamate, *p*-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (MsZ), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(*p*-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-

methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, m-chloro-p-acyloxybenzyl carbamate, p-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolymethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcro), m-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, o-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(o-nitrophenyl)methyl carbamate, phenothiazinyl-(10)-carbonyl derivative, N'-p-toluenesulfonylaminocarbonyl derivative, N'-phenylaminothiocarbonyl derivative, t-amyl carbamate, S-benzyl thiocarbamate, p-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p-decyloxybenzyl carbamate, 2,2-dimethoxycarbonylvinyl carbamate, o-(N,N-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(N,N-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isoborynl carbamate, isobutyl carbamate, isonicotinyl carbamate, p-(p'-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(p-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, p-(phenylazo)benzyl carbamate, 2,4,6-tri-t-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, 2,4,6-trimethylbenzyl carbamate, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, N-benzoylphenylalanyl derivative, benzamide, p-phenylbenzamide, o-nitrophenylacetamide, o-nitrophenoxycetamide, acetoacetamide, (N'-dithiobenzyloxycarbonylamino)acetamide, 3-(p-hydroxyphenyl)propanamide, 3-(o-nitrophenyl)propanamide, 2-methyl-2-(o-nitrophenoxycetamide), 2-methyl-2-(o-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, o-nitrocinnamide, N-acetylmethionine derivative, o-nitrobenzamide, o-(benzoyloxymethyl)benzamide, 4,5-diphenyl-3-oxazolin-2-one, N-phthalimide, N-dithiasuccinimide (Dts), N-2,3-diphenylmaleimide, N-2,5-dimethylpyrrole, N-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, N-methylamine, N-allylamine, N-[2-(trimethylsilyl)ethoxy]methylamine (SEM), N-3-acetoxypyrrolamine, N-(1-isopropyl-4-nitro-2-oxo-3-pyrroline-3-yl)amine, quaternary ammonium salts, N-benzylamine, N-di(4-methoxyphenyl)methylamine, N-5-dibenzosuberylamine, N-triphenylmethylamine (Tr), N-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), N-9-phenylfluorenylamine (PhF), N-2,7-dichloro-9-fluorenylmethylencamine, N-ferrocenylmethylamino (Fcm), N-2-picolylamino N'-oxide, N-1,1-dimethylthiomethyleneamine, N-benzylideneamine, N-p-methoxybenzylideneamine, N-

diphenylmethyleamine, N-[(2-pyridyl)mesityl]methyleamine, N-(N',N'-dimethylaminomethylene)amine, N,N'-isopropylidenediamine, N-p-nitrobenzylideneamine, N-salicylideneamine, N-5-chlorosalicylideneamine, N-(5-chloro-2-hydroxyphenyl)phenylmethyleamine, N-cyclohexylideneamine, N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, N-borane derivative, N-diphenylborinic acid derivative, N-[phenyl(pentacarbonylchromium- or tungsten)carbonyl]amine, N-copper chelate, N-zinc chelate, N-nitroamine, N-nitrosoamine, amine N-oxide, diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, 3-nitropyridinesulfenamide (Npys), p-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β -trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

[0062] Suitably protected carboxylic acids further include, but are not limited to, silyl-, alkyl-, alkenyl-, aryl-, and arylalkyl-protected carboxylic acids. Examples of suitable silyl groups include trimethylsilyl, triethylsilyl, t-butyltrimethylsilyl, t-butylphenylsilyl, triisopropylsilyl, and the like. Examples of suitable alkyl groups include methyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, trityl, t-butyl, tetrahydropyran-2-yl. Examples of suitable alkenyl groups include allyl. Examples of suitable aryl groups include optionally substituted phenyl, biphenyl, or naphthyl. Examples of suitable arylalkyl groups include optionally substituted benzyl (e.g., p-methoxybenzyl (MPM), 3,4-dimethoxybenzyl, O-nitrobenzyl, p-nitrobenzyl, p-halobenzy, 2,6-dichlorobenzy, p-cyanobenzy), and 2- and 4-picoly.

[0063] Suitable hydroxyl protecting groups include methyl, methoxymethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenylloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-

methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl, p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α -naphthylidiphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4''-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-tris(levulinoyloxyphenyl)methyl, 4,4',4''-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyl dimethylsilyl (TBDMS), t-butyl diphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), alkyl methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), alkyl ethyl carbonate, alkyl 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), alkyl isobutyl carbonate, alkyl vinyl carbonate, alkyl allyl carbonate, alkyl p-nitrophenyl carbonate, alkyl benzyl carbonate, alkyl p-methoxybenzyl carbonate, alkyl 3,4-dimethoxybenzyl carbonate, alkyl o-nitrobenzyl carbonate, alkyl p-nitrobenzyl carbonate, alkyl S-benzyl thiocarbonate, 4-ethoxy-1-naphthyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenate, o-(methoxycarbonyl)benzoate, α -naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-

dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzyisulfonate, and tosylate (Ts). For protecting 1,2- or 1,3-diols, the protecting groups include methylene acetal, ethylidene acetal, 1-*t*-butylethylidene ketal, 1-phenylethylidene ketal, (4-methoxyphenyl)ethylidene acetal, 2,2,2-trichloroethylidene acetal, acetonide, cyclopentylidene ketal, cyclohexylidene ketal, cycloheptylidene ketal, benzylidene acetal, *p*-methoxybenzylidene acetal, 2,4-dimethoxybenzylidene ketal, 3,4-dimethoxybenzylidene acetal, 2-nitrobenzylidene acetal, methoxymethylene acetal, ethoxymethylene acetal, dimethoxymethylene ortho ester, 1-methoxyethylidene ortho ester, 1-ethoxyethylidene ortho ester, 1,2-dimethoxyethylidene ortho ester, α -methoxybenzylidene ortho ester, 1-(*N,N*-dimethylamino)ethylidene derivative, α -(*N,N'*-dimethylamino)benzylidene derivative, 2-oxacyclopentylidene ortho ester, di-*t*-butylsilylene group (DTBS), 1,3-(1,1,3,3-tetraisopropylidisiloxanylidene) derivative (TIPDS), tetra-*t*-butoxydisiloxane-1,3-diylidene derivative (TBDS), cyclic carbonates, cyclic boronates, ethyl boronate, and phenyl boronate.

[0064] In some embodiments, a hydroxyl protecting group is acetyl, *t*-butyl, *t*butoxymethyl, methoxymethyl, tetrahydropyranyl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 2-trimethylsilylethyl, *p*-chlorophenyl, 2,4-dinitrophenyl, benzyl, benzoyl, *p*-phenylbenzoyl, 2,6-dichlorobenzyl, diphenylmethyl, *p*-nitrobenzyl, triphenylmethyl (trityl), 4,4'-dimethoxytrityl, trimethylsilyl, triethylsilyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl, triphenylsilyl, triisopropylsilyl, benzoylformate, chloroacetyl, trichloroacetyl, trifluoroacetyl, pivaloyl, 9-fluorenylmethyl carbonate, mesylate, tosylate, triflate, trityl, monomethoxytrityl (MMTr), 4,4'-dimethoxytrityl, (DMTr) and 4,4',4''-trimethoxytrityl (TMTr), 2-cyanoethyl (CE or Cne), 2-(trimethylsilyl)ethyl (TSE), 2-(2-nitrophenyl)ethyl, 2-(4-cyanophenyl)ethyl 2-(4-nitrophenyl)ethyl (NPE), 2-(4-nitrophenylsulfonyl)ethyl, 3,5-dichlorophenyl, 2,4-dimethylphenyl, 2-nitrophenyl, 4-nitrophenyl, 2,4,6-trimethylphenyl, 2-(2-nitrophenyl)ethyl, butylthiocarbonyl, 4,4',4''-tris(benzoyloxy)trityl, diphenylcarbamoyl, levuliny, 2-(dibromomethyl)benzoyl (Dbmb), 2-(isopropylthiomethoxymethyl)benzoyl (Ptmt), 9-phenylxanthene-9-yl (pixyl) or 9-(*p*-methoxyphenyl)xanthine-9-yl (MOX). In some embodiments, each of the hydroxyl protecting groups is, independently selected from acetyl, benzyl, *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl and 4,4'-dimethoxytrityl. In some embodiments, the hydroxyl protecting group is selected from the group consisting of trityl, monomethoxytrityl and 4,4'-dimethoxytrityl group. In some embodiments, a phosphorous linkage protecting group is a group attached to the phosphorous linkage (e.g., an internucleotidic linkage) throughout oligonucleotide synthesis. In some embodiments, a protecting group is attached to a sulfur atom of an phosphorothioate group. In some embodiments, a protecting group is attached to an oxygen atom of an internucleotide phosphorothioate linkage. In some embodiments, a protecting group is attached to an oxygen atom of the internucleotide phosphate linkage. In some embodiments a protecting group is 2-cyanoethyl (CE or Cne), 2-trimethylsilylethyl, 2-nitroethyl, 2-sulfonylethyl, methyl, benzyl, *o*-nitrobenzyl,

2-(p-nitrophenyl)ethyl (NPE or Npe), 2-phenylethyl, 3-(N-tert-butylcarboxamido)-1-propyl, 4-oxopentyl, 4-methylthio-1-butyl, 2-cyano-1,1-dimethylethyl, 4-N-methylaminobutyl, 3-(2-pyridyl)-1-propyl, 2-[N-methyl-N-(2-pyridyl)]aminoethyl, 2-(N-formyl,N-methyl)aminoethyl, or 4-[N-methyl-N-(2,2,2-trifluoroacetyl)amino]butyl.

[0065] Subject: As used herein, the term “subject” or “test subject” refers to any organism to which a compound (e.g., an oligonucleotide) or composition is administered in accordance with the present disclosure e.g., for experimental, diagnostic, prophylactic and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; etc.) and plants. In some embodiments, a subject is a human. In some embodiments, a subject may be suffering from and/or susceptible to a disease, disorder and/or condition.

[0066] Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. A base sequence which is substantially identical or complementary to a second sequence is not fully identical or complementary to the second sequence, but is mostly or nearly identical or complementary to the second sequence. In some embodiments, an oligonucleotide with a substantially complementary sequence to another oligonucleotide or nucleic acid forms duplex with the oligonucleotide or nucleic acid in a similar fashion as an oligonucleotide with a fully complementary sequence. In addition, one of ordinary skill in the biological and/or chemical arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” is therefore used herein to capture the potential lack of completeness inherent in many biological and/or chemical phenomena.

[0067] Sugar: The term “sugar” refers to a monosaccharide or polysaccharide in closed and/or open form. In some embodiments, sugars are monosaccharides. In some embodiments, sugars are polysaccharides. Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties. As used herein, the term “sugar” also encompasses structural analogs used in lieu of conventional sugar molecules, such as glycol, polymer of which forms the backbone of the nucleic acid analog, glycol nucleic acid (“GNA”), etc. As used herein, the term “sugar” also encompasses structural analogs used in lieu of natural or naturally-occurring nucleotides, such as modified sugars and nucleotide sugars. In some embodiments, a sugar is a RNA or DNA sugar (ribose or deoxyribose). In some embodiments, a sugar is a modified ribose or deoxyribose sugar, e.g., 2'-modified, 5'-modified, etc. As described herein, in some embodiments, when used in oligonucleotides and/or nucleic acids, modified sugars may provide one or more desired properties, activities, etc. In some embodiments, a sugar is optionally substituted ribose or deoxyribose. In some embodiments, a “sugar” refers to a sugar unit in an oligonucleotide or a nucleic acid.

[0068] Susceptible to: An individual who is “susceptible to” a disease, disorder and/or condition is one who has a higher risk of developing the disease, disorder and/or condition than does a member of the general public. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition is predisposed to have that disease, disorder and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not have been diagnosed with the disease, disorder and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may exhibit symptoms of the disease, disorder and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder and/or condition may not exhibit symptoms of the disease, disorder and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0069] Therapeutic agent: As used herein, the term “therapeutic agent” in general refers to any agent that elicits a desired effect (e.g., a desired biological, clinical, or pharmacological effect) when administered to a subject. In some embodiments, an agent is considered to be a therapeutic agent if it demonstrates a statistically significant effect across an appropriate population. In some embodiments, an appropriate population is a population of subjects suffering from and/or susceptible to a disease, disorder or condition. In some embodiments, an appropriate population is a population of model organisms. In some embodiments, an appropriate population may be defined by one or more criterion such as age group, gender, genetic background, preexisting clinical conditions, prior exposure to therapy. In some embodiments, a therapeutic agent is a substance that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms or features of a disease, disorder, and/or condition in a subject when administered to the subject in an effective amount. In some embodiments, a “therapeutic agent” is an agent that has been or is required to be approved by a government agency before it can be marketed for administration to humans. In some embodiments, a “therapeutic agent” is an agent for which a medical prescription is required for administration to humans. In some embodiments, a therapeutic agent is a provided compound, e.g., a provided oligonucleotide.

[0070] Therapeutically effective amount: As used herein, the term “therapeutically effective amount” means an amount of a substance (e.g., a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired

biological endpoint, the substance to be delivered, the target cell or tissue, etc. For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0071] Treat: As used herein, the term “treat,” “treatment,” or “treating” refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition. In some embodiments, treatment may be administered to a subject who exhibits only early signs of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[0072] Unsaturated: The term “unsaturated,” as used herein, means that a moiety has one or more units of unsaturation.

[0073] As those skilled in the art will appreciate, methods and compositions described herein relating to provided compounds (e.g., oligonucleotides) generally also apply to pharmaceutically acceptable salts of such compounds.

Description of Certain Embodiments

[0074] Certain embodiments are described below as examples.

[0075] Among other things, the present disclosure encompasses the recognition that traditional technologies for incorporating $-P(O)(OH)_2$ groups into compounds, e.g., utilization of protected groups such as $-P(O)(OMe)_2$, $-P(O)(OEt)_2$, etc., often utilize deprotection conditions that can reduce efficiency and/or increase cost of manufacturing. For example, when $-P(O)(OMe)_2$, $-P(O)(OEt)_2$, etc., are utilized to incorporate $-P(O)(OH)_2$ into oligonucleotides, removal of the methyl or ethyl groups may require extra steps and/or harsher reaction conditions which can lead to lower manufacturing efficiency, lower oligonucleotide yields and/or purity, and/or increased cost compared to manufacturing of reference oligonucleotides without $-P(O)(OH)_2$ (e.g., otherwise identical oligonucleotides without $-P(O)(OH)_2$).

[0076] In some embodiments, the present disclosure provides technologies for incorporating $-P(O)(OH)_2$ into compounds, e.g., oligonucleotides which technologies can provide higher yield, higher purity, higher efficiency, lower operation complexity, milder reaction condition, shorter preparation time, and/or lower cost, etc. when compared to using, e.g., $-P(O)(OMe)_2$, or $-P(O)(OEt)_2$. In some embodiments, the present disclosure provides protected $-P(O)(OH)_2$ groups which can be deprotected

under oligonucleotide synthesis conditions, e.g., during cleavage and/or deprotection, utilized for oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, provided technologies are useful for preparing stereorandom oligonucleotide compositions. In some embodiments, provided technologies are useful for stereoselective oligonucleotide synthesis. In some embodiments, a stereoselective oligonucleotide synthesis stereoselectively and independently prepares one stereochemical configuration of each of one or more or all chiral linkage phosphorus in an oligonucleotide over the other. In some embodiments, provided technologies are useful for preparing chirally controlled oligonucleotide compositions. In some embodiments, a chiral auxiliary is utilized during stereoselective oligonucleotide synthesis. Certain useful stereoselective oligonucleotide synthesis technologies including chiral auxiliaries, cycles, conditions, etc. are described in US 10167309, US 11643657, US 11718638, US 11608355 and US 20230089442, the entirety of each of which is independently incorporated herein by reference.

[0077] For example, in some embodiments, provided technologies utilizes $-P(O)(OR^{PG})_2$ as described herein for prepare compounds including oligonucleotides. In some embodiments, $-P(O)(OR^{PG})_2$ are converted into $-P(O)(OH)_2$ during manufacturing or after administration to a subject.

[0078] In some embodiments, the present disclosure provides technologies comprising $-P(O)(OR^{PG})_2$ wherein each R^{PG} is a group that is labile to oligonucleotide synthesis conditions, e.g., during cleavage, deprotection and/or chiral auxiliary removal, utilized for reference oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, each R^{PG} is a group that can be removed under oligonucleotide synthesis conditions for reference oligonucleotides without $-P(O)(OH)_2$ groups. In some embodiments, $-P(O)(OR^{PG})_2$ is a protected $-P(O)(OH)_2$ group that is converted to $-P(O)(OH)_2$ which may exist in a salt form, e.g., under oligonucleotide synthesis conditions. In some embodiments, as exemplified herein the P atom is bonded to a carbon atom.

[0079] In some embodiments, R^{PG} comprises 1) an alpha carbon atom bonded to the oxygen to which it is attached, wherein the alpha carbon atom is bonded to a hydrogen, and 2) a beta carbon atom, wherein the beta carbon atom is bonded to a group R^{PG11} that can facilitate removal of R^{PG} (e.g., compared to ethyl), such as an electron-withdrawing group. In some embodiments, R^{PG11} is an electron-withdrawing group (e.g., as described in US 20230089442). In some embodiments, R^{PG11} is R^s as described herein. In some embodiments, R^{PG11} is $-CN$. In some embodiments, R^{PG11} is $-L^s-R^{s11}$ wherein each L^s and R^{s11} is independently as described herein. In some embodiments, R^{PG} is $-CH_2-R^s$, wherein the $-CH_2-$ is independently optionally substituted and R^s is as described herein. In some embodiments, the $-CH_2-$ is optionally mono-substituted. In some embodiments, R^{PG} is R^{P1} as described herein. In some embodiments, R^{PG} is R^{P2} as described herein. In some embodiments, R^{PG} is $-O-CH_2CH_2CN$.

[0080] In some embodiments, R^{PG} is $-CH_2-O(CO)R^{PG12}$, wherein R^{PG12} is trisubstituted methyl and

the $-\text{CH}_2-$ is optionally substituted. In some embodiments, R^{PG} is $-\text{CH}_2-\text{O}(\text{CO})\text{R}^{\text{PG12}}$, wherein R^{PG12} is $-\text{C}(\text{R}^{\text{s11}})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, R^{PG} is R^{PG12} , wherein R^{PG12} is trisubstituted methyl. In some embodiments, R^{PG12} is $-\text{C}(\text{R}^{\text{s11}})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, the carbon of the trisubstituted methyl or $-\text{C}(\text{R}^{\text{s11}})_3$ is bonded to three carbon atoms. In some embodiments, R^{PG12} is optionally substituted t-butyl. In some embodiments, R^{PG12} is t-butyl. In some embodiments, R^{PG} is R^{P1} or R^{P2} as described herein. In some embodiments, R^{PG} is $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl. In some embodiments, R^{PG} is $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{tBu}$.

[0081] In some embodiments, $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ has the structure of $-\text{P}(\text{O})(\text{R}^{\text{P1}})(\text{R}^{\text{P2}})$ as described herein (e.g., in formula I or formula I'), wherein each of R^{P1} and R^{P2} is independently as described herein.

[0082] In some embodiments, R^{PG} is labile under a basic condition. In some embodiments, R^{PG} is labile under an acidic condition. In some embodiments, $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ is converted into $-\text{P}(\text{O})(\text{OH})_2$ under an acidic condition. In some embodiments, $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ is converted into $-\text{P}(\text{O})(\text{OH})_2$ under a basic condition. In some embodiments, a condition is utilized in oligonucleotide synthesis for a reference oligonucleotide which does not have a $-\text{P}(\text{O})(\text{OH})_2$ group but is otherwise identical. In some embodiments, a condition is a cleavage and/or deprotection condition in oligonucleotide synthesis. In some embodiments, a condition is a chiral auxiliary removal, cleavage and/or deprotection condition in oligonucleotide synthesis.

[0083] As those skilled in the art appreciate, acidic and/or basic groups, e.g., $-\text{P}(\text{O})(\text{OH})_2$, may exist in various forms including various salt forms. In some embodiments, an acidic and/or basic group, e.g., $-\text{P}(\text{O})(\text{OH})_2$, is in a salt form. In some embodiments, an acidic and/or basic group, e.g., $-\text{P}(\text{O})(\text{OH})_2$, is in a pharmaceutically acceptable salt form.

[0084] In some embodiments, as described below, the present disclosure provides technologies, e.g., compounds (e.g., nucleosides, phosphoramidites, oligonucleotides, reaction reagents, etc.), methods, etc., that are useful for, among other things, oligonucleotide synthesis.

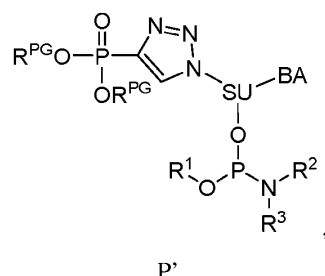
Phosphoramidites

[0085] Various phosphoramidites can be utilized for oligonucleotide preparation, e.g., those described in US 10167309, US 11643657, US 11718638, US 11608355, US 20230089442, etc. In some embodiments, the present disclosure provides phosphoramidites comprising $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ as described herein. In some embodiments, a phosphoramidite comprises $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ which is bonded to a sugar moiety of a nucleoside. In some embodiments, a phosphoramidite comprises $-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ which is bonded to a moiety, e.g., an optionally substituted triazole ring, that is bonded to a sugar moiety of a nucleoside. In some embodiments, a sugar is a natural sugar. In some embodiments, a sugar is a

modified sugar. In some embodiments, a nucleobase in a phosphoramidite is an optionally substituted natural nucleobase. In some embodiments, a nucleobase in a phosphoramidite is optionally protected A, T, C, G, U, or 5mC (e.g., for oligonucleotide synthesis). In some embodiments, a phosphoramidite is abasic.

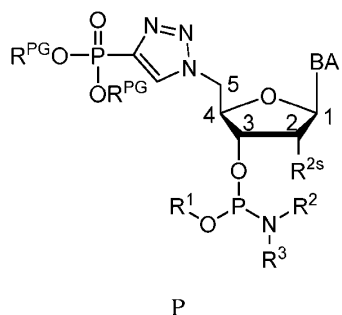
[0086] In some embodiments, the present disclosure provides a compound having the structure of $(R^{PG}O)_2P(O)-L^{5E}-SU(-BA)-OP(OR^1)N(R^2)(R^3)$ or a salt thereof, wherein L^{5E} is L as described herein, SU is a sugar as described herein, and each other variable is independently as described herein. In some embodiments, such a compound is utilized as a phosphoramidite for oligonucleotide synthesis. In some embodiments, L^{5E} is an optionally substituted triazole ring.

[0087] In some embodiments, the provided technology provides a compound of formula P' or a salt thereof:



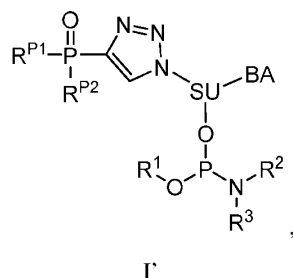
wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a phosphoramidite for oligonucleotide synthesis.

[0088] In some embodiments, the provided technology provides a compound of formula P or a salt thereof:



wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a phosphoramidite for oligonucleotide synthesis.

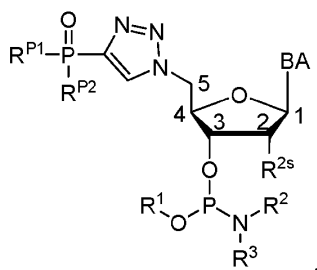
[0089] In some embodiments, the provided technology provides a compound of formula I' or a salt thereof:



I'

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a phosphoramidite for oligonucleotide synthesis.

[0090] In some embodiments, the provided technology provides a compound of formula I or a salt thereof:



I

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a phosphoramidite for oligonucleotide synthesis.

[0091] Certain embodiments of certain variables utilized in various formulae are described below.

R^{P1}

[0092] In some embodiments, R^{P1} is -O-CH₂CH₂CN. In some embodiments, R^{P1} is -O-CH₂-O-C(O)-R^P, wherein R^P is optionally substituted tert-butyl. In some embodiments, R^{P1} is -O-CH₂-O-C(O)-tBu.

R^{P2}

[0093] In some embodiments, R^{P2} is -O-CH₂CH₂CN. In some embodiments, R^{P2} is -O-CH₂-O-C(O)-R^P, wherein R^P is optionally substituted tert-butyl. In some embodiments, R^{P2} is -O-CH₂-O-C(O)-tBu.

[0094] In some embodiments, R^{P1} and R^{P2} are the same. In some embodiments, R^{P1} and R^{P2} are different. In some embodiments, each of R^{P1} and R^{P2} is -O-CH₂CH₂CN. In some embodiments, each of R^{P1} and R^{P2} is independently -O-CH₂-O-C(O)-R^P, wherein R^P is optionally substituted tert-butyl. In some embodiments, each of R^{P1} and R^{P2} is -O-CH₂-O-C(O)-tBu.

R^P

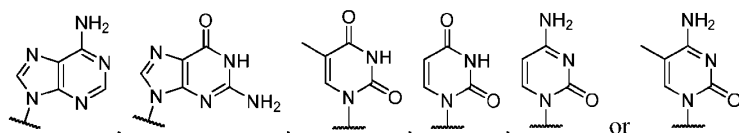
[0095] In some embodiments, R^P is substituted tert-butyl. In some embodiments, R^P is tert-butyl.

BA

[0096] In some embodiments, BA is hydrogen.

[0097] In some embodiments, BA is an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, BA is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, BA is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from nitrogen and oxygen. In some embodiments, BA is an optionally substituted ring having at least one nitrogen atom. In some embodiments, BA is an optionally substituted 5-15 membered ring. In some embodiments, BA is an optionally substituted 5-10 membered ring. In some embodiments, BA is an optionally substituted 5-9 membered ring. In some embodiments, BA is an optionally substituted 9-membered ring. In some embodiments, BA is an optionally substituted 10-membered ring. In some embodiments, an optionally substituted 5-membered ring. In some embodiments, BA is an optionally substituted 6-membered ring. In some embodiments, BA is an optionally substituted monocyclic ring. In some embodiments, BA is an optionally substituted bicyclic ring. In some embodiments, BA is bonded to C1 of a sugar at a nitrogen atom.

[0098] In some embodiments, BA is an optionally substituted group, wherein the group is

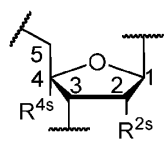


, or a tautomer thereof. In some embodiments, BA is an optionally protected nucleobase. In some embodiments, wherein BA is a protected nucleobase. In some embodiments, BA is an optionally protected nucleobase selected from A, T, C, G, U and 5mU. In some embodiments, protection is suitable for oligonucleotide synthesis.

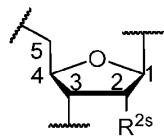
[0099] In some embodiments, BA is an optionally substituted or protected nucleobase. Various nucleobases are as described herein and can be utilized as BA.

SU

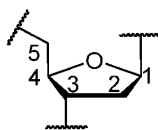
[0100] In some embodiments, SU is a sugar as described herein. In some embodiments, SU is



, wherein a nucleobase, BA, etc. is bonded at C1. In some embodiments, SU is



, wherein a nucleobase, BA, etc. as described herein, is bonded at C1. In some



embodiments, SU is optionally substituted , wherein a nucleobase, BA, etc. as described herein, is bonded at C1.

R^{2s}

[0101] In some embodiments, R^{s2} is $-H$. In some embodiments, R^{2s} is $-F$.

[0102] In some embodiments, R^{2s} is $-Cl$. In some embodiments, R^{2s} is $-Br$. In some embodiments, R^{2s} is $-I$. In some embodiments, R^{2s} is $-CN$. In some embodiments, R^{2s} is $-N_3$. In some embodiments, R^{2s} is $-NO$. In some embodiments, R^{2s} is $-NO_2$.

[0103] In some embodiments, R^{2s} is $-L^{2s}-R^{2a}$. In some embodiments, R^{s2} is R^{2a} . In some embodiments, R^{s2} is R . In some embodiments, R^{2s} is $-O-L^{2s'}-R^{2a}$, wherein $L^{2s'}$ is a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-4 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of $L^{2s'}$ are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$. In some embodiments, $L^{2s'}$ is a covalent bond. In some embodiments, $L^{2s'}$ is an optionally substituted bivalent C_{1-10} aliphatic group. In some embodiments, $L^{2s'}$ is a bivalent C_{1-10} aliphatic group. In some embodiments, $L^{2s'}$ is an optionally substituted bivalent linear C_{1-10} aliphatic group. In some embodiments, $L^{2s'}$ is a bivalent linear C_{1-10} aliphatic group. In some embodiments, $L^{2s'}$ is an optionally substituted C_{1-10} alkylene group. In some embodiments, $L^{2s'}$ is a C_{1-10} alkylene group. In some embodiments, $L^{2s'}$ is optionally substituted $-(CH_2)_{1-10}-$. In some embodiments, $L^{2s'}$ is $-(CH_2)_{1-10}-$. In some embodiments, $L^{2s'}$ is an optionally substituted bivalent C_{1-10} heteroaliphatic group having 1-4 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, one or more methylene units are independently replaced as described herein. For example, in some embodiments, R^{2s} is $-O(CH_2)_{0-10}R^{2a}$, wherein each $-CH_2-$ is optionally substituted. In some embodiments, R^{2a} is optionally substituted C_{1-10} aliphatic. In some embodiments,

R^{2a} is optionally substituted C_{1-10} alkyl. In some embodiments, R^{2a} is optionally substituted linear C_{1-10} alkyl. In some embodiments, R^{2a} is C_{1-10} alkyl. In some embodiments, R^{2a} is C_{1-10} linear alkyl. In some embodiments, R^{2s} is $-O(CH_2)_{0-15}CH_3$. In some embodiments, R^{2s} is $-O(CH_2)_{15}CH_3$.

[0104] In some embodiments, R^{2s} is $-L^{2s}-OR^{2a}$. In some embodiments, R^{2s} is $-OR^{2a}$. In some embodiments, R^{2s} is $-OMe$.

[0105] In some embodiments, R^{2s} is $-L^{2s}-SR^{2a}$. In some embodiments, R^{2s} is $-SR^{2a}$.

[0106] In some embodiments, R^{2s} is $-L^{2s}-N(R^{2a})_2$. In some embodiments, R^{2s} is $-N(R^{2a})_2$.

[0107] In some embodiments, R^{2s} is $-O-L^{2s}-OR^{2a}$. In some embodiments, R^{2s} is $-O-L^{2s}-SR^{2a}$. In some embodiments, R^{2s} is or $-O-L^{2s}-N(R^{2a})_2$. In some embodiments, L^{2s} is an optionally substituted bivalent C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent linear C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent linear C_{2-10} alkylene group. In some embodiments, R^{2s} is $-OCH_2CH_2OCH_3$.

[0108] In some embodiments, R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5 wherein L^s is L as described herein. For example, in some embodiments, L is $(C2)-O-(unsubstituted\ methylene)-$. In some embodiments, L is $(C2)-O-(substituted\ methylene)-$. In some embodiments, L is $(C2)-O-(substituted\ methylene)-$, wherein the methylene group is substituted with methyl. In some embodiments, L is $(C2)-O-(substituted\ methylene)-$, wherein the methylene group is substituted with ethyl. In some embodiments, the carbon atom of the methylene group is *R*. In some embodiments, the carbon atom of the methylene group is *S*.

[0109] In some embodiments, C2 and C4 are connected through $-O-CH_2-$, wherein the $-CH_2-$ is optionally substituted. In some embodiments, C2 and C4 are connected through $-O-CH_2-$. In some embodiments, C2 and C4 are connected through $-O-CH(CH_3)-$. In some embodiments, C2 and C4 are connected through $-O-CH(CH_2OMe)-$. In some embodiments, the configuration of the chiral carbon in L^{2s} is *R*. In some embodiments, the configuration of the chiral carbon in L^{2s} is *S*. In some embodiments, the $-O-$ is bonded to C2. In some embodiments, a sugar is a LNA sugar.

L^{2s}

[0110] In some embodiments, L^{2s} is a covalent bond. In some embodiments, L^{2s} is an optionally substituted bivalent C_{1-10} aliphatic group wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$. In some embodiments, L^{2s} is an optionally substituted bivalent C_{1-5} aliphatic group wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$. In some embodiments, L^{2s} is an optionally substituted bivalent C_{1-5} aliphatic

group wherein one or more methylene units of L are optionally and independently replaced by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{SC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. In some embodiments, L^{2s} is an optionally substituted bivalent C_{1-10} aliphatic group. In some embodiments, L^{2s} is an optionally substituted bivalent C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent linear C_{2-10} aliphatic group. In some embodiments, L^{2s} is a bivalent linear C_{1-10} alkylene group. In some embodiments, L^{2s} is a bivalent linear C_{2-10} alkylene group. In some embodiments, L^{2s} is optionally substituted $-\text{O}-\text{CH}_2-$.

R^{2a}

[0111] As defined herein, R^{2a} is R' as described herein. In some embodiments, R^{2a} is R as described herein. In some embodiments, R^{2a} is $-\text{H}$. In some embodiments, R^{2a} is C_{1-6} aliphatic. In some embodiments, R^{2a} is C_{1-6} alkyl. In some embodiments, R^{2a} is methyl.

[0112] In some embodiments, two or more R^{2a} are R' and are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from oxygen, nitrogen, sulfur. In some embodiments, two R^{2a} attached to the same atom are R' and are taken together with the atom to which they are attached to form an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, 3-20, 5-9, etc.) membered ring having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from oxygen, nitrogen, sulfur. In some embodiments, a formed ring is as described herein.

L

[0113] Certain embodiments for L are described below. Various variables, e.g., L^{2s} , L^s , etc., can be L , and embodiments for L can also be applied to such variables that can be L .

[0114] In some embodiments, L is a covalent bond. In some embodiments, L is a bivalent, optionally substituted C_{1-10} (e.g., C_{1-6} , C_{2-8} , C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 , C_8 , C_9 , C_{10} , etc.) aliphatic group wherein one or more methylene units of L are optionally and independently replaced by $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{SC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. In some embodiments, L is a bivalent, optionally substituted C_{1-6} aliphatic group wherein one or more methylene units of L are optionally and independently replaced by $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$, $-\text{SC}(\text{O})-$, or $-\text{C}(\text{O})\text{O}-$. In some embodiments, L is a bivalent, optionally substituted C_{1-5} aliphatic group wherein one or more methylene units of L are optionally and independently replaced by $-\text{Cy}-$, $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}')-$, $-\text{C}(\text{O})-$, $-\text{C}(\text{S})-$, $-\text{C}(\text{NR}')-$, $-\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{N}(\text{R}')-$, $-\text{N}(\text{R}')\text{C}(\text{O})\text{O}-$, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{S}(\text{O})_2\text{N}(\text{R}')-$,

–SC(O)–, or –C(O)O–. In some embodiments, each methylene unit are replaced. In some embodiments, at least one methylene unit is not replaced. In some embodiments, L comprises at least one chain carbon atom. In some embodiments, L is –O–. In some embodiments, L is –S–. In some embodiments, L is –N(R')–. In some embodiments, L is –C(O)–.

[0115] In some embodiments, no methylene unit is replaced. In some embodiments, L is a bivalent, optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) aliphatic group. In some embodiments, L is optionally substituted C₁₋₆ aliphatic group. In some embodiments, L is bivalent, optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) alkylene. In some embodiments, L is optionally substituted C₁₋₆ alkylene. In some embodiments, L is linear. In some embodiments, L is branched. In some embodiments, L is substituted. In some embodiments, L is not substituted.

[0116] In some embodiments, L is a bivalent, optionally substituted C₁₋₁₀ heteroaliphatic (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by –Cy–, –O–, –S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)₂–, –S(O)₂N(R')–, –SC(O)–, or –C(O)O–. In some embodiments, L is a bivalent, optionally substituted C₁₋₆ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by –Cy–, –O–, –S–, –N(R')–, –C(O)–, –C(S)–, –C(NR')–, –C(O)N(R')–, –N(R')C(O)N(R')–, –N(R')C(O)O–, –S(O)–, –S(O)₂–, –S(O)₂N(R')–, –SC(O)–, or –C(O)O–. In some embodiments, L is linear. In some embodiments, L is branched. In some embodiments, L is substituted. In some embodiments, L is not substituted.

[0117] In some embodiments, a methylene unit is replaced by –Cy– as described herein.

–Cy–

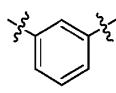
[0118] As used herein, –Cy– is an optionally substituted bivalent 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, –Cy– is an optionally substituted bivalent 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, –Cy– is monocyclic. In some embodiments, –Cy– is bicyclic. In some embodiments, –Cy– is polycyclic. In some

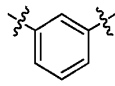
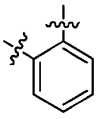
embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 5-8, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, a monocyclic ring unit is an optionally substituted saturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted partially unsaturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted aromatic ring. In some embodiments, a monocyclic ring unit is an optionally substituted phenyl ring. In some embodiments, a monocyclic ring unit is an optionally substituted 5-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is an optionally substituted 6-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. Each monocyclic ring unit is independently optionally substituted. In some embodiments, a monocyclic ring unit is unsaturated. In some embodiments, a monocyclic ring unit is saturated. In some embodiments, -Cy- is an optionally substituted saturated ring. In some embodiments, -Cy- is an optionally substituted partially unsaturated ring. In some embodiments, -Cy- is an optionally substituted aromatic ring.

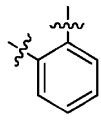
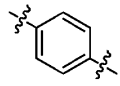
[0119] In some embodiments, -Cy- is an optionally substituted bivalent 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic cycloaliphatic ring. In some embodiments, -Cy- is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic cycloalkyl ring. In some embodiments, -Cy- is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic heteroaliphatic ring having 1-5 heteroatoms. In some embodiments, -Cy- is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic heteroalkyl ring having 1-5 heteroatoms. In some embodiments, -Cy- is an optionally substituted bivalent 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic cycloaliphatic group. In some embodiments, -Cy- is an optionally substituted bivalent 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic cycloalkyl group. In some embodiments, -Cy- is an optionally substituted 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic heteroaliphatic ring having 1-5 heteroatoms. In some embodiments, -Cy- is an optionally substituted 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic heterocyclyl ring having 1-5 heteroatoms. In some embodiments, a cycloaliphatic, cycloalkyl, heteroaliphatic or heteroalkyl ring is 3-membered. In some embodiments, it is 4-membered. In some embodiments, it is 5-membered. In some embodiments, it is 6-membered. In some embodiments, it is 7-membered. In some embodiments, it is 8-membered. In some embodiments, it is 9-membered. In some embodiments, it is 10-membered. In some embodiments, it is 11-membered. In some embodiments, it is 12-membered. In some embodiments, -Cy- is optionally

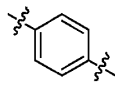
substituted phenylene. In some embodiments, –Cy– is an optionally substituted bivalent 10-membered bicyclic aryl ring. In some embodiments, –Cy– is an optionally substituted 5-membered heteroaryl ring having 1-4 heteroatoms. In some embodiments, –Cy– is an optionally substituted 6-membered heteroaryl ring having 1-4 heteroatoms. In some embodiments, –Cy– is an optionally substituted 9-membered bicyclic heteroaryl ring having 1-5 heteroatoms. In some embodiments, –Cy– is an optionally substituted 10-membered bicyclic heteroaryl ring having 1-5 heteroatoms. In some embodiments, a heteroaliphatic, heterocyclyl or heteroaryl ring contains no more than 1 heteroatom. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur.

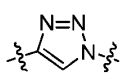
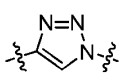
[0120] In some embodiments, –Cy– is an optionally substituted 4-7 membered ring having 0-3 heteroatoms. In some embodiments, –Cy– is an optionally substituted 6-membered aryl ring. In some embodiments, an aryl ring is substituted. In some embodiments, it is substituted with one or more halogen. In some embodiments, it is substituted with one or more –F. In some embodiments, it is not

substituted. In some embodiments, it is optionally substituted . In some embodiments, it is

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. In some embodiments, –Cy– is an optionally substituted 5-membered heteroaryl ring having 1-3 heteroatoms. In some embodiments, a heteroatom is nitrogen. In some embodiments, a heteroatom is oxygen. In some embodiments, a heteroatom is sulfur. In some embodiments, –Cy– is optionally

substituted . In some embodiments, –Cy– is .
 R^1

[0121] In some embodiments, R^1 is R' as described herein and is not –H. In some embodiments, R^1 is R as described herein. In some embodiments, R^1 is optionally substituted C_{1-6} aliphatic. In some embodiments, R^1 is optionally substituted methyl. In some embodiments, R^1 is optionally substituted ethyl. In some embodiments, R^1 is optionally substituted ethyl, wherein the methylene unit bonded to the oxygen is optionally monosubstituted, and the methyl is substituted with an electron-withdrawing group, e.g., –CN. Certain electron-withdrawing groups are described in US 10167309, US 11643657, US

11718638, US 11608355 or US 20230089442, the electron-withdrawing groups of each of which are incorporated herein by reference. In some embodiments, R^1 is $-\text{OCH}_2\text{CH}_2\text{CN}$.

R^2

[0122] In some embodiments, R^2 is R' as described herein and is not $-\text{H}$. In some embodiments, R^2 is R as described herein. In some embodiments, R^2 is not $-\text{H}$. In some embodiments, R^2 is optionally substituted C_{1-10} aliphatic. In some embodiments, R^2 is optionally substituted C_{1-10} alkyl. In some embodiments, R^2 is C_{1-6} aliphatic. In some embodiments, R^2 is C_{1-6} alkyl. In some embodiments, R^2 is isopropyl.

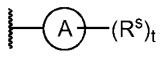
R^3

[0123] In some embodiments, R^3 is R' as described herein and is not $-\text{H}$. In some embodiments, R^3 is R as described herein. In some embodiments, R^3 is not $-\text{H}$. In some embodiments, R^3 is optionally substituted C_{1-10} aliphatic. In some embodiments, R^3 is optionally substituted C_{1-10} alkyl. In some embodiments, R^3 is C_{1-6} aliphatic. In some embodiments, R^3 is C_{1-6} alkyl. In some embodiments, R^3 is isopropyl.

[0124] In some embodiments, R^2 and R^3 are the same. In some embodiments, they are different.

[0125] In some embodiments, $-\text{P}(\text{OR}^1)\text{N}(\text{R}^2)(\text{R}^3)$ is $-\text{P}(\text{OCH}_2\text{CH}_2\text{CN})\text{N}[\text{CH}(\text{CH}_3)_2]_2$.

[0126] In some embodiments, two or three of R^1 , R^2 , and R^3 are taken together with their intervening

atoms to form  wherein each variable is independently as described herein. In some embodiments, R^1 and R^2 are taken together with their intervening atoms to form a ring as described herein. In some embodiments, R^2 and R^3 are taken together with their intervening atoms to form a ring as described herein. In some embodiments, R^1 , R^2 and R^3 are taken together with their intervening atoms to form a ring as described herein.

Ring A

[0127] As defined herein, Ring A is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having, in addition to the intervening atoms, 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, Ring A is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, Ring A has no additional heteroatoms. In some embodiments, Ring has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 additional heteroatoms.

[0128] In some embodiments, Ring A is not substituted (as appreciated by those skilled in the art,

not including R^s). In some embodiments, Ring A is substituted (as appreciated by those skilled in the art, not including R^s).

[0129] In some embodiments, Ring A is monocyclic. In some embodiments, Ring A is bicyclic. In some embodiments, Ring A is polycyclic. In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 4-6, 5-6, 5-8, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, each monocyclic ring unit is independently an optionally substituted 4-10 (e.g., 4, 5, 6, 7, 8, 9, 10, 5-7, 5-6) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 membered. In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-7 membered. In some embodiments, each monocyclic ring unit is independently an optionally substituted 4-6 membered. In some embodiments, each monocyclic ring unit is independently an optionally substituted 5-6 membered. In some embodiments, each monocyclic ring unit is independently an optionally substituted 5-membered. In some embodiments, a monocyclic ring unit is an optionally substituted saturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted partially unsaturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted aromatic ring. In some embodiments, a monocyclic ring unit is an optionally substituted phenyl ring. In some embodiments, a monocyclic ring unit is an optionally substituted 5-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is an optionally substituted 6-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. Each monocyclic ring unit is independently optionally substituted. In some embodiments, a monocyclic ring unit is unsaturated. In some embodiments, a monocyclic ring unit is saturated.

[0130] In some embodiments, a monocyclic ring unit comprising the nitrogen atom to which R² and R³ are bond is an optionally substituted saturated 4-10 (e.g., 4-8, 4-6, 4, 5, 6, 7, 8, 9, 10, etc.) membered ring having 0-2 heteroatoms in addition to the nitrogen atom independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit comprising the nitrogen atom to which R² and R³ are bond is an optionally substituted saturated 4-10 (e.g., 4-8, 4-6, 4, 5, 6, 7, 8, 9, 10, etc.) membered ring having no heteroatoms in addition to the nitrogen atom independently selected from nitrogen, oxygen and sulfur. In some embodiments, it is 4-membered. In some embodiments, it is 5-membered. In some embodiments, it is 6-membered. In some embodiments, it is 7-membered. In some

embodiments, it is 8-membered.

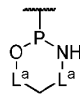
[0131] In some embodiments, Ring A is an optionally substituted saturated ring. In some embodiments, Ring A is an optionally substituted partially unsaturated ring. In some embodiments, Ring A is an optionally substituted aromatic ring.

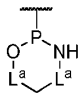
[0132] In some embodiments, Ring A is an optionally substituted monocyclic 3-10 (e.g., 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atom(s), 0-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, Ring A is an optionally substituted bicyclic or polycyclic 6-20 (e.g., 6-15, 6-10, 8-20, 8-15, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atom(s), 0-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, Ring A is 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10-membered. In some embodiments, Ring A is 3-9 membered. In some embodiments, Ring A is 3-7 membered. In some embodiments, Ring A is 4-10 membered. In some embodiments, Ring A is 4-7 membered. In some embodiments, Ring A is 5-10 membered. In some embodiments, Ring A is 5-7 membered. In some embodiments, Ring A is 3-membered. In some embodiments, Ring A is 4-membered. In some embodiments, Ring A is 5-membered. In some embodiments, Ring A is 6-membered. In some embodiments, Ring A is 7-membered. In some embodiments, Ring A is 8-membered. In some embodiments, Ring A is 9-membered. In some embodiments, Ring A is 10-membered. In some embodiments, Ring A is 11-membered. In some embodiments, Ring A is 12-membered. In some embodiments, Ring A is monocyclic. In some embodiments, Ring A is bicyclic. In some embodiments, Ring A is an optionally substituted bicyclic 7-12 membered ring. In some embodiments, Ring A is an optionally substituted bicyclic 8-10 membered ring. In some embodiments, Ring A is an optionally substituted bicyclic 7-membered ring. In some embodiments, Ring A is an optionally substituted bicyclic 8-membered ring. In some embodiments, Ring A is an optionally substituted bicyclic 9-membered ring. In some embodiments, Ring A is an optionally substituted bicyclic 10-membered ring. In some embodiments, Ring A is polycyclic. In some embodiments, Ring A has no additional heteroatoms. In some embodiments, Ring A has 0-6, e.g., 0, 1-6, 1-5, 1-3, or 1, 2, 3, 4, 5, or 6 additional heteroatoms. In some embodiments, Ring A comprises one or more aromatic ring. In some embodiments, Ring A is bicyclic or polycyclic, and each monocyclic ring unit is independently 3-10 (e.g., 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10) membered, saturated, partially unsaturated or aromatic and having 0-5 (e.g., 0, 1-5, 1-3, 1, 2, 3, 4, or 5) heteroatoms. In some embodiments, a monocyclic ring unit is saturated. In some embodiments, a monocyclic ring unit is partially unsaturated. In some embodiments, a monocyclic ring unit is aromatic. In some embodiments, a monocyclic ring unit

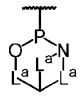
is heteroaromatic. Those skilled in the art appreciate that intervening atom(s), e.g., of groups taken together to form a ring, are typically atoms on the shortest path connecting such groups if multiple paths exist.

[0133] In some embodiments, a monocyclic ring unit comprising the phosphorus, nitrogen and oxygen atoms is saturated. In some embodiments, a monocyclic ring unit comprising the phosphorus, nitrogen and oxygen atoms is partially saturated. In some embodiments, it is 4-membered. In some embodiments, it is 5-membered. In some embodiments, it is 6-membered. In some embodiments, it is 7-membered. In some embodiments, it has one or more additional heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, the phosphorus, nitrogen and oxygen atoms are the only heteroatoms in that monocyclic ring unit.

[0134] In some embodiments, a monocyclic ring unit comprising the nitrogen atom to which R^2 and R^3 are bond is an optionally substituted partially unsaturated 4-10 (e.g., 4-8, 4-6, 4, 5, 6, 7, 8, 9, 10, etc.) membered ring having 0-2 heteroatoms in addition to the nitrogen atom independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit comprising the nitrogen atom to which R^2 and R^3 are bond is an optionally substituted partially unsaturated 4-10 (e.g., 4-8, 4-6, 4, 5, 6, 7, 8, 9, 10, etc.) membered ring having no heteroatoms in addition to the nitrogen atom independently selected from nitrogen, oxygen and sulfur. In some embodiments, it is 4-membered. In some embodiments, it is 5-membered. In some embodiments, it is 6-membered. In some embodiments, it is 7-membered. In some embodiments, it is 8-membered.

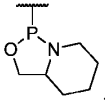
[0135] In some embodiments, Ring A comprises optionally substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH$. In some embodiments, Ring A

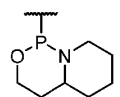
is optionally substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced

by $-O-$, $-S-$, or $-NH$. In some embodiments, Ring A comprises optionally substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH$. In some

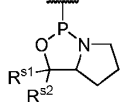
embodiments, Ring A is optionally substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH-$. In some embodiments, L^a is a covalent bond. In some embodiments, L^a is not a covalent bond. In some embodiments, L^a is $-CH_2-$. In some embodiments, L^a is $-(CH_2)_2-$. In some embodiments, L^a is $-(CH_2)_3-$. In some embodiments, L^a is $-(CH_2)_4-$. In some embodiments, L^a is $-(CH_2)_5-$. In some embodiments, a methylene unit is replaced with $-O-$. In some embodiments, a methylene unit is replaced with $-S-$. In some embodiments, a methylene unit is replaced with $-NH-$.

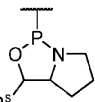
[0136] In some embodiments, Ring A is optionally substituted . In some embodiments,

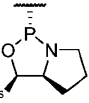
Ring A is optionally substituted . In some embodiments, Ring A is optionally substituted

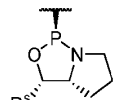


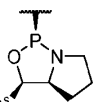
[0137] In some embodiments, an occurrence of R^s is bonded to a carbon atom bonded to the oxygen

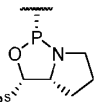
of $-P(OR^1)N(R^2)(R^3)$. In some embodiments, $-P(OR^1)N(R^2)(R^3)$ is , wherein each of R^{s1} and

R^{s2} is independently R^s as described herein. In some embodiments, $-P(OR^1)N(R^2)(R^3)$ is R^s . In

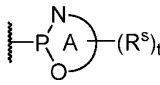
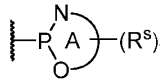
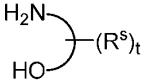
some embodiments, $-P(OR^1)N(R^2)(R^3)$ is R^s . In some embodiments, $-P(OR^1)N(R^2)(R^3)$ is

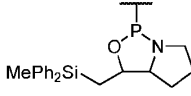
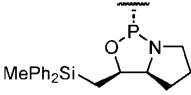
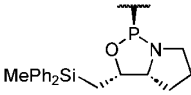
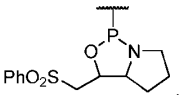
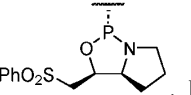
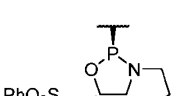
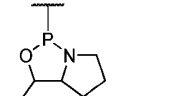
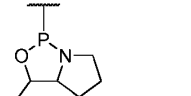
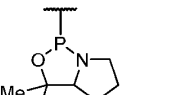
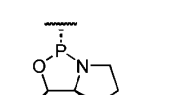


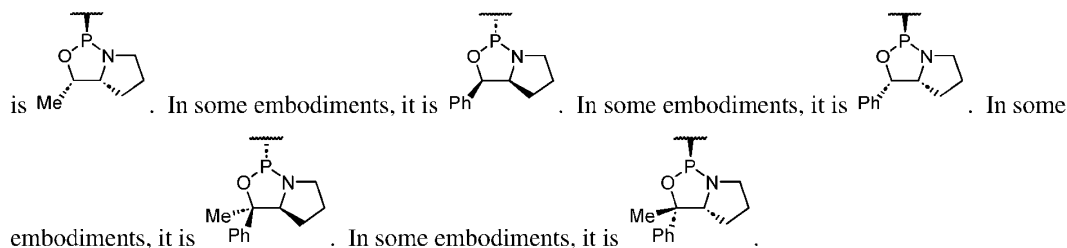
. In some embodiments, $-P(OR^1)N(R^2)(R^3)$ is R^s . In some embodiments,

$-P(OR^1)N(R^2)(R^3)$ is R^s .

[0138] In some embodiments, one of R^{s1} and R^{s2} is $-H$. In some embodiments, R^{s1} and R^{s2} are independently not $-H$. In some embodiments, R^{s1} is R as described herein. In some embodiments, R^{s1} is not hydrogen. In some embodiments, R^{s1} is hydrogen. In some embodiments, R^{s1} is hydrogen and R^{s2} is not hydrogen. In some embodiments, R^{s1} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{s1} is methyl. In some embodiments, R^{s1} is optionally substituted phenyl. In some embodiments, R^{s1} is phenyl. In some embodiments, R^{s2} is R as described herein. In some embodiments, R^{s2} is not hydrogen. In some embodiments, R^{s2} is hydrogen. In some embodiments, R^{s2} is optionally substituted C_{1-6} aliphatic. In some embodiments, wherein R^{s2} is methyl. In some embodiments, R^{s2} is optionally substituted phenyl. In some embodiments, wherein R^{s2} is phenyl.

[0139] In some embodiments, $-P(OR^1)N(R^2)(R^3)$ is  wherein each variable is independently as described herein. In some embodiments,  is of such a structure that  is a chiral auxiliary, e.g., described in US 10167309, US 11643657, US 11718638, US 11608355 or US 20230089442, the chiral auxiliaries of each of which are incorporated herein by

reference. For example, in some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it is . In some embodiments, it



t

[0140] In some embodiments, *t* is 0. In some embodiments, *t* is 1-5. In some embodiments, *t* is 1. In some embodiments, *t* is 2. In some embodiments, *t* is 3. In some embodiments, *t* is 4. In some embodiments, *t* is 5.

R^s

[0141] In some embodiments, *R^s* is -F. In some embodiments, *R^s* is -Cl. In some embodiments, *R^s* is -Br. In some embodiments, *R^s* is -I. In some embodiments, *R^s* is -CN. In some embodiments, *R^s* is -N₃. In some embodiments, *R^s* is -NO. In some embodiments, *R^s* is -NO₂.

[0142] In some embodiments, *R^s* is -L^s-R^{s11} wherein each of L^s and R^{s11} is independently as described herein. In some embodiments, *R^s* is R^{s11} as described herein.

[0143] In some embodiments, *R^s* is R' as described herein. For example, in some embodiments, R' is -C(O)R wherein R is as described herein. In some embodiments, R' is -CO₂R wherein R is as described herein. In some embodiments, R' is -S(O)₂R wherein R is as described herein. In some embodiments, R is C₁₋₆ aliphatic. In some embodiments, R is C₁₋₆ alkyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, *R^s* is -S(O)₂R wherein R is optionally substituted C₁₋₆ aliphatic. In some embodiments, *R^s* is -S(O)₂Me. In some embodiments, *R^s* is -S(O)₂t-Bu. In some embodiments, *R^s* is -S(O)₂R wherein R is optionally substituted phenyl. In some embodiments, *R^s* is -S(O)₂Ph.

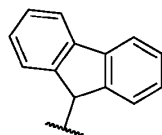
[0144] In some embodiments, *R^s* is R as described herein. For example, in some embodiments, R is not -H. In some embodiments, R is optionally substituted C₁₋₆ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ alkyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl.

[0145] In some embodiments, *R^s* is -L^s-OR^{s11} wherein each of L^s and R^{s11} is independently as described herein. In some embodiments, *R^s* is -OR^{s11} wherein R^{s11} is as described herein. In some embodiments, *R^s* is -CH₂OR^{s11} wherein R^{s11} is as described herein.

[0146] In some embodiments, *R^s* is -L^s-SR^{s11} wherein each of L^s and R^{s11} is independently as described herein. In some embodiments, *R^s* is -SR^{s11} wherein R^{s11} is as described herein. In some embodiments, *R^s* is -CH₂SR^{s11} wherein R^{s11} is as described herein.

[0147] In some embodiments, R^s is $-L^s-N(R^{s11})_2$ wherein each of L^s and R^{s11} is independently as described herein. In some embodiments, R^s is $-N(R^{s11})_2$ wherein each of L^s and R^{s11} is independently as described herein. In some embodiments, R^s is $-CH_2N(R^{s11})_2$ wherein each of L^s and R^{s11} is independently as described herein.

[0148] In some embodiments, R^s is $-C(R^{s11})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, R^s is $-CH(R^{s11})_2$ wherein each R^{s11} is independently as described herein. In some embodiments, two or more R^{s11} are taken together with the carbon atom to which they are attached to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.



In some embodiments, R^s is optionally substituted

[0149] In some embodiments, R^s is $-L^s-Si(R^{s11})_3$ wherein each R^{s11} is independently as described herein. In some embodiments, each R^{s11} is independently R' as described herein and is not $-H$. In some embodiments, each R^{s11} is independently R as described herein and is not $-H$. In some embodiments, at least one R^{s11} is optionally substituted C_{1-10} aliphatic. In some embodiments, at least one R^{s11} is optionally substituted C_{1-10} alkyl. In some embodiments, at least one R^{s11} is methyl. In some embodiments, at least one R^{s11} is optionally substituted phenyl. In some embodiments, at least one R^{s11} is phenyl. In some embodiments, each R^{s11} is independently an optionally substituted group selected from C_{1-10} aliphatic and 6-10 membered aryl. In some embodiments, each R^{s11} is independently an optionally substituted group selected from C_{1-10} alkyl and 6-10 membered aryl. In some embodiments, each R^{s11} is independently an optionally substituted group selected from C_{1-6} alkyl and 6-10 phenyl. In some embodiments, R^{s11} is $-SiPh_2Me$.

R^{s11}

[0150] As used herein, R^{s11} is R' as described herein. For example, in some embodiments, R' is R as described herein. In some embodiments, R' is $-C(O)R$ wherein R is as described herein. In some embodiments, R' is $-CO_2R$ wherein R is as described herein. In some embodiments, R' is $-S(O)_2R$ wherein R is as described herein. R^{s11} is $-H$. In some embodiments, R^{s11} is not $-H$. In some embodiments, two or more R^{s11} on the same atom are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. Certain embodiments of rings are described herein.

L^s

[0151] As used herein, L^s is L as described herein. For example, in some embodiments, L is a covalent bond. In some embodiments, L is optionally substituted $-\text{CH}_2-$. In some embodiments, L is $-\text{CH}_2-$. In some embodiments, L is monosubstituted $-\text{CH}_2-$.

R'

[0152] Certain embodiments for R' are described below. Various variables, e.g., R^{2a}, R¹, R², R³, R^s, R^{sl}, etc., can be R', and embodiments for R' can also be applied to such variables that can be R'.

[0153] In some embodiments, R' is R as described herein. In some embodiments, R' is $-\text{H}$. In some embodiments, R' is not $-\text{H}$.

[0154] In some embodiments, R' is $-\text{C}(\text{O})\text{R}$ wherein R is as described herein. In some embodiments, R' is $-\text{CO}_2\text{R}$ wherein R is as described herein. In some embodiments, R' is $-\text{S}(\text{O})_2\text{R}$ wherein R is as described herein. In some embodiments, as described herein, R is not $-\text{H}$.

[0155] In some embodiments, two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, two or more R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, two R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0156] In some embodiments, each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{CO}_2\text{R}$, or $-\text{S}(\text{O})_2\text{R}$, or two or more R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

Ring

[0157] Compounds of the present disclosure may contain various rings. In some embodiments, a variable, e.g., R or a variable that can be R, can be of a ring as described herein. In some embodiments, two variables, e.g., two R' groups, may be taken together with their intervening atom(s) to form a ring as

described herein. In some embodiments, a ring is monovalent. In some embodiments, a ring is bivalent. In some embodiments, a ring structure can be polyvalent. Rings are optionally substituted. In some embodiments, a ring is unsubstituted. In some embodiments, a ring is substituted.

[0158] Certain embodiments and features of rings are described below as examples.

[0159] In some embodiments, a ring is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, a ring is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic.

[0160] In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 5-8, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, a monocyclic ring unit is an optionally substituted saturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted partially unsaturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted aromatic ring. In some embodiments, a monocyclic ring unit is an optionally substituted phenyl ring. In some embodiments, a monocyclic ring unit is an optionally substituted 5-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is an optionally substituted 6-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. Each monocyclic ring unit is independently optionally substituted. In some embodiments, a monocyclic ring unit is unsaturated. In some embodiments, a monocyclic ring unit is saturated.

[0161] In some embodiments, a ring is an optionally substituted saturated ring. In some embodiments, a ring is an optionally substituted partially unsaturated ring. In some embodiments, a ring is an optionally substituted aromatic ring.

[0162] In some embodiments, a ring is an optionally substituted 3-10 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atom(s), 0-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a ring is 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10-membered. In some embodiments, a ring

is 3-9 membered. In some embodiments, a ring is 3-7 membered. In some embodiments, a ring is 4-10 membered. In some embodiments, a ring is 4-7 membered. In some embodiments, a ring is 5-10 membered. In some embodiments, a ring is 5-7 membered. In some embodiments, a ring is 3-membered. In some embodiments, a ring is 4-membered. In some embodiments, a ring is 5-membered. In some embodiments, a ring is 6-membered. In some embodiments, a ring is 7-membered. In some embodiments, a ring is 8-membered. In some embodiments, a ring is 9-membered. In some embodiments, a ring is 10-membered. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic. In some embodiments, a ring has no heteroatoms. In some embodiments, a ring has 1-6, e.g., 1-5, 1-3, or 1, 2, 3, 4, 5, or 6 heteroatoms. In some embodiments, a ring formed by two or more groups taken together with their intervening atom(s) has no heteroatoms in addition to the intervening atom(s). In some embodiments, a ring formed by two or more groups taken together with their intervening atom(s) has 1-6, e.g., 1-5, 1-3, or 1, 2, 3, 4, 5, or 6 heteroatoms in addition to the intervening atom(s). In some embodiments, a ring is saturated. In some embodiments, a ring is partially unsaturated. In some embodiments, a ring comprises one or more aromatic ring. In some embodiments, a ring is bicyclic or polycyclic, and each monocyclic ring unit is independently 3-10 (e.g., 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10) membered, saturated, partially unsaturated or aromatic and having 0-5 (e.g., 0, 1-5, 1-3, 1, 2, 3, 4, or 5) heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is saturated. In some embodiments, a monocyclic ring unit is partially unsaturated. In some embodiments, a monocyclic ring unit is aromatic. In some embodiments, a monocyclic ring unit is heteroaromatic. Those skilled in the art appreciate that intervening atom(s), e.g., of groups taken together to form a ring, are typically atoms on the shortest path connecting such groups if multiple paths exist.

[0163] In some embodiments, a ring has one or more heteroatoms. In some embodiments, a ring comprises a nitrogen atom. In some embodiments, a ring comprises an oxygen atom. In some embodiments, a ring comprises a sulfur atom.

R

[001] Various variables in the present disclosure can independently be *R*. Certain embodiments for *R* are described below as examples. Those skilled in the art reading the present disclosure appreciate that embodiments that are described for a variable that can be *R* and fall within the definition of *R* can be embodiments for *R* as well. Those skilled in the art reading the present disclosure further appreciate that embodiments for *R*, e.g., those described for *R*, those described for a variable that can be *R* and within the definition of *R*, etc., can be embodiments for a variable that can be *R*.

[002] In some embodiments, *R* is $-H$. In some embodiments, *R* is not $-H$. In some embodiments,

R is optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) aliphatic. In some embodiments, R is optionally substituted C₁₋₈ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ aliphatic. In some embodiments, R is optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) alkyl. In some embodiments, R is optionally substituted C₁₋₈ alkyl. In some embodiments, R is optionally substituted C₁₋₆ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ alkyl. In some embodiments, R is optionally substituted –CH₂–C₃₋₆ cycloaliphatic. In some embodiments, R is optionally substituted –CH₂–C₃₋₆ cycloaliphatic. In some embodiments, R is optionally substituted –CH₂–C₃₋₆ cycloalkyl. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, R is isopropyl. In some embodiments, R is –CF₃. In some embodiments, R is –CH₂CF₃. In some embodiments, R is butyl. In some embodiments, R is t-butyl.

[003] In some embodiments, R is optionally substituted 3-10 membered (e.g., 3-9, 3-8, 3-7, 3-6, 5-7, 4, 5, 6, 7, 8, 9, 10, etc.) cycloaliphatic. In some embodiments, R is optionally substituted C₃₋₁₀ cycloalkyl. In some embodiments, R is optionally substituted cyclopropyl. In some embodiments, R is optionally substituted cyclobutyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally substituted cyclohexyl.

[004] In some embodiments, R is optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted C₁₋₆ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted C₁₋₁₀ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted C₁₋₆ (e.g., C₁₋₆, C₂₋₈, C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted C₁₋₆ heteroaliphatic having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted C₁₋₆ heteroaliphatic having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted C₁₋₆ heteroaliphatic having 1 heteroatom independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is heteroalkyl.

[005] In some embodiments, R is optionally substituted 3-15 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 3-10, 3-7, 4-10, 4-6, etc.) membered heterocyclic ring having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted 3-15 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 3-10, 3-7, 4-

10, 4-6, etc.) membered heterocyclic ring having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 3-10 (e.g., 3-9, 3-8, 3-7, 3-6, 5-7, 4, 5, 6, 7, 8, 9, 10, etc.) membered heterocyclyl having 1-4 (e.g., 1, 2, 3, 4, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 3-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 3-membered heterocyclyl having one heteroatom independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 4-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 8-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 9-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 10-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, there is one carbon atom in a heterocyclyl ring. In some embodiments, there are two or more (e.g., 2-14, 2-9, 2-6, 2-4, 3-10, 3-5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, etc.) carbon atoms in a heterocyclyl ring.

[006] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is optionally substituted naphthyl. In some embodiments, R is optionally substituted 1-naphthyl. In some embodiments, R is optionally substituted 2-naphthyl. In some embodiments, R is naphthyl.

[007] In some embodiments, R is optionally substituted 5-14 (e.g., 5, 6, 9, 10, 14, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted 5-14 (e.g., 5, 6, 9, 10, 14, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 5-10 (e.g., 5, 6, 9, 10, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a heteroaryl ring is monocyclic and is 5-membered.

In some embodiments, a heteroaryl ring is monocyclic and is 6-membered. In some embodiments, a heteroaryl ring is bicyclic and is 9-membered. In some embodiments, a heteroaryl ring is bicyclic and is 10-membered. In some embodiments, a heteroaryl ring is tricyclic and is 14-membered. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 8-10 membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 9-membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 10-membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 9-membered aromatic ring having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 10-membered aromatic ring having 1 heteroatom nitrogen, oxygen and sulfur. In some embodiments, at least one heteroatom is nitrogen. In some embodiments, at least one heteroatom is oxygen. In some embodiments, at least one heteroatom is sulfur. In some embodiments, each heteroatom is the same. In some embodiments, at least one heteroatom is different from another heteroatom.

[008] As described herein, various groups may be optionally substituted. Substituents are routinely utilized in chemistry including in development of various therapeutics. Many substituents can be utilized in accordance with the present disclosure. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Substituents are preferably those that result in the formation of compounds for a desired property, activity, use, etc., as described herein. In some embodiments, compounds are stable for therapeutic use as described herein. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein. In some embodiments, a substituent is a hydrocarbon group. In some embodiments, a substituent comprises a heteroatom. In some embodiments, a substituent comprises multiple heteroatoms. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, sulfur, phosphorus and silicon. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, and sulfur. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, fluorine, chlorine, bromine, iodine, nitrogen, oxygen, and sulfur. In some

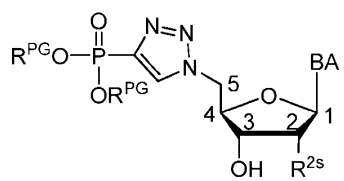
embodiments, the total number of carbon and non-halogen heteroatom(s) in a substituent is about or no more than about 1; in some embodiments, it is no more than about 2; in some embodiments, it is no more than about 3; in some embodiments, it is no more than about 4; in some embodiments, it is no more than about 5; in some embodiments, it is no more than about 6; in some embodiments, it is no more than about 7; in some embodiments, it is no more than about 8; in some embodiments, it is no more than about 9; in some embodiments, it is no more than about 10; in some embodiments, it is no more than about 11; in some embodiments, it is no more than about 12; in some embodiments, it is no more than about 13; in some embodiments, it is no more than about 14; in some embodiments, it is no more than about 15; in some embodiments, it is no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 15. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 10. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 6.

Nucleosides

[0164] Various nucleosides can be utilized for oligonucleotide preparation, e.g., those described in US 10167309, US 11643657, US 11718638, US 11608355, US 20230089442, etc. In some embodiments, the present disclosure provides nucleosides comprising $-P(O)(OR^{PG})_2$ as described herein. In some embodiments, a nucleoside comprises $-P(O)(OR^{PG})_2$ which is bonded to the sugar moiety of the nucleoside. In some embodiments, a phosphoramidite comprises $-P(O)(OR^{PG})_2$ which is bonded to a moiety, e.g., an optionally substituted triazole ring, that is bonded to the sugar moiety of the nucleoside. In some embodiments, a sugar is a natural sugar. In some embodiments, a sugar is a modified sugar. In some embodiments, a nucleobase is an optionally substituted natural nucleobase. In some embodiments, a nucleobase is optionally protected A, T, C, G, U, or 5mC (e.g., for oligonucleotide synthesis). In some embodiments, a nucleoside is abasic.

[0165] In some embodiments, the provided technology provides a compound of formula $(R^{PG})_2P(O)-L^{5E}-SU(-BA)-OH$ wherein each other variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation.

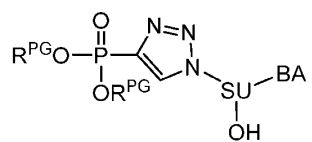
[0166] In some embodiments, the provided technology provides a compound of formula N or a salt thereof:



N

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation.

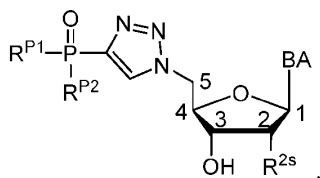
[0167] In some embodiments, the provided technology provides a compound of formula N' or a salt thereof:



N'

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation.

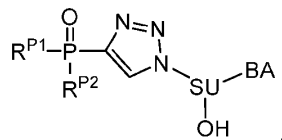
[0168] In some embodiments, the provided technology provides a compound of formula II or a salt thereof:



II

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation.

[0169] In some embodiments, the provided technology provides a compound of formula II' or a salt thereof:

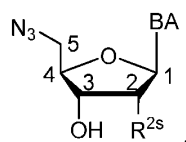


II'

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation.

[0170] In some embodiments, provided nucleosides can react with phosphorus-containing agents, e.g., compounds of formula V or salts thereof to provide phosphoramidites.

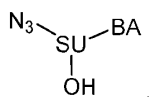
[0171] In some embodiments, the provided technology provides a compound of formula III or a salt thereof:



III

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation. In some embodiments, such a compound is utilized to prepare for, e.g., a compound of formula II or formula II', or a salt thereof.

[0172] In some embodiments, the provided technology provides a compound of formula III' or a salt thereof:



III'

wherein each variable is independently as described herein. In some embodiments, such a compound is utilized as a nucleoside, e.g., for phosphoramidite preparation. In some embodiments, such a compound is utilized to prepare for, e.g., a compound of formula II or formula II', or a salt thereof.

Nucleobases

[0173] Various nucleobases may be utilized in phosphoramidites, nucleosides, oligonucleotides, etc., in accordance with the present disclosure. In some embodiments, a nucleobase is a natural nucleobase, the most commonly occurring ones being A, T, C, G and U. In some embodiments, a nucleobase is a modified nucleobase in that it is not A, T, C, G or U. In some embodiments, a nucleobase is optionally substituted A, T, C, G or U, or a substituted tautomer of A, T, C, G or U. In some embodiments, a nucleobase is optionally substituted A, T, C, G or U, e.g., 5mC, 5-hydroxymethyl C, etc. In some embodiments, a nucleobase is alkyl-substituted A, T, C, G or U. In some embodiments, a nucleobase is A. In some embodiments, a nucleobase is T. In some embodiments, a nucleobase is C. In some embodiments, a nucleobase is G. In some embodiments, a nucleobase is U. In some embodiments, a nucleobase is 5mC. In some embodiments, a nucleobase is substituted A, T, C, G or U. In some embodiments, a nucleobase is a substituted tautomer of A, T, C, G or U. In some embodiments, substitution protects certain functional groups in nucleobases to minimize undesired reactions during oligonucleotide synthesis. Suitable

technologies for nucleobase protection in oligonucleotide synthesis are widely known in the art and may be utilized in accordance with the present disclosure.

[0174] In some embodiments, a nucleobase is a natural nucleobase or a modified nucleobase derived from a natural nucleobase. Examples include uracil, thymine, adenine, cytosine, and guanine optionally having their respective amino groups protected by acyl protecting groups, 2-fluorouracil, 2-fluorocytosine, 5-bromouracil, 5-iodouracil, 2,6-diaminopurine, azacytosine, pyrimidine analogs such as pseudoisocytosine and pseudouracil and other modified nucleobases such as 8-substituted purines, xanthine, or hypoxanthine (the latter two being the natural degradation products). Certain examples of modified nucleobases are disclosed in Chiu and Rana, RNA, 2003, 9, 1034-1048, Limbach et al. Nucleic Acids Research, 1994, 22, 2183-2196 and Revankar and Rao, Comprehensive Natural Products Chemistry, vol. 7, 313. In some embodiments, a modified nucleobase is substituted uracil, thymine, adenine, cytosine, or guanine. In some embodiments, a modified nucleobase is a functional replacement, e.g., in terms of hydrogen bonding and/or base pairing, of uracil, thymine, adenine, cytosine, or guanine. In some embodiments, a nucleobase is optionally substituted uracil, thymine, adenine, cytosine, 5-methylcytosine, or guanine. In some embodiments, a nucleobase is uracil, thymine, adenine, cytosine, 5-methylcytosine, or guanine.

[0175] In some embodiments, a modified base is optionally substituted adenine, cytosine, guanine, thymine, or uracil, or a tautomer thereof. In some embodiments, a modified nucleobase is a modified adenine, cytosine, guanine, thymine or uracil, modified by one or more modifications by which:

a nucleobase is modified by one or more optionally substituted groups independently selected from acyl, halogen, amino, azide, alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocyclyl, heteroaryl, carboxyl, hydroxyl, biotin, avidin, streptavidin, substituted silyl, and combinations thereof;

one or more atoms of a nucleobase are independently replaced with a different atom selected from carbon, nitrogen and sulfur;

one or more double bonds in a nucleobase are independently hydrogenated; or

one or more aryl or heteroaryl rings are independently inserted into a nucleobase.

[0176] In some embodiments, a modified nucleobase is a modified nucleobase reported in, e.g., WO2017/210647. In some embodiments, modified nucleobases are expanded-size nucleobases in which one or more aryl and/or heteroaryl rings, such as phenyl rings, have been added. Certain examples of modified nucleobases, including nucleobase replacements, are described in the Glen Research catalog (Glen Research, Sterling, Virginia); Krueger AT et al., Acc. Chem. Res., 2007, 40, 141-150; Kool, ET, Acc. Chem. Res., 2002, 35, 936-943; Benner S.A., et al., Nat. Rev. Genet., 2005, 6, 553-543; Romesberg, F.E., et al., Curr. Opin. Chem. Biol., 2003, 7, 723-733; or Hirao, I., Curr. Opin. Chem. Biol., 2006, 10, 622-627.

In some embodiments, an expanded-size nucleobase is an expanded-size nucleobase described in, e.g., WO2017/210647. In some embodiments, modified nucleobases are moieties such as corrin- or porphyrin-derived rings. Certain porphyrin-derived base replacements have been described in, e.g., Morales-Rojas, H and Kool, ET, Org. Lett., 2002, 4, 4377-4380. In some embodiments, a porphyrin-derived ring is a porphyrin-derived ring described in, e.g., WO2017/219647. In some embodiments, a modified nucleobase is a modified nucleobase described in, e.g., WO2017/219647. In some embodiments, a modified nucleobase is fluorescent. Examples of such fluorescent modified nucleobases include phenanthrene, pyrene, stillbene, isoxanthine, isozanthopterin, terphenyl, terthiophene, benzoterthiophene, coumarin, lumazine, tethered stillbene, benzo-uracil, naphtho-uracil, etc., and those described in e.g., WO2017/210647. In some embodiments, a nucleobase or modified nucleobase is selected from: C5-propyne T, C5-propyne C, C5-Thiazole, phenoxazine, 2-thio-thymine, 5-triazolylphenyl-thymine, diaminopurine, and N2-aminopropylguanine.

[0177] In some embodiments, a modified nucleobase is selected from 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain embodiments, modified nucleobases are selected from 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyladenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoylguanine, 2-N-isobutyrylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. In some embodiments, modified nucleobases are tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one or 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). In some embodiments, modified nucleobases are those in which the purine or pyrimidine base is replaced with other heterocycles, for example, 7-deazaadenine, 7-deazaguanosine, 2-aminopyridine or 2-pyridone. In some embodiments, modified nucleobases are those disclosed in US 3687808, The Concise Encyclopedia Of Polymer Science And Engineering, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, Antisense Research and Applications, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; or in Chapters 6 and 15, Antisense Drug Technology, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

[0178] In some embodiments, modified nucleobases and methods thereof are those described in US

20030158403, US 3687808, US 4845205, US 5130302, US 5134066, US 5 175273, US 5367066, US 5432272, US 5434257, US 5457187, US 5459255, US 5484908, US 5502177, US 5525711, US 5552540, US 5587469, US 5594 121, US 5596091, US 5614617, US 5645985, US 5681941, US 5750692, US 5763588, US 5830653, or US 6005096.

[0179] In some embodiments, a modified nucleobase is substituted. In some embodiments, a modified nucleobase is substituted such that it contains, e.g., heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. In some embodiments, a modified nucleobase is a “universal base” that is not a nucleobase in the most classical sense, but that functions similarly to a nucleobase. One example of a universal base is 3-nitropyrrole.

[0180] In some embodiments, nucleosides that can be utilized in provided technologies comprise modified nucleobases and/or modified sugars, e.g., 4-acetylcytidine; 5-(carboxyhydroxymethyl)uridine; 2'-O-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2'-O-methylpseudouridine; beta.D-galactosylqueosine; 2'-O-methylguanosine; N⁶-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; 1-methylinosine; 2,2-dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N⁷-methylguanosine; 3-methyl-cytidine; 5-methylcytidine; 5-hydroxymethylcytidine; 5-formylcytosine; 5-carboxylcytosine; N⁶-methyladenosine; 7-methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N⁶-isopentenyladenosine; N-((9-beta,D-ribofuranosyl-2-methylthiopurine-6-yl)carbamoyl)threonine; N-((9-beta,D-ribofuranosylpurine-6-yl)-N-methylcarbamoyl)threonine; uridine-5-oxyacetic acid methylester; uridine-5-oxyacetic acid (v); pseudouridine; queosine; 2-thiocytidine; 5-methyl-2-thiouridine; 2-thiouridine; 4-thiouridine; 5-methyluridine; 2'-O-methyl-5-methyluridine; and 2'-O-methyluridine.

[0181] In some embodiments, a nucleobase, e.g., a modified nucleobase comprises one or more biomolecule binding moieties such as e.g., antibodies, antibody fragments, biotin, avidin, streptavidin, receptor ligands, or chelating moieties. In other embodiments, a nucleobase is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In some embodiments, a nucleobase comprises substitution with a fluorescent or biomolecule binding moiety. In some embodiments, a substituent is a fluorescent moiety. In some embodiments, a substituent is biotin or avidin.

[0182] Certain examples of nucleobases and related methods are described in US 3687808, 4845205, US 513030, US 5134066, US 5175273, US 5367066, US 5432272, US 5457187, US 5457191, US 5459255, US 5484908, US 5502177, US 5525711, US 5552540, US 5587469, US 5594121, US 5596091, US 5614617, US 5681941, US 5750692, US 6015886, US 6147200, US 6166197, US 6222025, US 6235887, US 6380368, US 6528640, US 6639062, US 6617438, US 7045610, US 7427672, US or US 7495088.

[0183] In some embodiments, an oligonucleotide comprises a nucleobase, sugar, nucleoside, and/or

internucleotidic linkage which is described in any of: Gryaznov, S; Chen, J.-K. J. Am. Chem. Soc. 1994, 116, 3143; Hendrix et al. 1997 Chem. Eur. J. 3: 110; Hyrup et al. 1996 Bioorg. Med. Chem. 4: 5; Jepsen et al. 2004 Oligo. 14: 130-146; Jones et al. J. Org. Chem. 1993, 58, 2983; Koizumi et al. 2003 Nuc. Acids Res. 12: 3267-3273; Koshkin et al. 1998 Tetrahedron 54: 3607-3630; Kumar et al. 1998 Bioorg. Med. Chem. Lett. 8: 2219-2222; Lauritsen et al. 2002 Chem. Comm. 5: 530-531; Lauritsen et al. 2003 Bioorg. Med. Chem. Lett. 13: 253-256; Mesmaeker et al. Angew. Chem., Int. Ed. Engl. 1994, 33, 226; Morita et al. 2001 Nuc. Acids Res. Suppl. 1: 241-242; Morita et al. 2002 Bioorg. Med. Chem. Lett. 12: 73-76; Morita et al. 2003 Bioorg. Med. Chem. Lett. 2211-2226; Nielsen et al. 1997 Chem. Soc. Rev. 73; Nielsen et al. 1997 J. Chem. Soc. Perkins Transl. 1: 3423-3433; Obika et al. 1997 Tetrahedron Lett. 38 (50): 8735-8; Obika et al. 1998 Tetrahedron Lett. 39: 5401-5404; Pallan et al. 2012 Chem. Comm. 48: 8195-8197; Petersen et al. 2003 TRENDS Biotech. 21: 74-81; Rajwanshi et al. 1999 Chem. Commun. 1395-1396; Schultz et al. 1996 Nucleic Acids Res. 24: 2966; Seth et al. 2009 J. Med. Chem. 52: 10-13; Seth et al. 2010 J. Med. Chem. 53: 8309-8318; Seth et al. 2010 J. Org. Chem. 75: 1569-1581; Seth et al. 2012 Bioorg. Med. Chem. Lett. 22: 296-299; Seth et al. 2012 Mol. Ther-Nuc. Acids. 1, e47; Seth, Punit P; Siwkowski, Andrew; Allerson, Charles R; Vasquez, Guillermo; Lee, Sam; Prakash, Thazha P; Kinberger, Garth; Migawa, Michael T; Gaus, Hans; Bhat, Balkrishen; et al. From Nucleic Acids Symposium Series (2008), 52(1), 553-554; Singh et al. 1998 Chem. Comm. 1247-1248; Singh et al. 1998 J. Org. Chem. 63: 10035-39; Singh et al. 1998 J. Org. Chem. 63: 6078-6079; Sorensen 2003 Chem. Comm. 2130-2131; Ts'o et al. Ann. N. Y. Acad. Sci. 1988, 507, 220; Van Aerschot et al. 1995 Angew. Chem. Int. Ed. Engl. 34: 1338; Vasseur et al. J. Am. Chem. Soc. 1992, 114, 4006; WO 2007090071; or WO 2016/079181.

[0184] In some embodiments, an oligonucleotide comprises a modified nucleobase, nucleoside or nucleotide which is described in any of: Feldman et al. 2017 J. Am. Chem. Soc. 139: 11427-11433, Feldman et al. 2017 Proc. Natl. Acad. Sci. USA 114: E6478-E6479, Hwang et al. 2009 Nuc. Acids Res. 37: 4757-4763, Hwang et al. 2008 J. Am. Chem. Soc. 130: 14872-14882, Lavergne et al. 2012 Chem. Eur. J. 18: 1231-1239, Lavergne et al. 2013 J. Am. Chem. Soc. 135: 5408-5419, Ledbetter et al. 2018 J. Am. Chem. Soc. 140: 758-765, Malyshev et al. 2009 J. Am. Chem. Soc. 131: 14620-14621, Seo et al. 2009 Chem. Bio. Chem. 10: 2394-2400, e.g., d3FB, d2Py analogs, d2Py, d3MPy, d4MPy, d5MPy, d34DMPy, d35DMPy, d45DMPy, d5FM, d5PrM, d5SICS, dFEMO, dMMO2, dNaM, dNM01, dTPT3, nucleotides with 2'-azido, 2'-chloro, 2'-amino or arabinose sugars, isocarbostyryl-, naphthyl- and azaindole-nucleotides, and modifications and derivatives and functionalized versions thereof, e.g., those in which the sugar comprises a 2'-modification and/or other modification, and dMMO2 derivatives with meta-chlorine, -bromine, -iodine, -methyl, or -propinyl substituents.

[0185] In some embodiments, a nucleobase comprises at least one optionally substituted ring which comprises a heteroatom ring atom. In some embodiments, a nucleobase comprises at least one optionally

substituted ring which comprises a nitrogen ring atom. In some embodiments, such a ring is aromatic. In some embodiments, a nucleobase is bonded to a sugar through a heteroatom. In some embodiments, a nucleobase is bonded to a sugar through a nitrogen atom. In some embodiments, a nucleobase is bonded to a sugar through a ring nitrogen atom.

[0186] In some embodiments, an oligonucleotide comprises a nucleobase or modified nucleobase as described in US 10167309, US 11643657, US 11718638, US 11608355 and US 20230089442.

[0187] In some embodiments, a nucleobase is an optionally substituted purine base residue. In some embodiments, a nucleobase is a protected purine base residue. In some embodiments, a nucleobase is an optionally substituted adenine residue. In some embodiments, a nucleobase is a protected adenine residue. In some embodiments, a nucleobase is an optionally substituted guanine residue. In some embodiments, a nucleobase is a protected guanine residue. In some embodiments, a nucleobase is an optionally substituted cytosine residue. In some embodiments, a nucleobase is a protected cytosine residue. In some embodiments, a nucleobase is an optionally substituted thymine residue. In some embodiments, a nucleobase is a protected thymine residue. In some embodiments, a nucleobase is an optionally substituted uracil residue. In some embodiments, a nucleobase is a protected uracil residue. In some embodiments, a nucleobase is an optionally substituted 5-methylcytosine residue. In some embodiments, a nucleobase is a protected 5-methylcytosine residue.

[0188] In some embodiments, a provided oligonucleotide comprises a modified nucleobase described in, e.g., US 5552540, US 6222025, US 6528640, US 4845205, US 5681941, US 5750692, US 6015886, US 5614617, US 6147200, US 5457187, US 6639062, US 7427672, US 5459255, US 5484908, US 7045610, US 3687808, US 5502177, US 5525711 6235887, US 5175273, US 6617438, US 5594121, US 6380368, US 5367066, US 5587469, US 6166197, US 5432272, US 7495088, US 5134066, or US 5596091. In some embodiments, a nucleobase is described in WO 2020/154344, WO 2020/154343, WO 2020/154342, WO 2020/165077, WO 2020/201406, WO 2020/216637, or WO 2020/252376, and can be utilized in accordance with the present disclosure.

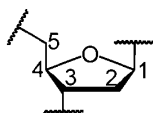
[0189] In some embodiments, a nucleobase is a protected base residue as used in oligonucleotide preparation. In some embodiments, a nucleobase is a base residue illustrated in US 2011/0294124, US 2015/0211006, US 2015/0197540, WO 2015/107425, WO 2017/192679, WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, or WO 2020/191252.

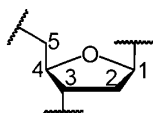
Sugars

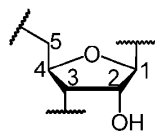
[0190] Various sugars, including modified sugars, can be utilized in accordance with the present

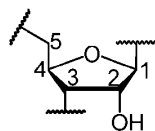
disclosure in, e.g., phosphoramidites, nucleosides, oligonucleotides, etc.

[0191] The most common naturally occurring nucleosides comprise ribose sugars (e.g., in RNA) or deoxyribose sugars (e.g., in DNA) linked to the nucleobases adenosine (A), cytosine (C), guanine (G), thymine (T) or uracil (U). In some embodiments, a sugar, e.g., in a phosphoramidite, a nucleoside, an oligonucleotide, etc., is a natural DNA sugar (in DNA nucleic acids or oligonucleotides, having the structure



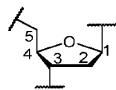
of , wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotide linkages (as appreciated by those skilled in the art, if at the 5'-end of oligonucleotide, the 5' position may be connected to a 5'-end group, and if at the 3'-end of an oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., $-\text{OH}$). In some embodiments, a sugar is a natural RNA sugar (in RNA nucleic acids or oligonucleotides, having the structure of

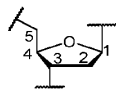


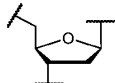
, wherein a nucleobase is attached to the 1' position, and the 3' and 5' positions are connected to internucleotide linkages (as appreciated by those skilled in the art, if at the 5'-end of an oligonucleotide, the 5' position may be connected to a 5'-end group (e.g., $-\text{OH}$), and if at the 3'-end of an oligonucleotide, the 3' position may be connected to a 3'-end group (e.g., $-\text{OH}$). In some embodiments, a sugar is a modified sugar in that it is not a natural DNA sugar or a natural RNA sugar. Among other things, modified sugars may provide improved stability. In some embodiments, modified sugars can be utilized to alter and/or optimize one or more hybridization characteristics. In some embodiments, modified sugars can be utilized to alter and/or optimize target nucleic acid recognition. In some embodiments, modified sugars can be utilized to optimize T_m . In some embodiments, modified sugars can be utilized to improve oligonucleotide activities.

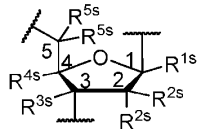
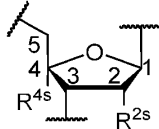
[0192] Sugars can be bonded to internucleotide linkages at various positions. For example, internucleotide linkages can be bonded to the 2', 3', 4' or 5' positions of sugars. In some embodiments, as most commonly in natural nucleic acids, an internucleotide linkage connects with one sugar at the 5' position and another sugar at the 3' position unless otherwise indicated.

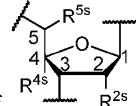
[0193] In some embodiments, a sugar is an optionally substituted natural DNA or RNA sugar. In some

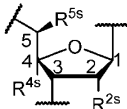


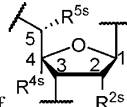
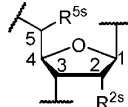
embodiments, a sugar is optionally substituted . In some embodiments, the 2' position is

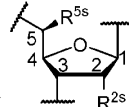
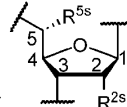
optionally substituted. In some embodiments, a sugar is . In some embodiments, a sugar has

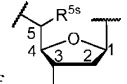
the structure of  or , wherein each of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} is independently $-H$, a suitable substituent or suitable sugar modification (e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, or WO 2022/099159, the substituents, sugar modifications, descriptions of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} , and modified sugars of each of which are independently incorporated herein by reference). In some embodiments, each of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} is independently R^s , wherein each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R'$, $-L^s-OR'$, $-L^s-SR'$, $-L^s-N(R')_2$, $-O-L^s-OR'$, $-O-L^s-SR'$, or $-O-L^s-N(R')_2$, wherein each R' is independently as described herein, and each L^s is independently a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms; or two R^s are taken together to form a bridge $-L^s-$. In some embodiments, R' is optionally

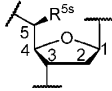
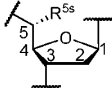
substituted C_{1-10} aliphatic. In some embodiments, a sugar has the structure of . In some

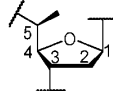
embodiments, a sugar has the structure of . In some embodiments, a sugar has the structure

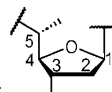
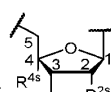
of . In some embodiments, a sugar has the structure of . In some embodiments,

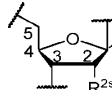
a sugar has the structure of . In some embodiments, a sugar has the structure of 

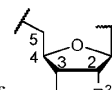
. In some embodiments, a sugar has the structure of . In some embodiments, a sugar has the

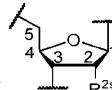
structure of . In some embodiments, a sugar has the structure of . In some embodiments, R^{5s} is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{5s} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{5s} is optionally substituted methyl. In some embodiments,

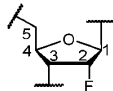
R^{5s} is methyl. In some embodiments, a sugar has the structure of . In some embodiments, a

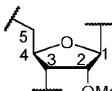
sugar has the structure of . In some embodiments, a sugar has the structure of . In

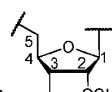
some embodiments, a sugar has the structure of . In some embodiments, a 2'-modified sugar

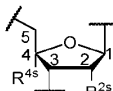
has the structure of  R^{2s} , wherein R^{2s} is a 2'-modification. In some embodiments, a sugar has the

structure of  R^{2s} , wherein R^{2s} is $-H$, halogen, or $-OR$, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{2s} is $-H$. In some embodiments, R^{2s} is $-F$. In some embodiments, R^{2s} is $-OMe$. In some embodiments, a modified nucleoside is mA, mT, mC, m5mC, mG, mU, etc., in which R^{2s} is $-OMe$. In some embodiments, R^{2s} is $-OCH_2CH_2OMe$. In some embodiments, R^{2s} is $-OCH_2CH_2OH$.

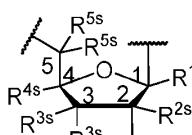
In some embodiments, a 2'-F modified sugar has the structure of . In some embodiments, a 2'-

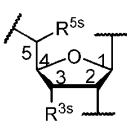
OMe modified sugar has the structure of . In some embodiments, a 2'-MOE modified sugar

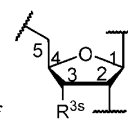
has the structure of  $OCH_2CH_2OCH_3$.

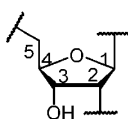
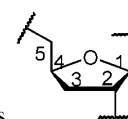
[0194] In some embodiments, a sugar has the structure of  R^{4s} and R^{2s} , wherein R^{2s} and R^{4s} are taken together to form $-L^s-$, wherein L^s is a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms. In some embodiments, each heteroatom is independently selected

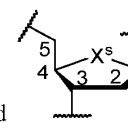
from nitrogen, oxygen or sulfur). In some embodiments, L^s is optionally substituted $C2-O-CH_2-C4$. In some embodiments, L^s is $C2-O-CH_2-C4$. In some embodiments, L^s is $C2-O-(R)-CH(CH_2CH_3)-C4$. In some embodiments, L^s is $C2-O-(S)-CH(CH_2CH_3)-C4$.

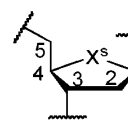
[0195] In some embodiments, a sugar has the structure of , wherein each variable

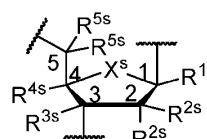
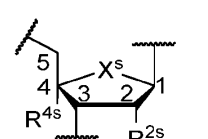
is independently as described herein. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{5s} is $-H$. In some

embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{3s} is $-OH$. In some embodiments, R^{3s} is $-H$. In some

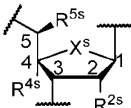
embodiments, a sugar is . In some embodiments, a sugar is .

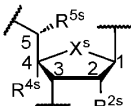
[0196] In some embodiments, a sugar is optionally substituted , wherein X^s is $-S-$, $-Se-$, or optionally substituted $-CH_2-$. In some embodiments, the 2' position is optionally substituted. In

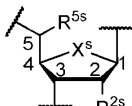
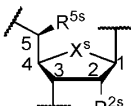
some embodiments, a sugar is . In some embodiments, a sugar has the structure of

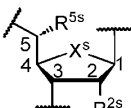
 or , wherein each of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} is independently $-H$, a suitable substituent or suitable sugar modification (e.g., those described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO

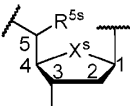
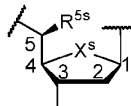
2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the substituents, descriptions of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} , and modified sugars of each of which are independently incorporated herein by reference). In some embodiments, each of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} is independently R^s , wherein each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R'$, $-L^s-OR'$, $-L^s-SR'$, $-L^s-N(R')_2$, $-O-L^s-OR'$, $-O-L^s-SR'$, or $-O-L^s-N(R')_2$, wherein each R' is independently as described herein, and each L^s is independently a covalent bond or optionally substituted bivalent C_{1-6} aliphatic or heteroaliphatic having 1-4 heteroatoms; or two R^s are taken together to form a bridge $-L^s-$. In some embodiments, R' is optionally substituted C_{1-10} aliphatic. In some embodiments, a

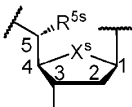
sugar has the structure of  . In some embodiments, a sugar has the structure of .

In some embodiments, a sugar has the structure of  . In some embodiments, a sugar has the

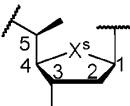
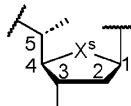
structure of  . In some embodiments, a sugar has the structure of  . In some

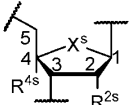
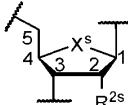
embodiments, a sugar has the structure of  . In some embodiments, a sugar has the structure

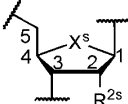
of  . In some embodiments, a sugar has the structure of  . In some embodiments,

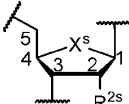
a sugar has the structure of  . In some embodiments, R^{5s} is optionally substituted C_{1-6} aliphatic.

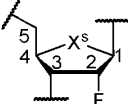
In some embodiments, R^{5s} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{5s} is optionally substituted methyl. In some embodiments, R^{5s} is methyl. In some embodiments, a sugar has the structure

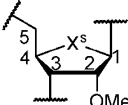
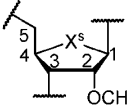
of  . In some embodiments, a sugar has the structure of  . In some embodiments,

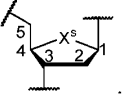
a sugar has the structure of . In some embodiments, a sugar has the structure of .

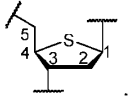
. In some embodiments, a 2'-modified sugar has the structure of , wherein R^{2s} is a 2'-

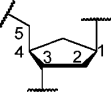
modification. In some embodiments, a sugar has the structure of , wherein R^{2s} is -H, halogen, or -OR, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, R^{2s} is -H. In some embodiments, R^{2s} is -F. In some embodiments, R^{2s} is -OMe. In some embodiments, R^{2s} is -OCH₂CH₂OMe. In some embodiments, R^{2s} is -OCH₂CH₂OH. In some embodiments, a modified sugar

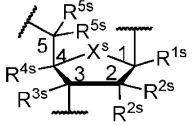
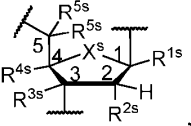
has the structure of . In some embodiments, a modified sugar has the structure of

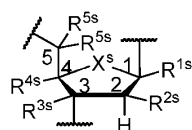
. In some embodiments, a modified sugar having the structure of .

In some embodiments, a modified sugar having the structure of . In some embodiments, X^s is -S-. In some embodiments, X^s is optionally substituted -CH₂-. In some embodiments, X^s is -CH₂-.

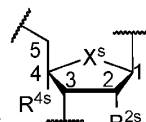
In some embodiments, a modified sugar having the structure of . In some embodiments, a

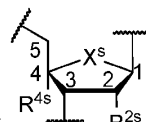
modified sugar having the structure of .

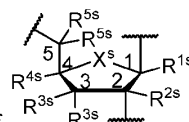
[0197] In some embodiments, a sugar has the structure of , or .

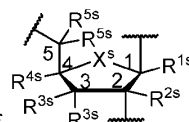


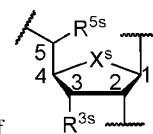
, wherein each R^{2s} is independently –H, –F, –OH or –OR^{ak}, wherein R^{ak} is optionally substituted C₁₋₆ aliphatic, and each of the other variables is independently as described herein. In some embodiments, each of R^{1s}, R^{3s}, R^{4s}, and R^{5s} is independently –H. In some embodiments, each of R^{1s}, R^{3s} and R^{4s}, and one of R^{5s}, are independently –H, and the other R^{5s} is independently C₁₋₆ aliphatic. In some embodiments, an occurrence of R^{5s} is C₁₋₆ aliphatic, e.g., methyl. In some embodiments, R^{2s} is –H. In some embodiments, R^{2s} is –F. In some embodiments, R^{2s} is –OR^{ak}. In some embodiments, R^{2s} is –OMe. In some embodiments, R^{2s} is –OCH₂CH₂CH₃. In some embodiments, at least one occurrence of R^{2s} is –H. In some embodiments, at least one occurrence of R^{2s} is not –H. In some embodiments, X^s is –O–. In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted –CH₂–. In some embodiments, X^s is –CH₂–.

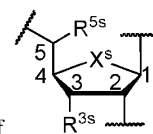


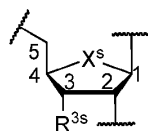
[0198] In some embodiments, a sugar has the structure of , wherein R^{2s} and R^{4s} are taken together to form –L^s–, wherein L^s is a covalent bond or optionally substituted bivalent C₁₋₆ aliphatic or heteroaliphatic having 1-4 heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen or sulfur). In some embodiments, L^s is optionally substituted C2–O–CH₂–C4. In some embodiments, L^s is C2–O–CH₂–C4. In some embodiments, L^s is C2–O–(R)–CH(CH₂CH₃)–C4. In some embodiments, L^s is C2–O–(S)–CH(CH₂CH₃)–C4. In some embodiments, X^s is –S–. In some embodiments, X^s is optionally substituted –CH₂–. In some embodiments, X^s is –CH₂–. In some embodiments, X^s is –Se–.

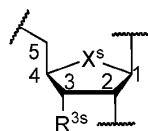


[0199] In some embodiments, a sugar has the structure of , wherein each variable



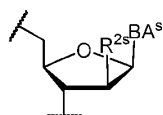
is independently as described herein. In some embodiments, a sugar has the structure of , wherein each variable is independently as described herein. In some embodiments, R^{5s} is –H. In some



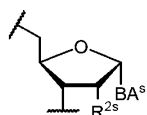
embodiments, a sugar has the structure of , wherein each variable is independently as

described herein. In some embodiments, R^{3s} is $-\text{OH}$. In some embodiments, R^{3s} is $-\text{H}$. In some embodiments, X^s is $-\text{S}-$. In some embodiments, X^s is optionally substituted $-\text{CH}_2-$. In some embodiments, X^s is $-\text{CH}_2-$.

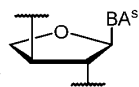
[0200] In some embodiments, a nucleoside comprising a modified sugar has the structure of



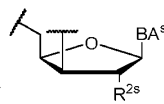
or a salt form thereof, wherein BA^s is $-\text{H}$ or an optionally substituted or protected nucleobase (c.g., BA), and R^{2s} is as described herein. In some embodiments, R^{2s} is $-\text{OH}$, halogen, or optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, BA^s is $-\text{H}$. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, R^{2s} is $-\text{F}$. In some embodiments, a nucleoside comprising a modified sugar has the structure of



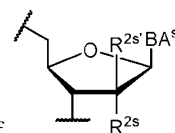
or a salt form thereof, wherein each variable is independently as described herein. In some embodiments, R^{2s} is $-\text{H}$, $-\text{OH}$, halogen, or optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, R^{2s} is $-\text{H}$. In some embodiments, R^{2s} is $-\text{F}$. In some embodiments, a nucleoside comprising a modified sugar



has the structure of , wherein each variable is as described herein. In some embodiments, a

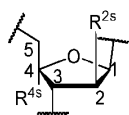


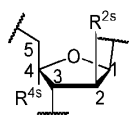
nucleoside comprising a modified sugar has the structure of or a salt form thereof, wherein each variable is independently as described herein. In some embodiments, R^{2s} is $-\text{H}$, $-\text{OH}$, halogen, or optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, R^{2s} is $-\text{H}$. In some embodiments, R^{2s} is $-\text{F}$.

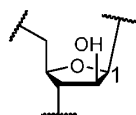


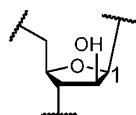
In some embodiments, a nucleoside comprising a modified sugar has the structure of or a salt form thereof, wherein $R^{2s'}$ is R^s , and each of R^s , R^{2s} and BA^s is independently as described herein. In some embodiments, each of R^{2s} and $R^{2s'}$ is independently $-\text{H}$, $-\text{OH}$, halogen, or optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, R^{2s} is $-\text{H}$. In some embodiments, R^{2s} is $-\text{OH}$. In some embodiments, R^{2s} is halogen. In some embodiments, R^{2s} is $-\text{F}$. In some embodiments, R^{2s} is optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, $R^{2s'}$ is $-\text{H}$. In some embodiments, $R^{2s'}$ is $-\text{OH}$. In some embodiments, $R^{2s'}$ is halogen. In some embodiments, $R^{2s'}$ is $-\text{F}$. In some embodiments, $R^{2s'}$ is optionally substituted $\text{C}_1\text{-C}_6$ alkoxy. In some embodiments, BA^s is $-\text{H}$. In some embodiments, BA^s is an optionally substituted or

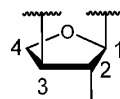
protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. Certain such nucleosides including sugars and nucleobases and uses thereof are described in WO 2020/154342. In some embodiments, an oligonucleotide comprises arabinoside, 2'-deoxy-2'-fluoro-arabinoside, 2'-OR arabinoside, adeoxycytidine, DNA-abasic, RNA-abasic, or 2'-OR abasic, wherein R is not hydrogen (e.g., optionally substituted C₁₋₆ aliphatic). In some embodiments, 2'-OR is 2'-OMe. In some embodiments, 2'-OR is 2'-MOE. In some embodiments, an oligonucleotide comprises 2'-O-methyl-arabinocytidine (amC). In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, monomers comprise such nucleosides. In some embodiments, phosphoramidites comprise such nucleosides (in some embodiments, one connecting site (e.g., a -CH₂- connecting site) is bonded to an optionally substituted -OH, e.g., (-ODMTr), and one connecting site (e.g., a ring connecting site) is bonded to O which is also bonded to P of a phosphoramidite). In some embodiments, one or more or each of a 5' immediate nucleoside (e.g., N₁), an opposite nucleoside (N₀) and a 3' immediate nucleoside (e.g., N₁) is independently such a nucleoside. In some embodiments, 5'-N₁N₀N₁-3' is amCCA. In some embodiments, a sugar has

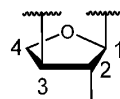


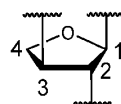
the structure of , wherein each variable is as described herein and C1' is bonded to a nucleobase. In some embodiments, a sugar is an arabinose. In some embodiments, a sugar has the structure

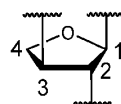


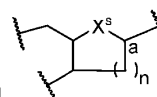
of , wherein C1' is bonded to a nucleobase.

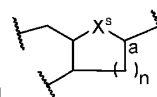


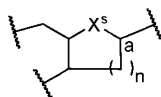
[0201] In some embodiments, a sugar is optionally substituted , wherein a nucleobase is

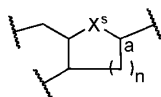
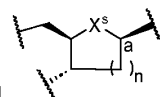


bonded at position 1'. In some embodiments, a sugar is , wherein a nucleobase is bonded at position 1'.

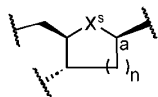


[0202] In some embodiments, a sugar is optionally substituted , wherein position a is bonded to a nucleobase, X^s is -O-, -S-, -Sc- or optionally substituted -CH₂-. In some embodiments, a



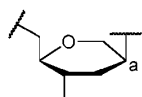
sugar is . In some embodiments, a sugar is optionally substituted , wherein

position a is bonded to a nucleobase, X^s is $-O-$, $-S-$, $-Se-$ or optionally substituted $-CH_2-$. In some

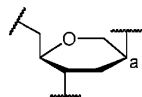


embodiments, a sugar is . In some embodiments, X^s is $-O-$. In some embodiments, X^s is $-S-$. In some embodiments, X^s is $-Se-$. In some embodiments, X^s is optionally substituted $-CH_2-$. In some embodiments, X^s is $-CH_2-$. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

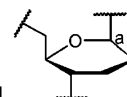
[0203] In some embodiments, a modified sugar comprises an optionally substituted 6-membered ring having 0-1 oxygen atom. In some embodiments, a modified sugar comprises an optionally substituted 6-membered ring having an oxygen atom. For example, in some embodiments, a modified sugar has the



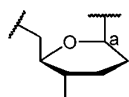
structure of optionally substituted , wherein position a is bonded to a nucleobase. In some



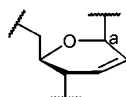
embodiments, a modified sugar has the structure of , wherein position a is bonded to a



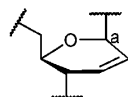
nucleobase. In some embodiments, a modified sugar has the structure of optionally substituted , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure



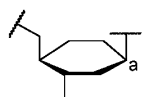
of , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has



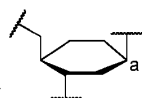
the structure of optionally substituted , wherein position a is bonded to a nucleobase. In some



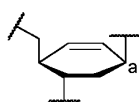
embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of optionally substituted

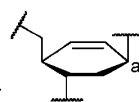


, wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has

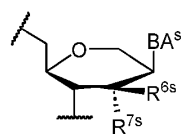


the structure of , wherein position a is bonded to a nucleobase. In some embodiments, a

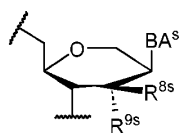
modified sugar has the structure of optionally substituted , wherein position a is bonded to a

nucleobase. In some embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase.

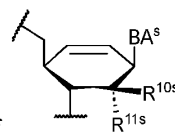
[0204] In some embodiments, a nucleoside comprising a modified sugar has the structure of



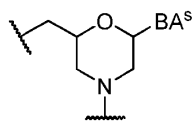
or a salt form thereof, wherein each of R^{6s} and R^{7s} is independently R^s , BA^s is $-H$ or an optionally substituted or protected nucleobase (e.g., BA), and R^s is independently as described herein. In some embodiments, R^{6s} is $-H$, $-OH$ or halogen, and R^{7s} is $-H$, $-OH$, halogen or optionally substituted C_1 - C_6 alkoxy. In some embodiments, BA^s is $-H$. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, a nucleoside comprising



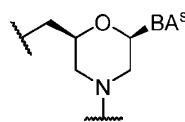
a modified sugar has the structure of or a salt form thereof, wherein each of R^{8s} and R^{9s} is independently R^s , and each of R^s and BA^s is independently as described herein. In some embodiments, R^{8s} is $-H$ or halogen, and R^{9s} is $-H$, $-OH$, halogen, or optionally substituted C_1 - C_6 alkoxy. In some



embodiments, a nucleoside comprising a modified sugar has the structure of or a salt form thereof, wherein each of R^{10s} and R^{11s} is independently R^s , and each of R^s and BA^s is independently as described herein. In some embodiments, R^{10s} is $-H$ or halogen, and R^{11s} is $-H$, $-OH$, halogen, or optionally substituted C_1 - C_6 alkoxy. In some embodiments, a nucleoside comprising a modified sugar has the structure

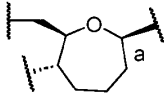


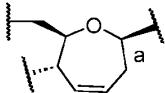
of or a salt form thereof, wherein BA^s is as described herein. In some embodiments, a

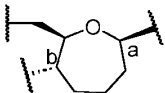


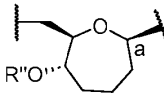
nucleoside comprising a modified sugar has the structure of or a salt form thereof, wherein BA^s is as described herein. Those skilled in the art appreciate that in some embodiments, the

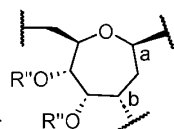
nitrogen may be directly bonded to linkage phosphorus. In some embodiments, a halogen is $-F$. In some embodiments, BA^s is $-H$. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. In some embodiments, an oligonucleotide comprises alpha-homo-DNA, beta-homo-DNA moieties. In some embodiments, an oligonucleotide comprises an alpha- or beta-homo-DNA sugar. In some embodiments, an oligonucleotide comprises an alpha-homo-DNA sugar. In some embodiments, an oligonucleotide comprises a beta-homo-DNA sugar. Certain such nucleosides including sugars and nucleobases and uses thereof are described in WO 2020/154343. In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, monomers comprise such nucleosides. In some embodiments, phosphoramidites comprise such nucleosides (in some embodiments, one connecting site (e.g., a $-CH_2-$ connecting site) is bonded to an optionally substituted $-OH$, e.g., $-ODMTr$, and one connecting site (e.g., a ring connecting site) is bonded to P of a phosphoramidite (e.g., when the connecting ring atom is N) or to O which is also bonded to P of a phosphoramidite (e.g., when the connecting ring atom is C)). In some embodiments, one or more or each of a 5' immediate nucleoside (e.g., N_1), an opposite nucleoside (N_0) and a 3' immediate nucleoside (e.g., N_{-1}) is independently such a nucleoside.

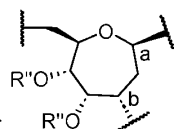
[0205] In some embodiments, a modified sugar has the structure of , wherein position

a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure of , wherein position a is bonded to a nucleobase. In some embodiments, a modified sugar has the structure

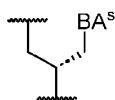
of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleotidic linkage and R'' is $-H$ or optionally substituted C_{1-6} aliphatic. In some embodiments, a modified sugar has

the structure of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleotidic linkage and R'' is $-H$ or C_{1-6} aliphatic. In some embodiments, a modified sugar has the

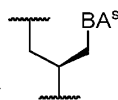


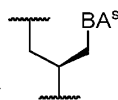
structure of , wherein position a is bonded to a nucleobase, position b is bonded to an internucleotidic linkage and R'' is -H or C₁₋₆ aliphatic. In some embodiments, R'' is methyl.

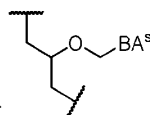
[0206] In some embodiments, a nucleoside comprising a modified sugar has the structure of

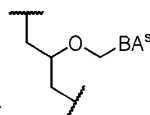


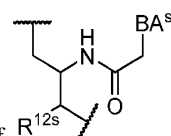
or a salt form thereof, wherein each variable is as described herein. In some embodiments, a

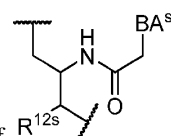


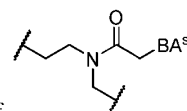
nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleoside comprising a modified sugar has

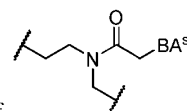


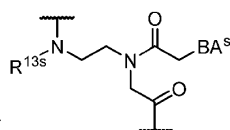
the structure of  or a salt form thereof, wherein each variable is as described herein. In some

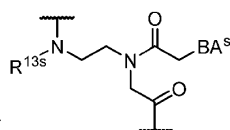


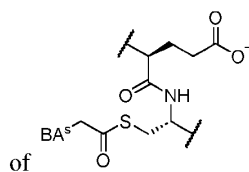
embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein R^{12s} is R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{12s} is -H, -OH, halogen, optionally substituted C₁₋₆ alkyl, optionally substituted C₁₋₆ heteroalkyl, or optionally substituted C₁₋₆ alkoxy. In some embodiments, a halogen is -F. In some



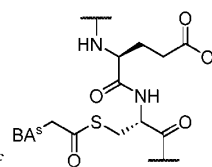
embodiments, a nucleoside comprising a modified sugar has the structure of  or a salt form thereof, wherein each variable is as described herein. In some embodiments, a nucleotide comprising



a modified sugar has the structure of  or a salt form thereof, wherein R^{13s} is R^s, and each of R^s and BA^s is independently as described herein. In some embodiments, R^{13s} is -H or optionally substituted C₁₋₆ alkyl. In some embodiments, a nucleoside comprising a modified sugar has the structure

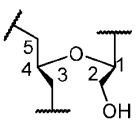


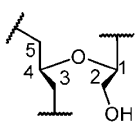
of or a salt form thereof, wherein each variable is as described herein. In some

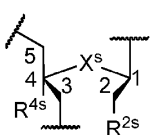
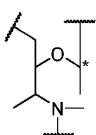


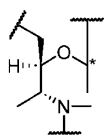
embodiments, a nucleotide comprising a modified sugar has the structure of or a salt form thereof, wherein each variable is as described herein. In some embodiments, a linkage is an amide linkage. In some embodiments, BA^s is $-H$. In some embodiments, BA^s is an optionally substituted or protected nucleobase. In some embodiments, BA^s is BA. In some embodiments, nucleobases such as BA are optionally substituted or protected for oligonucleotide synthesis. Certain such nucleosides and nucleotides including sugars and nucleobases and uses thereof are described in WO 2020/154344. In some embodiments, oligonucleotides comprise such nucleosides. In some embodiments, oligonucleotides comprise such nucleosides (in some embodiments, one connecting site (e.g., a $-CH_2-$ connecting site) is bonded to an optionally substituted $-OH$, e.g., $(-ODMTr)$, and one connecting site (e.g., a ring connecting site) is bonded to O which is also bonded to P of a phosphoramidite. In some embodiments, one or more or each of a 5' immediate nucleoside (e.g., N_1), an opposite nucleoside (N_0) and a 3' immediate nucleoside (e.g., N_{-1}) is independently such a nucleoside.

[0207] In some embodiments, a sugar is an acyclic sugar, e.g. a UNA sugar. In some embodiments, a

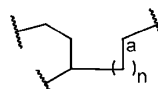
sugar is optionally substituted . In some embodiments, the 2' position is optionally

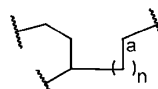
substituted. In some embodiments, a sugar is . In some embodiments, a sugar has the

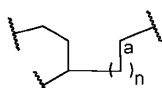
structure of . In some embodiments, R^{2s} is $-OH$. In some embodiments, a sugar is , wherein “*” indicates the carbon atom bonded to a nucleobase. In some embodiments, a sugar is

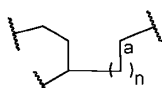


, wherein “*” indicates the carbon atom bonded to a nucleobase. In some embodiments, the carbon atom bonded to a nitrogen atom of a nucleobase and is of *R* configuration (e.g., sm18). In some embodiments, an oligonucleotide comprises a sugar described herein.



[0208] In some embodiments, a sugar is optionally substituted , wherein position a is bonded to a nucleobase, X^s is $-O-$, $-S-$, $-Se-$ or optionally substituted $-CH_2-$. In some embodiments, a



sugar is . In some embodiments, X^s is $-O-$. In some embodiments, X^s is $-S-$. In some embodiments, X^s is $-Se-$. In some embodiments, X^s is optionally substituted $-CH_2-$. In some embodiments, X^s is $-CH_2-$. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

[0209] In some embodiments, a sugar is connected not through 5' and 3' positions. Those skilled in the art appreciate that for such sugars, 5' can refer to the side/direction toward 5'-end of an oligonucleotide, and 3' can refer to the side/direction toward 3'-end of an oligonucleotide.

[0210] In some embodiments, each of R^{1s} , R^{2s} , R^{3s} , R^{4s} , and R^{5s} is independently R^s , wherein R^s is independently $-H$, halogen, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R'$, $-L^s-Si(R')_3$, $-L^s-OR'$, $-L^s-SR'$, $-L^s-N(R')_2$, $-O-L^s-R'$, $-O-L^s-Si(R)_3$, $-O-L^s-OR'$, $-O-L^s-SR'$, or $-O-L^s-N(R')_2$; wherein L^s is L^B as described herein, and each other variable is independently as described herein. In some embodiments, each of R^{1s} and R^{2s} is independently R^s . In some embodiments, R^s is $-H$. In some embodiments, R^s is not $-H$. In some embodiments, L^s is a covalent bond. In some embodiments, each of R^{2s} and R^{4s} are independently $-H$, $-F$, $-OR$, $-N(R)_2$. In some embodiments, R^{2s} is $-H$, $-F$, $-OR$, $-N(R)_2$. In some embodiments, R^{4s} is $-H$. In some embodiments, R^{2s} and R^{4s} form $2'-O-L^s-$, wherein L^s is optionally substituted C_{1-6} alkylene. In some embodiments, L^s is optionally substituted $-CH_2-$. In some embodiments, L^s is optionally substituted $-CH_2-$.

[0211] In some embodiments, R is hydrogen. In some embodiments, R is not hydrogen. In some embodiments, R is an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0212] In some embodiments, R is optionally substituted C₁₋₁₀ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ alkyl. In some embodiments, R is optionally substituted hexyl, pentyl, butyl, propyl, ethyl or methyl. In some embodiments, R is optionally substituted hexyl. In some embodiments, R is optionally substituted pentyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is optionally substituted propyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is hexyl. In some embodiments, R is pentyl. In some embodiments, R is butyl. In some embodiments, R is propyl. In some embodiments, R is ethyl. In some embodiments, R is methyl. In some embodiments, R is isopropyl. In some embodiments, R is n-propyl. In some embodiments, R is tert-butyl. In some embodiments, R is sec-butyl. In some embodiments, R is n-butyl. In some embodiments, R is $-(CH_2)_2OCH_3$.

[0213] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl.

[0214] In some embodiments, R^{2s} is a 2'-modification as described in the present disclosure, and R^{4s} is -H. In some embodiments, R^{2s} is -OR, wherein R is not hydrogen. In some embodiments, R^{2s} is -F. In some embodiments, R^{2s} is -OMe. In some embodiments, R^{2s} is -OCH₂CH₂CH₃. In some embodiments, R^{2s} is selected from -H, -F, and -OR, wherein R is optionally substituted C₁₋₆ alkyl. In some embodiments, R^{2s} is selected from -H, -F, and -OMe.

[0215] In some embodiments, a sugar is a bicyclic sugar, e.g., sugars wherein R^{2s} and R^{4s} are taken to form an optionally substituted ring as described in the present disclosure. In some embodiments, a sugar is selected from LNA sugars, BNA sugars, cEt sugars, etc. In some embodiments, a bridge is between the 2' and 4'-carbon atoms (corresponding to R^{2s} and R^{4s} taken together with their intervening atoms to form an optionally substituted ring as described herein). In some embodiments, a bridge is 2'-L^a-L^b-4', wherein L^a is -O-, -S- or N(R), and L^b is an optionally substituted C₁₋₄ bivalent aliphatic chain, e.g., methylene.

[0216] In some embodiments, a sugar is a 2'-OMe, 2'-MOE, 2'-F, a LNA (locked nucleic acid) sugar, an ENA (ethylene bridged nucleic acid) sugar, a BNA(NMe) (Methylamino bridged nucleic acid) sugar, 2'-F ANA (2'-F arabinose), alpha-DNA (alpha-D-ribose), 2'/5' ODN (e.g., 2'/5' linked oligonucleotide), Inv (inverted sugar, e.g., inverted desoxyribose), AmR (Amino-Ribose), ThioR (Thio-ribose), HNA (hexose nucleic acid), CeNA (cyclohexene nucleic acid), or MOR (Morpholino) sugar.

[0217] Those skilled in the art after reading the present disclosure will appreciate that various types of sugar modifications are known and can be utilized in accordance with the present disclosure. In some embodiments, a sugar modification is a 2'-modification (e.g., R^{2s}). In some embodiments, a 2'-modification is 2'-F. In some embodiments, a 2'-modification is 2'-OR, wherein R is not hydrogen. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C₁₋₆ aliphatic. In some embodiments, a 2'-modification is 2'-OR, wherein R is optionally substituted C₁₋₆ alkyl. In some

embodiments, a 2'-modification is 2'-OMe. In some embodiments, a 2'-modification is 2'-MOE. In some embodiments, a 2'-modification is $-O-L^b-$ or $-L^b-L^b-$ which connects the 2'-carbon of a sugar moiety to another carbon of a sugar moiety. In some embodiments, a 2'-modification is 2'- $O-L^b-4'$ or 2'- L^b-L^b-4' which connects the 2'-carbon of a sugar moiety to the 4'-carbon of a sugar moiety. In some embodiments, a 2'-modification is S-cEt. In some embodiments, a modified sugar is an LNA sugar. In some embodiments, $-L^b-$ is $-C(R)_2-$. In some embodiments, a 2'-modification is $(C2-O-C(R)_2-C4)$, wherein each R is independently as described in the present disclosure. In some embodiments, a 2'-modification is a LNA sugar modification $(C2-O-CH_2-C4)$. In some embodiments, a 2'-modification is $(C2-O-CHR-C4)$, wherein R is as described in the present disclosure. In some embodiments, a 2'-modification is $(C2-O-(R)-CHR-C4)$, wherein R is as described in the present disclosure and is not hydrogen. In some embodiments, a 2'-modification is $(C2-O-(S)-CHR-C4)$, wherein R is as described in the present disclosure and is not hydrogen. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is unsubstituted C_{1-6} alkyl. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, a 2'-modification is $(C2-O-CHR-C4)$, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is $(C2-O-CHR-C4)$, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is $(C2-O-CHR-C4)$, wherein R is methyl. In some embodiments, a 2'-modification is $(C2-O-CHR-C4)$, wherein R is ethyl. In some embodiments, a 2'-modification is $(C2-O-(R)-CHR-C4)$, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is $(C2-O-(R)-CHR-C4)$, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is $(C2-O-(R)-CHR-C4)$, wherein R is methyl. In some embodiments, a 2'-modification is $(C2-O-(R)-CHR-C4)$, wherein R is ethyl. In some embodiments, a 2'-modification is $(C2-O-(S)-CHR-C4)$, wherein R is optionally substituted C_{1-6} aliphatic. In some embodiments, a 2'-modification is $(C2-O-(S)-CHR-C4)$, wherein R is optionally substituted C_{1-6} alkyl. In some embodiments, a 2'-modification is $(C2-O-(S)-CHR-C4)$, wherein R is methyl. In some embodiments, a 2'-modification is $(C2-O-(S)-CHR-C4)$, wherein R is ethyl. In some embodiments, a 2'-modification is $C2-O-(R)-CH(CH_2CH_3)-C4$. In some embodiments, a 2'-modification is $C2-O-(S)-CH(CH_2CH_3)-C4$. In some embodiments, a sugar is a natural DNA sugar. In some embodiments, a sugar is a natural RNA sugar. In some embodiments, a sugar is an optionally substituted natural DNA sugar. In some embodiments, a sugar is a natural DNA sugar optionally substituted at 2'. In some embodiments, a sugar is a natural DNA sugar substituted at 2' (2'-modification). In some embodiments, a sugar is a natural DNA sugar modified at 2' (2'-modification).

[0218] In some embodiments, a sugar is an optionally substituted ribose or deoxyribose. In some embodiments, a sugar is an optionally modified ribose or deoxyribose, wherein one or more hydroxyl

groups of the ribose or deoxyribose moiety is optionally and independently replaced by halogen, R', -N(R')₂, -OR', or -SR', wherein each R' is as described herein. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with halogen, R', -N(R')₂, -OR', or -SR', wherein each R' is independently described in the present disclosure. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with halogen. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with one or more -F. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with -OR', wherein each R' is independently described in the present disclosure. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with -OR', wherein each R' is independently optionally substituted C₁-C₆ aliphatic. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with -OR', wherein each R' is independently an optionally substituted C₁-C₆ alkyl. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with -OMe. In some embodiments, a sugar is an optionally substituted deoxyribose, wherein the 2' position of the deoxyribose is optionally substituted with -O-methoxyethyl.

[0219] In some embodiments, provided oligonucleotides comprise one or more modified sugars. In some embodiments, provided oligonucleotides comprise one or more modified sugars and one or more natural sugars.

[0220] Examples of bicyclic sugars include sugars of alpha-L-methyleneoxy (4'-CH₂-O-2') LNA, beta-D-methyleneoxy (4'-CH₂-O-2') LNA, ethyleneoxy (4'-(CH₂)₂-O-2') LNA, aminooxy (4'-(CH₂-O-N(R)-2') LNA, and oxyamino (4'-CH₂-N(R)-O-2') LNA. In some embodiments, a bicyclic sugar, e.g., a LNA or BNA sugar, is sugar having at least one bridge between two sugar carbons. In some embodiments, a bicyclic sugar in a nucleoside may have the stereochemical configurations of alpha-L-ribofuranose or beta-D-ribofuranose.

[0221] In some embodiments, a bicyclic sugar may be further defined by isomeric configuration. For example, a sugar comprising a 4'-(CH₂)-O-2' bridge may be in the alpha-L configuration or in the beta-D configuration. In some embodiments, a 4' to 2' bridge is a -L-4'-(CH₂)-O-2', b-D-4'-CH₂-O-2', 4'-(CH₂)₂-O-2', 4'-CH₂-O-N(R')-2', 4'-CH₂-N(R')-O-2', 4'-CH(R')-O-2', 4'-CH(CH₃)-O-2', 4'-CH₂-S-2', 4'-CH₂-N(R')-2', 4'-CH₂-CH(R')-2', 4'-CH₂-CH(CH₃)-2', and 4'-(CH₂)₃-2', wherein each R' is as described in the present disclosure. In some embodiments, R' is -H, a protecting group or optionally substituted C₁-C₁₂ alkyl. In some embodiments, R' is -H or optionally substituted C₁-C₁₂ alkyl.

[0222] In some embodiments, a bicyclic sugar is a sugar of alpha-L-methyleneoxy (4'-CH₂-O-2') BNA, beta-D-methyleneoxy (4'-CH₂-O-2') BNA, ethyleneoxy (4'-(CH₂)₂-O-2') BNA, aminooxy (4'-CH₂-O-N(R)-2') BNA, oxyamino (4'-CH₂-N(R)-O-2') BNA, methyl(methyleneoxy) (4'-CH(CH₃)-O-2') BNA (also referred to as constrained ethyl or cEt), methylene-thio (4'-CH₂-S-2') BNA, methylene-amino (4'-CH₂-N(R)-2') BNA, methyl carbocyclic (4'-CH₂-CH(CH₃)-2') BNA, propylene carbocyclic (4'-(CH₂)₃-2') BNA, or vinyl BNA.

[0223] In some embodiments, a sugar modification is a modification described in US 9006198. In some embodiments, a modified sugar is described in US 9006198. In some embodiments, a sugar modification is a modification described in US 9394333, US 9744183, US 9605019, US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/022473, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/032612, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the sugar modifications and modified sugars of each of which are independently incorporated herein by reference.

[0224] In some embodiments a modified sugar is one described in US 5658873, US 5118800, US 5393878, US 5514785, US 5627053, US 7034133;7084125, US 7399845, US 5319080, US 5591722, US 5597909, US 5466786, US 6268490, US 6525191, US 5519134, US 5576427, US 6794499, US 6998484, US 7053207, US 4981957, US 5359044, US 6770748, US 7427672, US 5446137, US 6670461, US 7569686, US 7741457, US 8022193, US 8030467, US 8278425, US 5610300, US 5646265, US 8278426, US 5567811, US 5700920, US 8278283, US 5639873, US 5670633, US 8314227, US 2008/0039618, US 2009/0012281, WO 2021/030778, WO 2020/154344, WO 2020/154343, WO 2020/154342, WO 2020/165077, WO 2020/201406, WO 2020/216637, or WO 2020/252376.

[0225] In some embodiments, a sugar modification is 2'-OMe, 2'-MOE, 2'-LNA, 2'-F, 5'-vinyl, or S-cEt. In some embodiments, a modified sugar is a sugar of FRNA, FANA, or morpholino. In some embodiments, an oligonucleotide comprises a nucleic acid analog, e.g., GNA, LNA, PNA, TNA, F-HNA (F-THP or 3'-fluoro tetrahydropyran), MNA (mannitol nucleic acid, e.g., Leumann 2002 Bioorg. Med. Chem. 10: 841-854), ANA (anitol nucleic acid), or morpholino, or a portion thereof. In some embodiments, a sugar is as in flexible nucleic acids or serinol nucleic acids. In some embodiments, a sugar modification replaces a natural sugar with another cyclic or acyclic moiety. Examples of such moieties are widely known in the art, e.g., those used in morpholino, glycol nucleic acids, etc. and may be utilized in accordance with the present disclosure. As appreciated by those skilled in the art, when utilized with modified sugars, in some embodiments internucleotidic linkages may be modified, e.g., as in morpholino, PNA, etc. In some embodiments, a sugar is a (R)-GNA sugar. In some embodiments, a sugar is a (S)-GNA sugar. In some

embodiments, a nucleoside having a GNA sugar is utilized as N_{-1} , N_0 and/or N_1 . In some embodiments, N_0 is a nucleoside having a GNA sugar. In some embodiments, a sugar is bicyclic sugar. In some embodiments, a sugar is a LNA sugar. In some embodiments, a sugar is an acyclic sugar. In some embodiments, a sugar is a UNA sugar. In some embodiments, a nucleoside having a UNA sugar is utilized as N_{-1} , N_0 and/or N_1 . In some embodiments, N_0 is a nucleoside having a UNA sugar. In some embodiments, a nucleoside is abasic. In some embodiments, an abasic sugar is utilized as N_{-1} , N_0 and/or N_1 . In some embodiments, N_0 is a nucleoside having an abasic sugar.

[0226] In some embodiments, a sugar is a 6'-modified bicyclic sugar that have either (R) or (S)-chirality at the 6-position, e.g., those described in US 7399845. In some embodiments, a sugar is a 5'-modified bicyclic sugar that has either (R) or (S)-chirality at the 5-position, e.g., those described in US 20070287831.

[0227] In some embodiments, a modified sugar contains one or more substituents at the 2' position (typically one substituent, and often at the axial position) independently selected from -F; -CF₃, -CN, -N₃, -NO, -NO₂, -OR', -SR', or -N(R')₂, wherein each R' is independently described in the present disclosure; -O-(C₁-C₁₀ alkyl), -S-(C₁-C₁₀ alkyl), -NH-(C₁-C₁₀ alkyl), or -N(C₁-C₁₀ alkyl)₂; -O-(C₂-C₁₀ alkenyl), -S-(C₂-C₁₀ alkenyl), -NH-(C₂-C₁₀ alkenyl), or -N(C₂-C₁₀ alkenyl)₂; -O-(C₂-C₁₀ alkynyl), -S-(C₂-C₁₀ alkynyl), -NH-(C₂-C₁₀ alkynyl), or -N(C₂-C₁₀ alkynyl)₂; or -O-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), -O-(C₁-C₁₀ alkylene)-NH-(C₁-C₁₀ alkyl) or -O-(C₁-C₁₀ alkylene)-NH(C₁-C₁₀ alkyl)₂, -NH-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), or -N(C₁-C₁₀ alkyl)-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), wherein each of the alkyl, alkylene, alkenyl and alkynyl is independently and optionally substituted. In some embodiments, a substituent is -O(CH₂)_nOCH₃, -O(CH₂)_nNH₂, MOE, DMAOE, or DMAEOE, wherein n is from 1 to about 10. In some embodiments, a modified sugar is one described in WO 2001/088198; and Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504. In some embodiments, a modified sugar comprises one or more groups selected from a substituted silyl group, an RNA cleaving group, a reporter group, a fluorescent label, an intercalator, a group for improving the pharmacokinetic properties of a nucleic acid, a group for improving the pharmacodynamic properties of a nucleic acid, or other substituents having similar properties. In some embodiments, modifications are made at one or more of the 2', 3', 4', or 5' positions, including the 3' position of the sugar on the 3'-terminal nucleoside or in the 5' position of the 5'-terminal nucleoside.

[0228] In some embodiments, the 2'-OH of a ribose is replaced with a group selected from -H, -F; -CF₃, -CN, -N₃, -NO, -NO₂, -OR', -SR', or -N(R')₂, wherein each R' is independently described in the present disclosure; -O-(C₁-C₁₀ alkyl), -S-(C₁-C₁₀ alkyl), -NH-(C₁-C₁₀ alkyl), or -N(C₁-C₁₀ alkyl)₂; -O-(C₂-C₁₀ alkenyl), -S-(C₂-C₁₀ alkenyl), -NH-(C₂-C₁₀ alkenyl), or -N(C₂-C₁₀ alkenyl)₂; -O-(C₂-C₁₀ alkynyl), -S-(C₂-C₁₀ alkynyl), -NH-(C₂-C₁₀ alkynyl), or -N(C₂-C₁₀ alkynyl)₂; or -O-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), -O-(C₁-C₁₀ alkylene)-NH-(C₁-C₁₀ alkyl) or -O-(C₁-C₁₀ alkylene)-NH(C₁-C₁₀

alkyl)₂, -NH-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), or -N(C₁-C₁₀ alkyl)-(C₁-C₁₀ alkylene)-O-(C₁-C₁₀ alkyl), wherein each of the alkyl, alkylene, alkenyl and alkynyl is independently and optionally substituted. In some embodiments, the 2'-OH is replaced with -H (deoxyribose). In some embodiments, the 2'-OH is replaced with -F. In some embodiments, the 2'-OH is replaced with -OR'. In some embodiments, the 2'-OH is replaced with -OMe. In some embodiments, the 2'-OH is replaced with -OCH₂CH₂OMe.

[0229] In some embodiments, a sugar modification is a 2'-modification. Commonly used 2'-modifications include but are not limited to 2'-OR, wherein R is not hydrogen and is as described in the present disclosure. In some embodiments, a modification is 2'-OR, wherein R is optionally substituted C₁₋₆ aliphatic. In some embodiments, a modification is 2'-OR, wherein R is optionally substituted C₁₋₆ alkyl. In some embodiments, a modification is 2'-OMe. In some embodiments, a modification is 2'-MOE. In some embodiments, a 2'-modification is S-cEt. In some embodiments, a modified sugar is an LNA sugar. In some embodiments, a 2'-modification is -F. In some embodiments, a 2'-modification is FANA. In some embodiments, a 2'-modification is FRNA. In some embodiments, a sugar modification is a 5'-modification, e.g., 5'-Me. In some embodiments, a sugar modification changes the size of the sugar ring. In some embodiments, a sugar modification is the sugar moiety in FHNA.

[0230] In some embodiments, a sugar modification replaces a sugar moiety with another cyclic or acyclic moiety. Examples of such moieties are widely known in the art, including but not limited to those used in morpholino (optionally with its phosphorodiamidate linkage), glycol nucleic acids, etc.

[0231] Modified sugars include cyclobutyl or cyclopentyl moieties in place of a pentofuranosyl sugar. Representative examples of such modified sugars include those described in US 4,981,957, US 5,118,800, US 5,319,080, or US 5,359,044. In some embodiments, the oxygen atom within the ribose ring is replaced by nitrogen, sulfur, selenium, or carbon. In some embodiments, -O- is replaced with -N(R')-, -S-, -Se- or -C(R')₂-. In some embodiments, a modified sugar is a modified ribose wherein the oxygen atom within the ribose ring is replaced with nitrogen, and wherein the nitrogen is optionally substituted with an alkyl group (e.g., methyl, ethyl, isopropyl, etc.).

[0232] An example of modified sugars is glycerol, which is part of glycerol nucleic acids (GNAs), e.g., as described in Zhang, R et al., J. Am. Chem. Soc., 2008, 130, 5846-5847; Zhang L, et al., J. Am. Chem. Soc., 2005, 127, 4174-4175 and Tsai CH et al., PNAS, 2007, 14598-14603.

[0233] A flexible nucleic acid (FNA) is based on a mixed acetal amination of formyl glycerol, e.g., as described in Joyce GF et al., PNAS, 1987, 84, 4398-4402 and Heuberger BD and Switzer C, J. Am. Chem. Soc., 2008, 130, 412-413.

[0234] In some embodiments, one or more hydroxyl group in a sugar is optionally and independently replaced with halogen, R' -N(R')₂, -OR', or -SR', wherein each R' is independently described in the present disclosure.

[0235] In some embodiments, a modified nucleoside is a modified nucleoside described in WO 2018/022473, WO 2018/098264, WO 2018/223056, WO 2018/223073, WO 2018/223081, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, and/or WO 2022/099159, the modified nucleosides of each of which are independently incorporated herein by reference.

[0236] In some embodiments, a sugar modification is 5'-vinyl (R or S), 5'-methyl (R or S), 2'-SH, 2'-F, 2'-OCH₃, 2'-OCH₂CH₃, 2'-OCH₂CH₂F or 2'-O(CH₂)₂₀CH₃. In some embodiments, a substituent at the 2' position, e.g., a 2'-modification, is allyl, amino, azido, thio, -O-allyl, -O-C₁-C₁₀ alkyl, -OCF₃, -OCH₂F, -O(CH₂)₂SCH₃, -O(CH₂)₂ON(R')₂, -OCH₂C(O)N(R')₂, and -OCH₂C(O)N(R')(CH₂)₂N(R')₂, wherein each allyl, amino and alkyl is optionally substituted, and each R' is independently as described in the present disclosure. In some embodiments, each R' is independently -H or optionally substituted C₁-C₁₀ alkyl.

[0237] In some embodiments, bicyclic sugars comprise a bridge, e.g., -L^b-L^b-, -L-, etc. between two sugar carbons, e.g., between the 4' and the 2' ribosyl ring carbon atoms. In some embodiments, a bridge is 4'-(CH₂)-O-2' (e.g., LNA sugars), 4'-(CH₂)-S-2', 4'-(CH₂)₂-O-2' (e.g., ENA sugars), 4'-CH(R')-O-2' (e.g., 4'-CH(CH₃)-O-2', 4'-CH(CH₂OCH₃)-O-2', and examples in US 7399845, etc.), 4'-CH(R')₂-O-2' (e.g., 4'-C(CH₃)(CH₃)-O-2' and examples in WO 2009006478, etc.), 4'-CH₂-N(OR')-2' (e.g., 4'-CH₂-N(OCH₃)-2', examples in WO 2008150729, etc.), 4'-CH₂-O-N(R')-2' (e.g., 4'-CH₂-O-N(CH₃)-2', examples in US 20040171570, etc.), 4'-CH₂-N(R')-O-2' [e.g., wherein R is -H, C₁-C₁₂ alkyl, or a protecting group (e.g., see US 7427672)], 4'-C(R')₂-C(H)(R')-2' (e.g., 4'-CH₂-C(H)(CH₃)-2', examples in Chattopadhyaya et al., J. Org. Chem., 2009, 74, 118-134, etc.), or 4'-C(R')₂-C(=C(R')₂)-2' (e.g., 4'-CH₂-C(=CH₂)-2', examples in WO 2008154401, etc.).

[0238] In some embodiments, a sugar is a tetrahydropyran or THP sugar. In some embodiments, a modified nucleoside is tetrahydropyran nucleoside or THP nucleoside which is a nucleoside having a six-membered tetrahydropyran sugar substituted for a pentofuranosyl residue in typical natural nucleosides. THP sugars and/or nucleosides include those used in hexitol nucleic acid (HNA), anitol nucleic acid (ANA), mannitol nucleic acid (MNA) (e.g., Leumann, Bioorg. Med. Chem., 2002, 10, 841-854) or fluoro HNA (F-HNA).

[0239] In some embodiments, sugars comprise rings having more than 5 atoms and/or more than one heteroatom, e.g., morpholino sugars which are described in e.g., Braasch et al., Biochemistry, 2002, 41, 4503-4510; US 5698685; US 5166315; US 5185444; US 5034506; etc.).

[0240] In some embodiments, a nucleoside has a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Example cyclohexenyl nucleosides and preparation and uses thereof are described in, e.g., WO 2010036696; Robeyns et al., J. Am. Chem. Soc.,

2008, 130(6), 1979-1984; Horvath et al., *Tetrahedron Letters*, 2007, 48, 3621-3623; Nauwelaerts et al., *J. Am. Chem. Soc.*, 2007, 129(30), 9340-9348; Gu et al., *Nucleosides, Nucleotides & Nucleic Acids*, 2005, 24(5-7), 993-998; Nauwelaerts et al., *Nucleic Acids Research*, 2005, 33(8), 2452-2463; Robeyns et al., *Acta Crystallographica, Section F: Structural Biology and Crystallization Communications*, 2005, F61(6), 585-586; Gu et al., *Tetrahedron*, 2004, 60(9), 2111-2123; Gu et al., *Oligonucleotides*, 2003, 13(6), 479-489; Wang et al., *J. Org. Chem.*, 2003, 68, 4499-4505; Verbeure et al., *Nucleic Acids Research*, 2001, 29(24), 4941-4947; Wang et al., *J. Org. Chem.*, 2001, 66, 8478-82; Wang et al., *Nucleosides, Nucleotides & Nucleic Acids*, 2001, 20(4-7), 785-788; Wang et al., *J. Am. Chem.*, 2000, 122, 8595-8602; WO 2006047842; WO 2001049687; etc.

[0241] Many monocyclic, bicyclic and tricyclic ring systems are suitable as sugar surrogates (modified sugars) and may be utilized in accordance with the present disclosure. See, e.g., Leumann, *Christian J. Bioorg. & Med. Chem.*, 2002, 10, 841-854. Such ring systems can undergo various additional substitutions to further enhance their properties and/or activities.

[0242] In some embodiments, a 2'-modified sugar is a furanosyl sugar modified at the 2' position. In some embodiments, a 2'-modification is halogen, -R' (wherein R' is not -H), -OR' (wherein R' is not -H), -SR', -N(R')₂, optionally substituted -CH₂-CH=CH₂, optionally substituted alkenyl, or optionally substituted alkynyl. In some embodiments, a 2'-modification is selected from -O[(CH₂)_nO]_mCH₃, -O(CH₂)_nNH₂, -O(CH₂)_nCH₃, -O(CH₂)_nF, -O(CH₂)_nONH₂, -OCH₂C(=O)N(H)CH₃, and -O(CH₂)_nON[(CH₂)_nCH₃]₂, wherein each n and m is independently from 1 to about 10. In some embodiments, a 2'-modification is optionally substituted C₁-C₁₂ alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted alkaryl, optionally substituted aralkyl, optionally substituted -O-alkaryl, optionally substituted -O-aralkyl, -SH, -SCH₃, -OCN, -Cl, -Br, -CN, -F, -CF₃, -OCF₃, -SOCH₃, -SO₂CH₃, -ONO₂, -NO₂, -N₃, -NH₂, optionally substituted heterocycloalkyl, optionally substituted heterocycloalkaryl, optionally substituted aminoalkylamino, optionally substituted polyalkylamino, substituted silyl, a reporter group, an intercalator, a group for improving pharmacokinetic properties, a group for improving the pharmacodynamic properties, and other substituents. In some embodiments, a 2'-modification is a 2'-MOE modification (e.g., see Baker et al., *J. Biol. Chem.*, 1997, 272, 11944-12000). In some cases, a 2'-MOE modification has been reported as having improved binding affinity compared to unmodified sugars and to some other modified nucleosides, such as 2'-O-methyl, 2'-O-propyl, and 2'-O-aminopropyl. Oligonucleotides having the 2'-MOE modification have also been reported to be capable of inhibiting gene expression with promising features for in vivo use (see, e.g., Martin, *Helv. Chim. Acta*, 1995, 78, 486-504; Altmann et al., *Chimia*, 1996, 50, 168-176; Altmann et al., *Biochem. Soc. Trans.*, 1996, 24, 630-637; and Altmann et al., *Nucleosides Nucleotides*, 1997, 16, 917-926; etc.).

[0243] In some embodiments, a 2'-modified or 2'-substituted sugar or nucleoside is a sugar or nucleoside comprising a substituent at the 2' position of the sugar which is other than -H (typically not considered a substituent) or -OH. In some embodiments, a 2'-modified sugar is a bicyclic sugar comprising a bridge connecting two carbon atoms of the sugar ring one of which is the 2' carbon. In some embodiments, a 2'-modification is non-bridging, e.g., allyl, amino, azido, thio, optionally substituted -O-allyl, optionally substituted -O-C₁-C₁₀ alkyl, -OCF₃, -O(CH₂)₂OCH₃, 2'-O(CH₂)₂SCH₃, -O(CH₂)₂ON(R_m)(R_n), or -OCH₂C(=O)N(R_m)(R_n), where each R_m and R_n is independently -H or optionally substituted C₁-C₁₀ alkyl.

[0244] Certain modified sugars, their preparation and uses are described in US 4981957, US 5118800, US 5319080, US 5359044, US 5393878, US 5446137, US 5466786, US 5514785, US 5519134, US 5567811, US 5576427, US 5591722, US 5597909, US 5610300, US 5627053, US 5639873, US 5646265, US 5670633, US 5700920, US 5792847, US 6600032 and WO 2005121371.

[0245] In some embodiments, a sugar is the sugar of N-methanocarba, LNA, cMOE BNA, cEt BNA, α-L-LNA or related analogs, HNA, Me-ANA, MOE-ANA, Ara-FHNA, FHNA, R-6'-Me-FHNA, S-6'-Me-FHNA, ENA, or c-ANA. In some embodiments, a modified internucleotidic linkage is C3-amide (e.g., sugar that has the amide modification attached to the C3', Mutisya et al. 2014 Nucleic Acids Res. 2014 Jun 1; 42(10): 6542-6551), formacetal, thioformacetal, MMI [e.g., methylene(methylimino), Peoc'h et al. 2006 Nucleosides and Nucleotides 16 (7-9)], a PMO (phosphorodiamidate linked morpholino) linkage (which connects two sugars), or a PNA (peptide nucleic acid) linkage. In some embodiments, examples of internucleotidic linkages and/or sugars are described in Allerson et al. 2005 J. Med. Chem. 48: 901-4; BMCL 2011 21: 1122; BMCL 2011 21: 588; BMCL 2012 22: 296; Chattopadhyaya et al. 2007 J. Am. Chem. Soc. 129: 8362; Chem. Bio. Chem. 2013 14: 58; Curr. Prot. Nucl. Acids Chem. 2011 1.24.1; Egli et al. 2011 J. Am. Chem. Soc. 133: 16642; Hendrix et al. 1997 Chem. Eur. J. 3: 110; Hyrup et al. 1996 Bioorg. Med. Chem. 4: 5; Imanishi 1997 Tet. Lett. 38: 8735; J. Am. Chem. Soc. 1994, 116, 3143; J. Med. Chem. 2009 52: 10; J. Org. Chem. 2010 75: 1589; Jepsen et al. 2004 Oligo. 14: 130-146; Jones et al. J. Org. Chem. 1993, 58, 2983; Jung et al. 2014 ACIEE 53: 9893; Kodama et al. 2014 AGDS; Koizumi 2003 BMC 11: 2211; Koizumi et al. 2003 Nuc. Acids Res. 12: 3267-3273; Koshkin et al. 1998 Tetrahedron 54: 3607-3630; Kumar et al. 1998 Bioorg. Med. Chem. Lett. 8: 2219-2222; Lauritsen et al. 2002 Chem. Comm. 5: 530-531; Lauritsen et al. 2003 Bioorg. Med. Chem. Lett. 13: 253-256; Lima et al. 2012 Cell 150: 883-894; Mesmaeker et al. Angew. Chem., Int. Ed. Engl. 1994, 33, 226; Migawa et al. 2013 Org. Lett. 15: 4316; Mol. Ther. Nucl. Acids 2012 1: e47; Morita et al. 2001 Nucl. Acids Res. Supp. 1: 241-242; Morita et al. 2002 Bioorg. Med. Chem. Lett. 12: 73-76; Morita et al. 2003 Bioorg. Med. Chem. Lett. 2211-2226; Murray et al. 2012 Nucl. Acids Res. 40: 6135; Nielsen et al. 1997 Chem. Soc. Rev. 73; Nielsen et al. 1997 J. Chem. Soc. Perkins Transl. 1: 3423-3433; Obika et al. 1997 Tetrahedron Lett. 38 (50): 8735-8; Obika et al. 1998

Tetrahedron Lett. 39: 5401-5404; Obika et al. 2008 J. Am. Chem. Soc. 130: 4886; Obika et al. 2011 Org. Lett. 13: 6050; Oestergaard et al. 2014 JOC 79: 8877; Pallan et al. 2012 Biochem. 51: 7; Pallan et al. 2012 Chem. Comm. 48: 8195-8197; Petersen et al. 2003 TRENDS Biotech. 21: 74-81; Prakash et al. 2010 J. Med. Chem. 53: 1636; Prakash et al. 2015 Nucl. Acids Res. 43: 2993-3011; Prakash et al. 2016 Bioorg. Med. Chem. Lett. 26: 2817-2820; Rajwanshi et al. 1999 Chem. Commun. 1395-1396; Schultz et al. 1996 Nucleic Acids Res. 24: 2966; Seth et al. 2008 Nucl. Acid Sym. Ser. 52: 553; Seth et al. 2009 J. Med. Chem. 52: 10-13; Seth et al. 2010 J. Am. Chem. Soc. 132: 14942; Seth et al. 2010 J. Med. Chem. 53: 8309-8318; Seth et al. 2010 J. Org. Chem. 75: 1569-1581; Seth et al. 2011 BMCL 21: 4690; Seth et al. 2012 Bioorg. Med. Chem. Lett. 22: 296-299; Seth et al. 2012 Mol. Ther-Nuc. Acids. 1, e47; Seth et al., Nucleic Acids Symposium Series (2008), 52(1), 553-554; Singh et al. 1998 Chem. Comm. 1247-1248; Singh et al. 1998 J. Org. Chem. 63: 10035-39; Singh et al. 1998 J. Org. Chem. 63: 6078-6079; Sorensen 2003 Chem. Comm. 2130-2131; Starrup et al. 2010 Nucl. Acids Res. 38: 7100; Swayze et al. 2007 Nucl. Acids Res. 35: 687; Ts'o et al. Ann. N. Y. Acad. Sci. 1988, 507, 220; Van Aerschot et al. 1995 Angew. Chem. Int. Ed. Engl. 34: 1338; Vasseur et al. J. Am. Chem. Soc. 1992, 114, 4006; WO 2007090071; WO 2016079181; US 6326199; US 6066500; or US 6440739.

[0246] Various additional sugars useful for preparing oligonucleotides or analogs thereof are known in the art and may be utilized in accordance with the present disclosure.

Internucleotidic linkages

[0247] Among other things, the present disclosure provides various internucleotidic linkages, including various modified internucleotidic linkages, that may be utilized together with other structural elements, e.g., various sugars as described herein, to provide oligonucleotides and compositions thereof.

[0248] Various internucleotidic linkages may be utilized in oligonucleotides in accordance with the present disclosure. In some embodiments, an oligonucleotide comprises one or more types of internucleotidic linkage. In some embodiments, an oligonucleotide comprises two or more types of internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least three types of internucleotidic linkages. In some embodiments, a linkage contains a linkage phosphorus atom bonded to an oxygen atom which oxygen atom is not bonded to or is not part of a backbone sugar ("a PO linkage", e.g., a natural phosphate linkage). In some embodiments, a linkage contains a linkage phosphorus atom bonded to a sulfur atom which sulfur atom is not bonded to or is not part of a backbone sugar ("a PS linkage", e.g., a phosphorothioate internucleotidic linkage). In some embodiments, a linkage contains a linkage phosphorus atom bonded to a nitrogen atom which nitrogen atom is not bonded to or is not part of a backbone sugar ("a PN linkage", e.g., n001). In some embodiments, an oligonucleotide comprises one or more PS linkages. In some embodiments, an oligonucleotide comprises one or more PO linkages. In

some embodiments, an oligonucleotide comprises one or more PN linkages. In some embodiments, an oligonucleotide comprises one or more PS and one or more PO linkages. In some embodiments, an oligonucleotide comprises one or more PS and one or more PN linkages. In some embodiments, an oligonucleotide comprises one or more PS, one or more PN and one or more PO linkages. In some embodiments, a PS linkage is a phosphorothioate linkage. In some embodiments, each PS linkage is independently a phosphorothioate linkage. In some embodiments, a PO linkage is a natural phosphate linkage. In some embodiments, each PO linkage is independently a natural phosphate linkage. In some embodiments, a PN linkage is a phosphoryl guanidine linkage. In some embodiments, each PN linkage is independently a phosphoryl guanidine linkage.

[0249] In some embodiments, provided oligonucleotides comprise both one or more modified internucleotidic linkages and one or more natural phosphate linkages. As widely known by those skilled in the art, natural phosphate linkages are widely found in natural DNA and RNA molecules; they have the structure of --OP(O)(OH)O-- , connect sugars in the nucleosides in DNA and RNA, and may be in various salt forms, for example, at physiological pH (about 7.4), natural phosphate linkages are predominantly exist in salt forms with the anion being $\text{--OP(O)(O}^-\text{)O--}$. A modified internucleotidic linkage, or a non-natural phosphate linkage, is an internucleotidic linkage that is not natural phosphate linkage or a salt form thereof. Modified internucleotidic linkages, depending on their structures, may also be in their salt forms. For example, as appreciated by those skilled in the art, phosphorothioate internucleotidic linkages which have the structure of --OP(O)(SH)O-- may be in various salt forms, e.g., at physiological pH (about 7.4) with the anion being $\text{--OP(O)(S}^-\text{)O--}$.

[0250] In some embodiments, an oligonucleotide comprises an internucleotidic linkage which is a modified internucleotidic linkage, e.g., phosphorothioate, phosphorodithioate, methylphosphonate, phosphoroamidate, thiophosphate, 3'-thiophosphate, or 5'-thiophosphate. In some embodiments, a modified internucleotidic linkage is a PN linkage. In some embodiments, a modified internucleotidic linkage is a PS linkage. In some embodiments, a modified internucleotidic linkage is a PO linkage (e.g., other than a natural phosphate linkage). In some embodiments, each modified internucleotidic linkage is independently a PN internucleotidic linkage or a PS internucleotidic linkage. In some embodiments, an oligonucleotide comprises one or more PN internucleotidic linkages, one or more PS internucleotidic linkages, and one or more PO internucleotidic linkages. In some embodiments, one or more PN internucleotidic linkages are independently phosphoryl guanidine internucleotidic linkages. In some embodiments, one or more PN internucleotidic linkages are independently n001. In some embodiments, one or more PS internucleotidic linkages are independently phosphorothioate internucleotidic linkages. In some embodiments, each PS internucleotidic linkage is independently a phosphorothioate internucleotidic linkage. In some embodiments, one or more PO internucleotidic linkages are independently natural

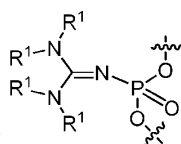
phosphate linkages. In some embodiments, each PO internucleotidic linkage is independently a natural phosphate linkage.

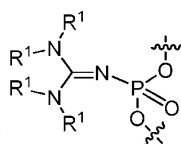
[0251] In some embodiments, a modified internucleotidic linkage is a chiral internucleotidic linkage which comprises a chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is a phosphorothioate linkage. In some embodiments, a chiral internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, a chiral internucleotidic linkage is chirally controlled with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is stereochemically pure with respect to its chiral linkage phosphorus. In some embodiments, a chiral internucleotidic linkage is not chirally controlled. In some embodiments, a pattern of backbone chiral centers comprises or consists of positions and linkage phosphorus configurations of chirally controlled internucleotidic linkages (*R_p* or *S_p*) and positions of achiral internucleotidic linkages (e.g., natural phosphate linkages).

[0252] In some embodiments, an internucleotidic linkage comprises a P-modification, wherein a P-modification is a modification at a linkage phosphorus. In some embodiments, a modified internucleotidic linkage is a moiety which does not comprise a phosphorus but serves to link two sugars or two moieties that each independently comprises a nucleobase, e.g., as in peptide nucleic acid (PNA).

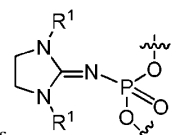
[0253] In some embodiments, an internucleotidic linkage is described in US 10167309, US 11643657, US 11718638, US 11608355, US 20230089442, US 20220306573, or US 20230203087, the internucleotidic linkages of each of which incorporated herein by reference.

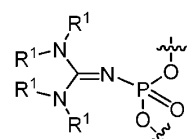
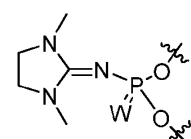
[0254] In some embodiments, a modified internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage) comprises optionally substituted triazolyl. In some embodiments, a modified internucleotidic linkage (e.g., a non-negatively charged internucleotidic linkage) comprises optionally substituted alkynyl. In some embodiments, a modified internucleotidic linkage comprises a triazole or alkyne moiety. In some embodiments, a triazole moiety, e.g., a triazolyl group, is optionally substituted. In some embodiments, a triazole moiety, e.g., a triazolyl group) is substituted. In some embodiments, a triazole moiety is unsubstituted. In some embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine moiety. In some embodiments, a modified internucleotidic linkage

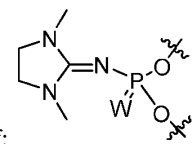
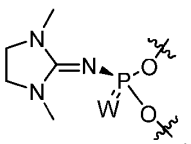
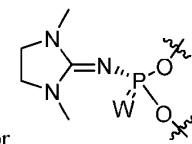


has the structure of  and is optionally chirally controlled, wherein *R*¹ is $-L-R'$, wherein each *L* and *R'* is independently as described herein. In some embodiments, each *R*¹ is independently *R'*. In some embodiments, each *R'* is independently *R*. In some embodiments, two *R*¹ are *R* and are taken

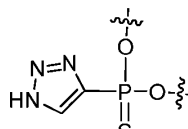
together to form a ring as described herein. In some embodiments, two R¹ on two different nitrogen atoms are R and are taken together to form a ring as described herein. In some embodiments, R¹ is independently optionally substituted C₁₋₆ aliphatic as described herein. In some embodiments, R¹ is methyl. In some embodiments, two R¹ on the same nitrogen atom are R and are taken together to form a ring as described

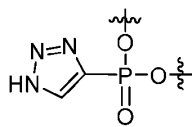
herein. In some embodiments, a modified internucleotidic linkage has the structure of  and

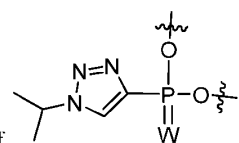
 is . In some embodiments, a modified internucleotidic linkage comprises an optionally substituted cyclic guanidine

moiety and has the structure of: , , or , wherein W is O or S. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a non-negatively charged internucleotidic linkage is stereochemically controlled.

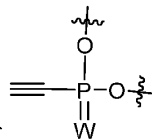
[0255] In some embodiments, a non-negatively charged internucleotidic linkage or a neutral internucleotidic linkage is an internucleotidic linkage comprising a triazole moiety. In some embodiments, a non-negatively charged internucleotidic linkage or a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, an internucleotidic linkage comprising a triazole moiety (e.g., an optionally substituted triazolyl group) has the structure of

. In some embodiments, an internucleotidic linkage comprising a triazole moiety has

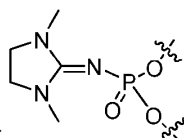
the structure of . In some embodiments, an internucleotidic linkage comprising a

triazole moiety has the formula of , where W is O or S. In some embodiments, an

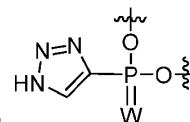
internucleotidic linkage comprising an alkyne moiety (e.g., an optionally substituted alkynyl group) has the



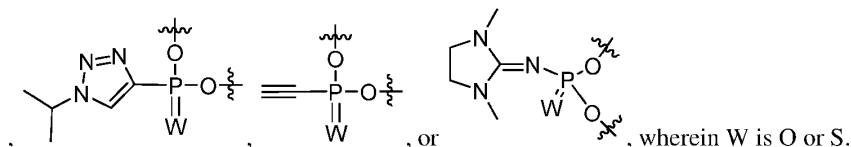
formula of $\text{P}(=\text{W})(\text{O}-\text{wavy})(\text{O}-\text{wavy})-\text{C}\equiv\text{C}-\text{wavy}$, wherein W is O or S. In some embodiments, an internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, a neutral internucleotidic linkage, comprises a cyclic guanidine moiety. In some embodiments, an internucleotidic linkage comprising a cyclic guanidine moiety



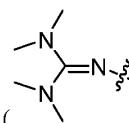
has the structure of $\text{cyclic guanidine}-\text{N}=\text{N}-\text{P}(=\text{O})(\text{O}-\text{wavy})(\text{O}-\text{wavy})$. In some embodiments, a non-negatively charged internucleotidic



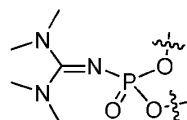
linkage, or a neutral internucleotidic linkage, is or comprising a structure selected from



, , or , wherein W is O or S.



[0256] In some embodiments, an internucleotidic linkage comprises a Tmg group ($\text{cyclic guanidine}-\text{N}=\text{N}-\text{P}(=\text{O})(\text{O}-\text{wavy})(\text{O}-\text{wavy})$). In some embodiments, an internucleotidic linkage comprises a Tmg group and has the structure of

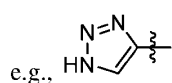


(the “Tmg internucleotidic linkage”). In some embodiments, neutral internucleotidic linkages include internucleotidic linkages of PNA and PMO, and an Tmg internucleotidic linkage.

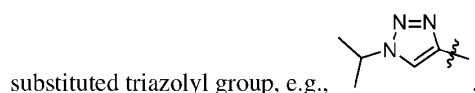
[0257] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 3-20 membered heterocyclyl or heteroaryl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, such a heterocyclyl or heteroaryl group is of a 5-membered ring. In some embodiments, such a heterocyclyl or heteroaryl group is of a 6-membered ring.

[0258] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms. In some embodiments,

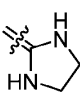
a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heteroaryl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heteroaryl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heteroaryl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a heteroaryl group is directly bonded to a linkage phosphorus. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted triazolyl group. In some embodiments, a non-negatively charged internucleotidic linkage comprises an unsubstituted triazolyl group,

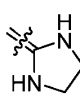


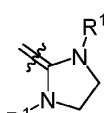
In some embodiments, a non-negatively charged internucleotidic linkage comprises a



[0259] In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-20 membered heterocyclyl group having 1-10 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-6 membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, a non-negatively charged internucleotidic linkage comprises an optionally substituted 5-membered heterocyclyl group having 1-4 heteroatoms, wherein at least one heteroatom is nitrogen. In some embodiments, at least two heteroatoms are nitrogen. In some embodiments, a heterocyclyl group is directly bonded to a linkage phosphorus. In some embodiments, a heterocyclyl group is bonded to a linkage phosphorus through a linker, e.g., =N– when the heterocyclyl group is part of a guanidine moiety who directed bonded to a linkage phosphorus through its =N–. In some embodiments, a non-negatively charged

internucleotidic linkage comprises an optionally substituted  group. In some embodiments, a non-

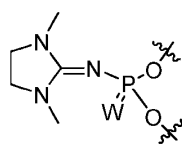
negatively charged internucleotidic linkage comprises an substituted  group. In some

embodiments, a non-negatively charged internucleotidic linkage comprises a  group, wherein

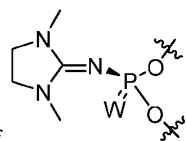
each R^1 is independently $-L-R$. In some embodiments, each R^1 is independently optionally substituted C_{1-6} alkyl. In some embodiments, each R^1 is independently methyl.

[0260] In some embodiments, a modified internucleotidic linkage, e.g., a non-negatively charged internucleotidic linkage, comprises a triazole or alkyne moiety, each of which is optionally substituted. In some embodiments, a modified internucleotidic linkage comprises a triazole moiety. In some embodiments, a modified internucleotidic linkage comprises an unsubstituted triazole moiety. In some embodiments, a modified internucleotidic linkage comprises a substituted triazole moiety. In some embodiments, a modified internucleotidic linkage comprises an alkyl moiety. In some embodiments, a modified internucleotidic linkage comprises an optionally substituted alkynyl group. In some embodiments, a modified internucleotidic linkage comprises an unsubstituted alkynyl group. In some embodiments, a modified internucleotidic linkage comprises a substituted alkynyl group. In some embodiments, an alkynyl group is directly bonded to a linkage phosphorus.

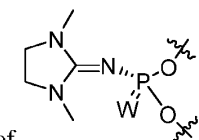
[0261] In some embodiments, a non-negatively charged internucleotidic linkage has the structure of



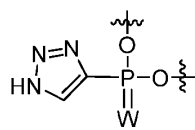
. In some embodiments, a non-negatively charged internucleotidic linkage has the



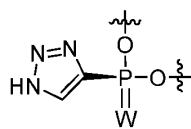
structure of



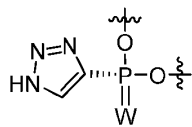
has the structure of



linkage has the structure of

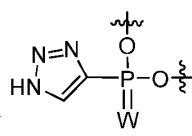


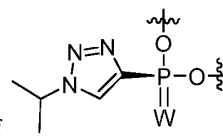
internucleotidic linkage has the structure of

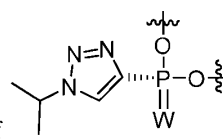


charged internucleotidic linkage has the structure of

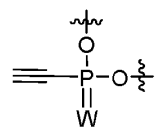
. In some embodiments, a non-

negatively charged internucleotidic linkage has the structure of . In some embodiments,

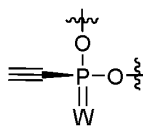
a non-negatively charged internucleotidic linkage has the structure of . In some

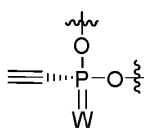
embodiments, a non-negatively charged internucleotidic linkage has the structure of .

In some embodiments, a non-negatively charged internucleotidic linkage has the structure of



In some embodiments, a non-negatively charged internucleotidic linkage has the structure

of . In some embodiments, a non-negatively charged internucleotidic linkage has the

structure of . In some embodiments, W is O. In some embodiments, W is S. In some embodiments, a neutral internucleotidic linkage is a non-negatively charged internucleotidic linkage described above.

[0262] In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage. In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled internucleotidic linkage which is not the neutral internucleotidic linkage. In some embodiments, an oligonucleotide comprises a neutral internucleotidic linkage and a chirally controlled phosphorothioate internucleotidic linkage. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more non-negatively charged internucleotidic linkages and one or more phosphorothioate internucleotidic linkages, wherein each phosphorothioate internucleotidic linkage in the oligonucleotide is independently a chirally controlled internucleotidic linkage. In some embodiments, the present disclosure provides an oligonucleotide comprising one or more neutral internucleotidic linkages and one or more phosphorothioate internucleotidic linkage, wherein each phosphorothioate internucleotidic linkage in the oligonucleotide is independently a

chirally controlled internucleotidic linkage. In some embodiments, an oligonucleotide comprises at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more chirally controlled phosphorothioate internucleotidic linkages. In some embodiments, non-negatively charged internucleotidic linkage is chirally controlled. In some embodiments, non-negatively charged internucleotidic linkage is not chirally controlled. In some embodiments, a neutral internucleotidic linkage is chirally controlled. In some embodiments, a neutral internucleotidic linkage is not chirally controlled. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled chiral internucleotidic linkages. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled non-negatively charged internucleotidic linkages (in some embodiments, each of which is independently n001). In some embodiments, a neutral internucleotidic linkage is chirally controlled. In some embodiments, a neutral internucleotidic linkage is not chirally controlled. In some embodiments, an oligonucleotide comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) chirally controlled and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) non-chirally controlled neutral internucleotidic linkages (in some embodiments, each of which is independently n001).

[0263] In some embodiments, an internucleotidic linkage has the structure of $-O-P(=W)-N(R')_2-O-$, wherein W is O or S, and each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-O-P(=W)(-NHR')-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-O-P(=W)(-NHSO_2R)-O-$, wherein each variable is independently as described herein. In some embodiments, R is methyl. In some embodiments, an internucleotidic linkage is $-O-P(=O)(-NHSO_2CH_3)-O-$. In some embodiments, an internucleotidic linkage has the structure of $-O-P(=W)[-N=C(R')_2]-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-O-P(=W)[-N=C(N(R')_2)_2]-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=W)(-N=C(R')_2)-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-OP(=W)(-N(R')_2)-O-$, wherein each variable is independently as described herein. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, such an internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, such an internucleotidic linkage is a neutral internucleotidic linkage.

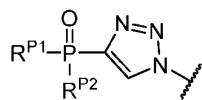
[0264] In some embodiments, an internucleotidic linkage has the structure of $-P(=W)[-N(R')_2]-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)(-NHR')-O-$, wherein each variable is independently as described

herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)(-NHSO_2R)-O-$, wherein each variable is independently as described herein. In some embodiments, R is methyl. In some embodiments, an internucleotidic linkage is $-P(=O)(-NHSO_2CH_3)-O-$. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)[-N=C(-L^L-R')_2]-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)[-N=C[N(R')_2]_2]-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)(-N=C(R')_2)-O-$, wherein each variable is independently as described herein. In some embodiments, an internucleotidic linkage has the structure of $-P(=W)(-N(R')_2)-O-$, wherein each variable is independently as described herein. In some embodiments, W is O. In some embodiments, W is S. In some embodiments, such an internucleotidic linkage is a non-negatively charged internucleotidic linkage. In some embodiments, such an internucleotidic linkage is a neutral internucleotidic linkage. In some embodiments, P of such an internucleotidic linkage is bonded to N of a sugar.

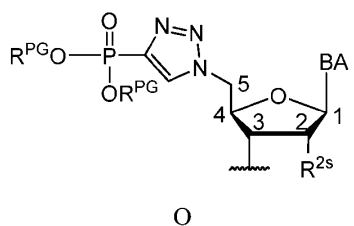
[0265] In some embodiments, a linkage is a phosphoryl guanidine internucleotidic linkage. In some embodiments, a linkage is a thio-phosphoryl guanidine internucleotidic linkage.

Oligonucleotides

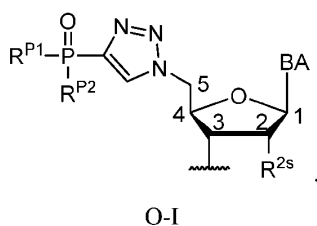
[0266] In some embodiments, provided technologies are useful for preparing or delivering oligonucleotides comprising $-P(O)(OH)_2$ which may exist in various salt forms. In some embodiments, the present disclosure provides oligonucleotides comprising $-P(O)(OR^{PG})_2$ as described herein. In some embodiments, oligonucleotides comprising $-P(O)(OR^{PG})_2$ as described herein are administered to a subject for preventing or treating conditions, disorders or diseases. In some embodiments, oligonucleotides comprising $-P(O)(OR^{PG})_2$ as described herein are utilized for preparing oligonucleotides comprising $-P(O)(OH)_2$ which may exist in various salt forms. For example, in some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising



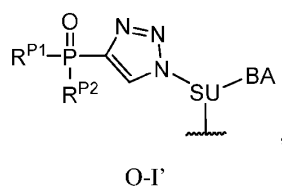
, wherein each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl. In some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising a moiety of formula O or a salt thereof:



wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I or a salt thereof:



wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I' or a salt thereof:



wherein each variable is independently as described herein.

[0267] As those skilled in the art appreciate, oligonucleotides may comprise various sugars, nucleobases, internucleotidic linkages as described herein. In some embodiments, an oligonucleotide comprises a PS linkage. In some embodiments, an oligonucleotide comprises a PN linkage. In some embodiments, an oligonucleotide comprises a PO linkage. In some embodiments, an oligonucleotide comprises a PS linkage and a PN linkage. In some embodiments, an oligonucleotide comprises a PS linkage and a PO linkage. In some embodiments, an oligonucleotide comprises a PO linkage, a PS linkage and a PN linkage. In some embodiments, the number of PS internucleotidic linkages in an oligonucleotide is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more. In some embodiments, the number of PN internucleotidic linkages in an oligonucleotide is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 or more. In some embodiments, the number of PO internucleotidic linkages in an oligonucleotide is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,

17, 18, 19 or 20 or more. In some embodiments, each PS linkage is a phosphorothioate internucleotidic linkage. In some embodiments, each PN linkage is independently a non-negatively charged internucleotidic linkage. In some embodiments, each PN linkage is independently a neutral internucleotidic linkage. In some embodiments, each PN linkage is independently a phosphoryl guanidine internucleotidic linkage. In some embodiments, each PN linkage is independently n001.

[0268] In some embodiments, each PS internucleotidic linkage is independently chirally controlled. In some embodiments, each PN internucleotidic linkage is independently chirally controlled. In some embodiments, each chiral linkage phosphorus is independently chirally controlled.

[0269] In some embodiments, an oligonucleotide has a length of about 10-200 (e.g., about 10-20, 10-30, 10-40, 10-50, 10-60, 10-70, 10-80, 10-90, 10-100, 10-120, 10-150, 20-30, 20-40, 20-50, 20-60, 20-70, 20-80, 20-90, 20-100, 20-120, 20-150, 20-200, 25-30, 25-40, 25-50, 25-60, 25-70, 25-80, 25-90, 25-100, 25-120, 25-150, 25-200, 30-40, 30-50, 30-60, 30-70, 30-80, 30-90, 30-100, 30-120, 30-150, 30-200, 10, 20, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 60, etc.) nucleobases. In some embodiments, the base sequence of an oligonucleotide is about 10-60 nucleobases in length. In some embodiments, a base sequence is about 15-50 nucleobases in length. In some embodiments, a base sequence is from about 15 to about 35 nucleobases in length. In some embodiments, an oligonucleotide is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more nucleobases in length. In some embodiments, each nucleobase counted in the length is independently optionally substituted or protected A, T, C, G, U or a tautomer thereof.

[0270] In some embodiments, oligonucleotides are provided as salt forms. In some embodiments, oligonucleotides are provided as salts comprising negatively-charged internucleotidic linkages (e.g., phosphorothioate internucleotidic linkages, natural phosphate linkages, etc.) existing as their salt forms. In some embodiments, oligonucleotides are provided as pharmaceutically acceptable salts. In some embodiments, oligonucleotides are provided as metal salts. In some embodiments, oligonucleotides are provided as sodium salts. In some embodiments, oligonucleotides are provided as ammonium salts. In some embodiments, oligonucleotides are provided as metal salts, e.g., sodium salts, wherein each negatively-charged internucleotidic linkage is independently in a salt form (e.g., for sodium salts, $-\text{O}-\text{P}(\text{O})(\text{SNa})-\text{O}-$ for a phosphorothioate internucleotidic linkage, $-\text{O}-\text{P}(\text{O})(\text{ONa})-\text{O}-$ for a natural phosphate linkage, etc.).

[0271] In some embodiments, oligonucleotides are chirally controlled, comprising one or more chirally controlled internucleotidic linkages. In some embodiments, provided oligonucleotides are stereochemically pure. In some embodiments, provided oligonucleotides or compositions thereof are substantially pure of other stereoisomers. In some embodiments, the present disclosure provides chirally controlled

oligonucleotide compositions.

[0272] As described herein, oligonucleotides of the present disclosure can be provided in high purity (e.g., 50%-100%). In some embodiments, oligonucleotides of the present disclosure are of high stereochemical purity (e.g., 50%-100%). In some embodiments, oligonucleotides in provided compositions are of high stereochemical purity (e.g., high percentage (e.g., 50%-100%) of a stereoisomer compared to the other stereoisomers of the same oligonucleotide). In some embodiments, a percentage is at least or about 50%. In some embodiments, a percentage is at least or about 60%. In some embodiments, a percentage is at least or about 70%. In some embodiments, a percentage is at least or about 75%. In some embodiments, a percentage is at least or about 80%. In some embodiments, a percentage is at least or about 85%. In some embodiments, a percentage is at least or about 90%. In some embodiments, a percentage is at least or about 95%.

[0273] In some embodiments, oligonucleotides of the present disclosure are at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% stereochemically pure at linkage phosphorus of chiral internucleotidic linkages. In some embodiments, oligonucleotides of the present disclosure are prepared stereoselectively and are substantially free of stereoisomers. In some embodiments, in provided compositions comprising a plurality of oligonucleotides which share the same base sequence of the same pattern of chiral linkage phosphorus stereochemistry (e.g., comprising one or more of *R_p* and/or *S_p*, wherein each chiral linkage phosphorus is independently *R_p* or *S_p*), at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of all oligonucleotides in the composition that share the same base sequence as oligonucleotides of the plurality share the same pattern of chiral linkage phosphorus stereochemistry or are oligonucleotides of the plurality. In some embodiments, in provided compositions comprising a plurality of oligonucleotides which share the same base sequence of the same pattern of chiral linkage phosphorus stereochemistry, at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% of all oligonucleotides in the composition that share the same constitution as oligonucleotides of the plurality share the same pattern of chiral linkage phosphorus stereochemistry or are oligonucleotides of the plurality. In some embodiments, diastereomeric excess of each chiral phosphorus is independently about or at least about 90%. In some embodiments, diastereomeric excess of each chiral phosphorus is independently about or at least about 95%. In some embodiments, diastereomeric excess of each chiral phosphorus is independently about or at least about 97%. In some embodiments, diastereomeric excess of each chiral phosphorus is independently about or at least about 98%. In some embodiments, diastereomeric purity is about or at least about (DS)^{nc}, wherein DS is about 90-100%, and nc is the number of chiral linkage phosphorus. In some embodiments, DS is about 90% or more. In some embodiments, DS is about 95% or more. In some embodiments, DS is about 96% or more. In some embodiments, DS is about 97% or more. In some embodiments, DS is about 98% or more. In some embodiments, DS is about 99% or more. In some embodiments, diastereomeric purity is represented

as the product of the diastereopurity of each chiral linkage phosphorus.

Oligonucleotide Compositions

[0274] Among other things, the present disclosure provides various oligonucleotide compositions. In some embodiments, the present disclosure provides oligonucleotide compositions of oligonucleotides described herein. In some embodiments, an oligonucleotide composition comprises a plurality of oligonucleotides described in the present disclosure. In some embodiments, an oligonucleotide composition is chirally controlled. In some embodiments, an oligonucleotide composition is not chirally controlled (stereorandom).

[0275] Linkage phosphorus of natural phosphate linkages is achiral. Linkage phosphorus of many modified internucleotidic linkages, e.g., phosphorothioate internucleotidic linkages, are chiral. In some embodiments, during preparation of oligonucleotide compositions (e.g., in traditional phosphoramidite oligonucleotide synthesis), configurations of chiral linkage phosphorus are not purposefully designed or controlled, creating non-chirally controlled (stereorandom) oligonucleotide compositions (substantially racemic preparations) which are complex, random mixtures of various stereoisomers (diastereoisomers) - for oligonucleotides with n chiral internucleotidic linkages (linkage phosphorus being chiral), typically 2^n stereoisomers (e.g., when n is 10, $2^{10}=1,032$; when n is 20, $2^{20}=1,048,576$). These stereoisomers have the same constitution, but differ with respect to the pattern of stereochemistry of their linkage phosphorus.

[0276] In some embodiments, stereorandom oligonucleotide compositions have sufficient properties and/or activities for certain purposes and/or applications. In some embodiments, stereorandom oligonucleotide compositions can be cheaper, easier and/or simpler to produce than chirally controlled oligonucleotide compositions. However, stereoisomers within stereorandom compositions may have different properties, activities, and/or toxicities, resulting in inconsistent therapeutic effects and/or unintended side effects by stereorandom compositions, particularly compared to certain chirally controlled oligonucleotide compositions of oligonucleotides of the same constitution.

[0277] In some embodiments, the present disclosure encompasses technologies for designing and preparing chirally controlled oligonucleotide compositions. In some embodiments, a chirally controlled oligonucleotide composition comprises a controlled/pre-determined (not random as in stereorandom compositions) level of a plurality of oligonucleotides, wherein the oligonucleotides share the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages). In some embodiments, the oligonucleotides share the same pattern of backbone chiral centers (stereochemistry of linkage phosphorus). In some embodiments, a pattern of backbone chiral centers is as described in the present disclosure. In some embodiments, oligonucleotides of a plurality are structural identical.

[0278] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein the oligonucleotides share:

- 1) a common constitution, and
- 2) share the same linkage phosphorus stereochemistry at one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more) chiral internucleotidic linkages (chirally controlled internucleotidic linkages),

wherein the composition is enriched, relative to a substantially racemic preparation of oligonucleotides of the common constitution, for oligonucleotides of the plurality.

[0279] In some embodiments, the present disclosure provides a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein the oligonucleotides share:

- 1) a common constitution, and
- 2) share the same linkage phosphorus stereochemistry at one or more (e.g., 1-50, 1-40, 1-30, 1-25, 1-20, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more) chiral internucleotidic linkages (chirally controlled internucleotidic linkages),

wherein stereochemical purity of the linkage phosphorus of each chirally controlled internucleotidic linkage is independently 80%-100% (e.g., 85-100%, 90-100%, about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5%).

[0280] In some embodiments, at least one chiral internucleotidic linkage is chirally controlled. In some embodiments, at least 2 internucleotidic linkages are independently chirally controlled. In some embodiments, the number of chirally controlled internucleotidic linkages is at least 3. In some embodiments, it is at least 4. In some embodiments, it is at least 5. In some embodiments, it is at least 6. In some embodiments, it is at least 7. In some embodiments, it is at least 8. In some embodiments, it is at least 9. In some embodiments, it is at least 10. In some embodiments, it is at least 11. In some embodiments, it is at least 12. In some embodiments, it is at least 13. In some embodiments, it is at least 14. In some embodiments, it is at least 15. In some embodiments, it is at least 20. In some embodiments, it is at least 25. In some embodiments, it is at least 30.

[0281] In some embodiments, at least 5%-100% (e.g., about 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-80%, 50%-85%, 50%-90%, 50%-95%, 60%-80%, 60%-85%, 60%-90%, 60%-95%, 60%-100%, 65%-80%, 65%-85%, 65%-90%, 65%-95%, 65%-100%, 70%-80%, 70%-85%, 70%-90%, 70%-95%, 70%-100%, 75%-80%, 75%-85%, 75%-90%, 75%-95%, 75%-100%, 80%-85%, 80%-90%, 80%-95%, 80%-100%, 85%-90%, 85%-95%, 85%-100%, 90%-95%, 90%-100%, 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, etc.) of all chiral linkage phosphorus are chirally controlled. In some embodiments, at least 5%-100% (e.g., about 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-80%, 50%-85%, 50%-90%, 50%-95%, 60%-80%, 60%-85%, 60%-90%, 60%-95%, 60%-

100%, 65%-80%, 65%-85%, 65%-90%, 65%-95%, 65%-100%, 70%-80%, 70%-85%, 70%-90%, 70%-95%, 70%-100%, 75%-80%, 75%-85%, 75%-90%, 75%-95%, 75%-100%, 80%-85%, 80%-90%, 80%-95%, 80%-100%, 85%-90%, 85%-95%, 85%-100%, 90%-95%, 90%-100%, 10%, 20%, 30%, 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%, etc.) of all phosphorothioate internucleotidic linkages are chirally controlled. In some embodiments, each phosphorothioate internucleotidic linkage is independently chirally controlled. In some embodiments, a percentage is at least 50%. In some embodiments, a percentage is at least 60%. In some embodiments, a percentage is at least 70%. In some embodiments, a percentage is at least 80%. In some embodiments, a percentage is at least 90%. In some embodiments, each chiral internucleotidic linkage is chirally controlled. In some embodiments, each phosphorothioate internucleotidic linkage is chirally controlled.

[0282] In some embodiments, no more than 1-10, e.g., no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, chiral internucleotidic linkages are not chirally controlled. In some embodiments, no more than 1 chiral internucleotidic linkage is not chirally controlled. In some embodiments, no more than 2 chiral internucleotidic linkages are not chirally controlled. In some embodiments, no more than 3 chiral internucleotidic linkages are not chirally controlled. In some embodiments, no more than 4 chiral internucleotidic linkages are not chirally controlled. In some embodiments, no more than 5 chiral internucleotidic linkages are not chirally controlled. In some embodiments, the number of non-chirally controlled internucleotidic linkages is 1. In some embodiments, it is 2. In some embodiments, it is 3. In some embodiments, it is 4. In some embodiments, it is 5.

[0283] In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides, wherein each oligonucleotide of the plurality is independently a particular oligonucleotide or a salt thereof. In some embodiments, the present disclosure provides a composition comprising a plurality of oligonucleotides, wherein each oligonucleotide of the plurality is independently a particular oligonucleotide or a pharmaceutically acceptable salt thereof. In some embodiments, such a composition is enriched relative to a substantially racemic preparation of a particular oligonucleotide. As appreciated by those skilled in the art, oligonucleotides of the plurality share a common sequence which is the base sequence of the particular oligonucleotide. In some embodiments, at least about 5%-100%, 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-100%, 5%-90%, 10%-90%, 20-90%, 30%-90%, 40%-90%, 50%-90%, 5%-85%, 10%-85%, 20-85%, 30%-85%, 40%-85%, 50%-85%, 5%-80%, 10%-80%, 20-80%, 30%-80%, 40%-80%, 50%-80%, 5%-75%, 10%-75%, 20-75%, 30%-75%, 40%-75%, 50%-75%, 5%-70%, 10%-70%, 20-70%, 30%-70%, 40%-70%, 50%-70%, 5%-65%, 10%-65%, 20-65%, 30%-65%, 40%-65%, 50%-65%, 5%-60%, 10%-60%, 20-60%, 30%-60%, 40%-60%, 50%-60%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides

in the composition that share the base sequence of a the particular oligonucleotide are oligonucleotide of the plurality. In some embodiments, at least about 5%-100%, 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-100%, 5%-90%, 10%-90%, 20-90%, 30%-90%, 40%-90%, 50%-90%, 5%-85%, 10%-85%, 20-85%, 30%-85%, 40%-85%, 50%-85%, 5%-80%, 10%-80%, 20-80%, 30%-80%, 40%-80%, 50%-80%, 5%-75%, 10%-75%, 20-75%, 30%-75%, 40%-75%, 50%-75%, 5%-70%, 10%-70%, 20-70%, 30%-70%, 40%-70%, 50%-70%, 5%-65%, 10%-65%, 20-65%, 30%-65%, 40%-65%, 50%-65%, 5%-60%, 10%-60%, 20-60%, 30%-60%, 40%-60%, 50%-60%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition that share the constitution of the particular oligonucleotide or a salt thereof are oligonucleotide of the plurality. In some embodiments, a percentage is at least 10%. In some embodiments, a percentage is at least 20%. In some embodiments, a percentage is at least 30%. In some embodiments, a percentage is at least 40%. In some embodiments, a percentage is at least 50%. In some embodiments, it is at least 60%. In some embodiments, it is at least 70%. In some embodiments, it is at least 80%. In some embodiments, it is at least 90%. In some embodiments, it is at least 95%. In some embodiments, it is about 5-100%. In some embodiments, it is about 10-100%. In some embodiments, it is about 20-100%. In some embodiments, it is about 30-90%. In some embodiments, it is about 30-80%. In some embodiments, it is about 30-70%. In some embodiments, it is about 40-90%. In some embodiments, it is about 40-80%. In some embodiments, it is about 40-70%.

[0284] In some embodiments, an enrichment relative to a substantially racemic preparation is that at least about 5%-100%, 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-100%, 5%-90%, 10%-90%, 20-90%, 30%-90%, 40%-90%, 50%-90%, 5%-85%, 10%-85%, 20-85%, 30%-85%, 40%-85%, 50%-85%, 5%-80%, 10%-80%, 20-80%, 30%-80%, 40%-80%, 50%-80%, 5%-75%, 10%-75%, 20-75%, 30%-75%, 40%-75%, 50%-75%, 5%-70%, 10%-70%, 20-70%, 30%-70%, 40%-70%, 50%-70%, 5%-65%, 10%-65%, 20-65%, 30%-65%, 40%-65%, 50%-65%, 5%-60%, 10%-60%, 20-60%, 30%-60%, 40%-60%, 50%-60%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition, or all oligonucleotides in the composition that share the common base sequence of a plurality, or all oligonucleotides in the composition that share the common constitution of a plurality, are oligonucleotide of the plurality. In some embodiments, a percentage is at least 10%. In some embodiments, a percentage is at least 20%. In some embodiments, a percentage is at least 30%. In some embodiments, a percentage is at least 40%. In some embodiments, a percentage is at least 50%. In some embodiments, it is at least 60%. In some embodiments, it is at least 70%. In some embodiments, it is at least 80%. In some embodiments, it is at least 90%. In some embodiments, it is at least 95%. In some embodiments, it is about 5-100%. In some embodiments, it is about 10-100%. In some embodiments, it is about 20-100%. In some embodiments, it is about 30-90%. In some embodiments, it is

about 30-80%. In some embodiments, it is about 30-70%. In some embodiments, it is about 40-90%. In some embodiments, it is about 40-80%. In some embodiments, it is about 40-70%.

[0285] In some embodiments, at least about 5%-100%, 10%-100%, 20-100%, 30%-100%, 40%-100%, 50%-100%, 5%-90%, 10%-90%, 20-90%, 30%-90%, 40%-90%, 50%-90%, 5%-85%, 10%-85%, 20-85%, 30%-85%, 40%-85%, 50%-85%, 5%-80%, 10%-80%, 20-80%, 30%-80%, 40%-80%, 50%-80%, 5%-75%, 10%-75%, 20-75%, 30%-75%, 40%-75%, 50%-75%, 5%-70%, 10%-70%, 20-70%, 30%-70%, 40%-70%, 50%-70%, 5%-65%, 10%-65%, 20-65%, 30%-65%, 40%-65%, 50%-65%, 5%-60%, 10%-60%, 20-60%, 30%-60%, 40%-60%, 50%-60%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of all oligonucleotides in the composition that share the common base sequence of a plurality are oligonucleotide of the plurality. In some embodiments, a percentage is at least 10%. In some embodiments, a percentage is at least 20%. In some embodiments, a percentage is at least 30%. In some embodiments, a percentage is at least 40%. In some embodiments, a percentage is at least 50%. In some embodiments, it is at least 60%. In some embodiments, it is at least 70%. In some embodiments, it is at least 80%. In some embodiments, it is at least 90%. In some embodiments, it is at least 95%. In some embodiments, it is about 5-100%. In some embodiments, it is about 10-100%. In some embodiments, it is about 20-100%. In some embodiments, it is about 30-90%. In some embodiments, it is about 30-80%. In some embodiments, it is about 30-70%. In some embodiments, it is about 40-90%. In some embodiments, it is about 40-80%. In some embodiments, it is about 40-70%.

[0286] Levels of oligonucleotides of a plurality in chirally controlled oligonucleotide compositions are controlled. In contrast, in non-chirally controlled (or stereorandom, racemic) oligonucleotide compositions (or preparations), levels of oligonucleotides are random and not controlled. In some embodiments, an enrichment relative to a substantially racemic preparation is a level described herein.

[0287] In some embodiments, a level as a percentage (e.g., a controlled level, a pre-determined level, an enrichment) is or is at least $(DS)^{nc}$, wherein DS (diastereopurity of an individual internucleotidic linkage) is 90%-100%, and nc is the number of chiral linkage phosphorus as described in the present disclosure (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more). In some embodiments, a level as a percentage (e.g., a controlled level, a pre-determined level, an enrichment) is or is at least $(DS)^{nc}$, wherein DS (diastereopurity of an individual internucleotidic linkage) is 90%-100%, and nc is the number of chirally controlled internucleotidic linkages as described in the present disclosure (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more). In some embodiments, each chiral internucleotidic linkage is chirally controlled, and nc is the number of chiral internucleotidic linkage. In some embodiments, DS is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% or more. In some embodiments, DS is or is at least 90%. In some embodiments, DS is or is at least 91%. In some embodiments, DS is or is at least 92%. In

some embodiments, DS is or is at least 93%. In some embodiments, DS is or is at least 94%. In some embodiments, DS is or is at least 95%. In some embodiments, DS is or is at least 96%. In some embodiments, DS is or is at least 97%. In some embodiments, DS is or is at least 98%. In some embodiments, DS is or is at least 99%. In some embodiments, a level (e.g., a controlled level, a pre-determined level, an enrichment) is a percentage of all oligonucleotides in a composition that share the same constitution, wherein the percentage is or is at least $(DS)^{nc}$. For example, when DS is 99% and nc is 10, the percentage is or is at least 90% $((99\%)^{10} \approx 0.90 = 90\%)$. As appreciated by those skilled in the art, in a stereorandom preparation the percentage is typically about $1/2^{nc}$ - when nc is 10, the percentage is about $1/2^{10} \approx 0.001 = 0.1\%$. In some embodiments, an enrichment (e.g., relative to a substantially racemic preparation), a level, etc., is that at least about $(DS)^{nc}$ of all oligonucleotides in the composition, or all oligonucleotides in the composition that share the common base sequence of a plurality, or all oligonucleotides in the composition that share the common constitution of a plurality, are oligonucleotide of the plurality. In some embodiments, it is of all oligonucleotides in the composition. In some embodiments, it is of all oligonucleotides in the composition that share the common base sequence of a plurality. In some embodiments, it is of all oligonucleotides in the composition that share the common constitution of a plurality. In some embodiments, various forms (e.g., various salt forms) of an oligonucleotide may be properly considered to have the same constitution.

[0288] In some embodiments, oligonucleotides comprise one or more (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) chirally controlled chiral internucleotidic linkages the diastereomeric excess (d.e.) of whose linkage phosphorus is independently about or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, about or at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of all chiral internucleotidic linkages comprising a chiral linkage phosphorus are independently such a chirally controlled internucleotidic linkage. In some embodiments, about or at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of phosphorothioate internucleotidic linkages are independently such a chirally controlled internucleotidic linkage. In some embodiments, each phosphorothioate internucleotidic linkage is independently such a chirally controlled internucleotidic linkage. In some embodiments, each chiral internucleotidic linkage comprising a chiral linkage phosphorus is independently such a chirally controlled internucleotidic linkage. In some embodiments, d.e. is about or at least about 80%. In some embodiments, d.e. is about or at least about 85%. In some embodiments, d.e. is about or at least about 90%. In some embodiments, d.e. is about or at least about 95%. In some embodiments, d.e. is about or at least about 96%. In some embodiments, d.e. is about or at least about 97%. In some embodiments, d.e. is about or at least about 98%.

[0289] In some embodiments, an oligonucleotide composition (also referred to as an oligonucleotide composition) is a chirally controlled oligonucleotide composition comprising a plurality of

oligonucleotides, wherein the oligonucleotides share:

- a common base sequence,
- a common pattern of backbone linkages, and
- the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages),

wherein the percentage of the oligonucleotides of the plurality within all oligonucleotides in the composition that share the common base sequence and pattern of backbone linkages is at least $(DS)^{nc}$, wherein DS is 90%-100%, and nc is the number of chiral linkage phosphorus.

[0290] In some embodiments, an oligonucleotide composition (also referred to as an oligonucleotide composition) is a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein the oligonucleotides share:

- a common base sequence,
- a common pattern of backbone linkages, and
- the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages (chirally controlled internucleotidic linkages),

wherein the percentage of the oligonucleotides of the plurality within all oligonucleotides in the composition that share the common base sequence and pattern of backbone linkages is at least $(DS)^{nc}$, wherein DS is 90%-100%, and nc is the number of chirally controlled internucleotidic linkages.

[0291] In some embodiments, an oligonucleotide composition (also referred to as an oligonucleotide composition) is a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein the oligonucleotides share:

- a common base sequence,
- a common pattern of backbone linkages, and
- a common pattern of backbone chiral centers, which pattern comprises at least one Sp,

wherein the percentage of the oligonucleotides of the plurality within all oligonucleotides in the composition that share the common base sequence and pattern of backbone linkages is at least $(DS)^{nc}$, wherein DS is 90%-100%, and nc is the number of chiral linkage phosphorus.

[0292] In some embodiments, an oligonucleotide composition (also referred to as an oligonucleotide composition) is a chirally controlled oligonucleotide composition comprising a plurality of oligonucleotides, wherein the oligonucleotides share:

- a common base sequence,
- a common pattern of backbone linkages, and
- a common pattern of backbone chiral centers, which pattern comprises at least one Sp,

wherein the percentage of the oligonucleotides of the plurality within all oligonucleotides in the

composition that share the common base sequence and pattern of backbone linkages is at least (DS)^{nc}, wherein DS is 90%-100%, and nc is the number of chirally controlled internucleotidic linkages.

[0293] In some embodiments, level of a diastereopurity of a plurality of oligonucleotides in a composition can be determined as the product of the diastereopurity of each chirally controlled internucleotidic linkage in the oligonucleotides. In some embodiments, diastereopurity of an internucleotidic linkage connecting two nucleosides in an oligonucleotide (or nucleic acid) is represented by the diastereopurity of an internucleotidic linkage of a dimer connecting the same two nucleosides, wherein the dimer is prepared using comparable conditions, in some instances, identical synthetic cycle conditions (e.g., for the linkage between Nx and Ny in an oligonucleotideNxNy....., the dimer is NxNy).

[0294] In some embodiments, a chirally controlled oligonucleotide composition comprises two or more pluralities of oligonucleotides, wherein each plurality is independently a plurality of oligonucleotides as described herein (e.g., in various chirally controlled oligonucleotide compositions). For example, in some embodiments, each plurality independently shares a common base sequence, and the same linkage phosphorus stereochemistry at one or more chiral internucleotidic linkages, and each plurality is independently enriched compared to stereorandom preparation of that plurality or each plurality is independently of a level as described herein. In some embodiments, at least two pluralities or each plurality independently targets a different adenosine. In some embodiments, at least two pluralities or each plurality independently targets a different transcript of the same or different nucleic acids. In some embodiments, at least two pluralities or each plurality independently targets transcripts of a different gene. Among other things, such compositions may be utilized to target two or more targets, in some embodiments, simultaneously and in the same system.

[0295] As described herein, various variables can be R, e.g., R', R^L, etc. Various embodiments for R are described in the present disclosure (e.g., when describing variables that can be R). Such embodiments are generally useful for all variables that can be R. In some embodiments, R is hydrogen. In some embodiments, R is optionally substituted C₁₋₃₀ (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) aliphatic. In some embodiments, R is optionally substituted C₁₋₂₀ aliphatic. In some embodiments, R is optionally substituted C₁₋₁₀ aliphatic. In some embodiments, R is optionally substituted C₁₋₆ aliphatic. In some embodiments, R is optionally substituted alkyl. In some embodiments, R is optionally substituted C₁₋₆ alkyl. In some embodiments, R is optionally substituted methyl. In some embodiments, R is methyl. In some embodiments, R is optionally substituted ethyl. In some embodiments, R is optionally substituted propyl. In some embodiments, R is isopropyl. In some embodiments, R is optionally substituted butyl. In some embodiments, R is optionally substituted pentyl. In some embodiments, R is optionally substituted hexyl.

[0296] In some embodiments, R is optionally substituted 3-30 membered (e.g., 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) cycloaliphatic. In some embodiments, R is optionally substituted cycloalkyl. In some embodiments, cycloaliphatic is monocyclic, bicyclic, or polycyclic, wherein each monocyclic ring unit is independently saturated or partially saturated. In some embodiments, R is optionally substituted cyclopropyl. In some embodiments, R is optionally substituted cyclobutyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally substituted cyclohexyl. In some embodiments, R is optionally substituted adamantyl.

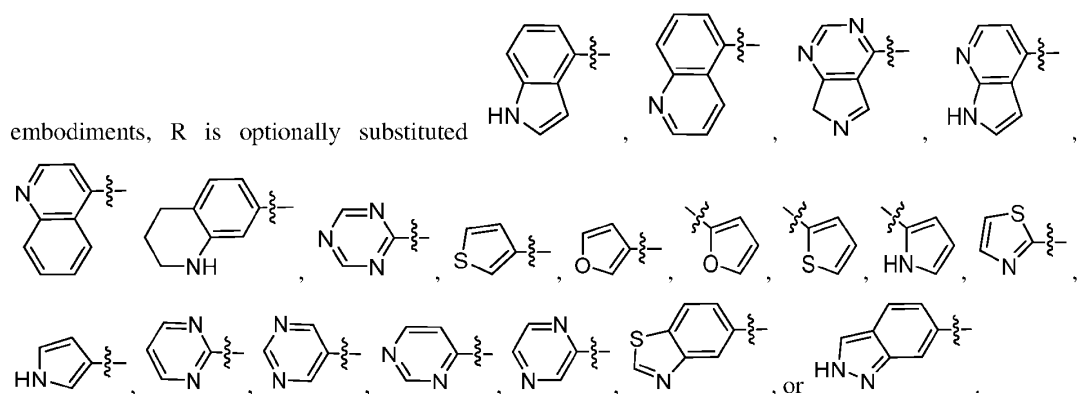
[0297] In some embodiments, R is optionally substituted C₁₋₃₀ (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) heteroaliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C₁₋₂₀ aliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C₁₋₁₀ aliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted C₁₋₆ aliphatic having 1-3 heteroatoms. In some embodiments, R is optionally substituted heteroalkyl. In some embodiments, R is optionally substituted C₁₋₆ heteroalkyl. In some embodiments, R is optionally substituted 3-30 membered (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) heterocycloaliphatic having 1-10 heteroatoms. In some embodiments, R is optionally substituted heterocycloalkyl. In some embodiments, heterocycloaliphatic is monocyclic, bicyclic, or polycyclic, wherein each monocyclic ring unit is independently saturated or partially saturated.

[0298] In some embodiments, R is optionally substituted C₆₋₃₀ aryl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is optionally substituted phenyl. In some embodiments, R is C₆₋₁₄ aryl. In some embodiments, R is optionally substituted bicyclic aryl. In some embodiments, R is optionally substituted polycyclic aryl. In some embodiments, R is optionally substituted C₆₋₃₀ arylaliphatic. In some embodiments, R is C₆₋₃₀ arylheteroaliphatic having 1-10 heteroatoms.

[0299] In some embodiments, R is optionally substituted 5-30 (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-10 membered heteroaryl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heteroaryl having one heteroatom. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-4 heteroatoms. In some

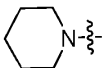
embodiments, R is optionally substituted 6-membered heteroaryl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 6-membered heteroaryl having one heteroatom. In some embodiments, R is optionally substituted monocyclic heteroaryl. In some embodiments, R is optionally substituted bicyclic heteroaryl. In some embodiments, R is optionally substituted polycyclic heteroaryl. In some embodiments, a heteroatom is nitrogen.

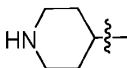
[0300] In some embodiments, R is optionally substituted 2-pyridinyl. In some embodiments, R is optionally substituted 3-pyridinyl. In some embodiments, R is optionally substituted 4-pyridinyl. In some

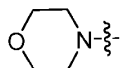


[0301] In some embodiments, R is optionally substituted 3-30 (3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 3-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 4-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-20 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-10 membered heterocyclyl having 1-10 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 5-membered heterocyclyl having one heteroatom. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-5 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-4 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-3 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-2 heteroatoms. In some embodiments, R is optionally substituted 6-membered heterocyclyl having one heteroatom. In some embodiments, R is optionally substituted monocyclic heterocyclyl. In some

embodiments, R is optionally substituted bicyclic heterocyclyl. In some embodiments, R is optionally substituted polycyclic heterocyclyl. In some embodiments, R is optionally substituted saturated heterocyclyl. In some embodiments, R is optionally substituted partially unsaturated heterocyclyl. In some

embodiments, a heteroatom is nitrogen. In some embodiments, R is optionally substituted . In

some embodiments, R is optionally substituted . In some embodiments, R is optionally

substituted .

[0302] In some embodiments, two R groups are optionally and independently taken together to form a covalent bond. In some embodiments, two or more R groups on the same atom are optionally and independently taken together with the atom to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the atom, 0-10 heteroatoms. In some embodiments, two or more R groups on two or more atoms are optionally and independently taken together with their intervening atoms to form an optionally substituted, 3-30 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atoms, 0-10 heteroatoms.

[0303] Various variables may comprises an optionally substituted ring, or can be taken together with their intervening atom(s) to form a ring. In some embodiments, a ring is 3-30 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30) membered. In some embodiments, a ring is 3-20 membered. In some embodiments, a ring is 3-15 membered. In some embodiments, a ring is 3-10 membered. In some embodiments, a ring is 3-8 membered. In some embodiments, a ring is 3-7 membered. In some embodiments, a ring is 3-6 membered. In some embodiments, a ring is 4-20 membered. In some embodiments, a ring is 5-20 membered. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently saturated, partially saturated or aromatic. In some embodiments, each monocyclic ring or each monocyclic ring unit in bicyclic or polycyclic rings is independently 3-10 membered and has 0-5 heteroatoms.

[0304] In some embodiments, each heteroatom is independently selected oxygen, nitrogen, sulfur, silicon, and phosphorus. In some embodiments, each heteroatom is independently selected oxygen, nitrogen, sulfur, and phosphorus. In some embodiments, each heteroatom is independently selected oxygen, nitrogen, and sulfur. In some embodiments, a heteroatom is in an oxidized form.

[0305] As described herein, various groups may be optionally substituted. Substituents are routinely utilized in chemistry including in development of various therapeutics. Many substituents can be utilized

in accordance with the present disclosure. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Substituents are preferably those that result in the formation of compounds (e.g., oligonucleotides, reagents, etc.) for a desired property, activity, use, etc., as described herein. In some embodiments, compounds are stable for therapeutic use as described herein. The term “stable,” as used herein, refers to oligonucleotides, agents, etc. that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein. In some embodiments, a substituent is a hydrocarbon group. In some embodiments, a substituent comprises a heteroatom. In some embodiments, a substituent comprises multiple heteroatoms. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, sulfur, phosphorus and silicon. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, and sulfur. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, fluorine, chlorine, bromine, iodine, nitrogen, oxygen, and sulfur. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in a substituent is about or no more than about 1; in some embodiments, it is no more than about 2; in some embodiments, it is no more than about 3; in some embodiments, it is no more than about 4; in some embodiments, it is no more than about 5; in some embodiments, it is no more than about 6; in some embodiments, it is no more than about 7; in some embodiments, it is no more than about 8; in some embodiments, it is no more than about 9; in some embodiments, it is no more than about 10; in some embodiments, it is no more than about 11; in some embodiments, it is no more than about 12; in some embodiments, it is no more than about 13; in some embodiments, it is no more than about 14; in some embodiments, it is no more than about 15; in some embodiments, it is no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 15. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 10. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 6.

Manufacturing

[0306] Among other things, the present disclosure provides technologies, e.g., compounds, methods, etc. that are useful for preparing oligonucleotides and compositions thereof including chirally controlled oligonucleotide compositions. Particularly, in some embodiments, the present disclosure provides technologies that are useful for manufacturing oligonucleotides comprising $-P(O)(OH)_2$ groups (e.g., at 5'-ends as in RNAi oligonucleotides) and compositions of. In some embodiments, the present disclosure

utilizes $-P(O)(OR^{PG})_2$ groups as described herein.

[0307] In some embodiments, the present disclosure provides a method, comprising converting $-P(O)(OR^{PG})_2$ of an oligonucleotide into $-P(O)(OH)_2$ (e.g., a salt form thereof). In some embodiments, such conversion is achieved under oligonucleotide synthesis conditions utilized for a reference oligonucleotide (e.g., an otherwise identical oligonucleotide but without $-P(O)(OH)_2$). In some embodiments, such conversion is achieved during cleavage (e.g., from solid support), deprotection (e.g., of nucleobases) and/or chiral auxiliary removal process of oligonucleotide synthesis. In some embodiments, an oligonucleotide comprising $-P(O)(OR^{PG})_2$ is a compound of formula O-I or a salt thereof. In some embodiments, an oligonucleotide comprising $-P(O)(OR^{PG})_2$ is a compound of formula O-I' or a salt thereof.

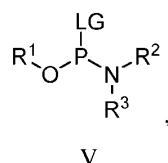
[0308] In some embodiments, the present disclosure provides a method for preparing an oligonucleotide, comprising coupling a phosphoramidite as described herein to an oligonucleotide. In some embodiments, the present disclosure provides a method for preparing an oligonucleotide, comprising coupling a compound of formula I or a salt thereof, to an oligonucleotide. In some embodiments, the present disclosure provides a method for preparing an oligonucleotide, comprising coupling a compound of formula I' or a salt thereof, to an oligonucleotide. In some embodiments, the present disclosure provides a method for manufacturing an oligonucleotide, comprising reacting a phosphoramidite as described herein with a compound comprising a $-OH$ group. In some embodiments, the present disclosure provides a method for preparing an oligonucleotide, comprising reacting a compound formula I or a salt thereof, with a compound comprising a $-OH$ group. In some embodiments, the present disclosure provides a method for preparing an oligonucleotide, comprising reacting a compound formula I' or a salt thereof, with a compound comprising a $-OH$ group. In some embodiments, coupling is to 5'-OH of an oligonucleotide. In some embodiments, oligonucleotide preparation is performed on solid support. In some embodiments, $-P(O)(OR^{PG})_2$ is converted to $-P(O)(OH)_2$ (e.g., a salt form thereof) during removal of chiral auxiliaries, deprotection of protected nucleobases and/or cleavage of oligonucleotides from solid support. In some embodiments, during preparation, e.g., before chiral auxiliary removal, cleavage, and/or deprotection, an oligonucleotide of formula O-I or a salt thereof is formed. In some embodiments, during preparation, e.g., before chiral auxiliary removal, cleavage, and/or deprotection, an oligonucleotide of formula O-I' or a salt thereof is formed. In some embodiments, after cleavage/deprotection, a $-P(O)(OH)_2$ group is formed. In some embodiments, $-P(O)(R^{P1})(R^{P2})$ is converted into $-P(O)(OH)_2$. In some embodiments, $-P(O)(OH)_2$ is in a salt form. In some embodiments, $-P(O)(OH)_2$ is in a pharmaceutically acceptable salt form.

[0309] In some embodiments, a phosphoramidite is a compound of formula P or a salt thereof as described herein. In some embodiments, a phosphoramidite is a compound of formula I or a salt thereof

as described herein. In some embodiments, a phosphoramidite is a compound of formula I' or a salt thereof as described herein.

[0310] In some embodiments, the present disclosure provides methods for preparing phosphoramidites, e.g., a compound of formula P or I, or salt thereof.

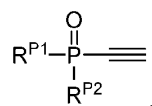
[0311] For example, in some embodiments, the present disclosure provides a method for preparing a compound of formula P or a salt thereof, comprising reacting a compound of formula N or a salt thereof with a compound of formula V or a salt thereof:



wherein LG is a leaving group and each other variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula P' or a salt thereof, comprising reacting a compound of formula N' or a salt thereof with a compound of formula V or a salt thereof. In some embodiments, the present disclosure provides a method for preparing a compound of formula I' or a salt thereof, comprising reacting a compound of formula II' or a salt thereof with a compound of formula V or a salt thereof. In some embodiments, the present disclosure provides a method for preparing a compound of formula I or a salt thereof, comprising reacting a compound of formula II or a salt thereof with a compound of formula V or a salt thereof. In some embodiments, a reaction is performed in the presence of a base, e.g., Et₃N.

[0312] Various LG groups can be utilized in accordance with the present disclosure. For example, in some embodiments, LG is -Cl.

[0313] In some embodiments, the present disclosure provides a method for preparing a compound of formula N or salt thereof, comprising reacting a compound of formula III with a compound having the structure of $\equiv\text{-P(O)(OR}^{\text{PG}}\text{)}_2$ or salt thereof, wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula N' or salt thereof, comprising reacting a compound of formula III' with a compound having the structure of $\equiv\text{-P(O)(OR}^{\text{PG}}\text{)}_2$ or salt thereof, wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula II or salt thereof, comprising reacting a compound of formula III with a compound having the structure of formula V or salt thereof:

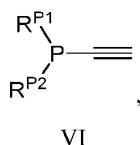


IV

wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula II' or salt thereof, comprising reacting a compound of formula III' with a compound having the structure of formula V or salt thereof

[0314] Various reaction conditions for azides and alkynes may be utilized in accordance with the present disclosure. In some embodiments, the reaction is in the presence of a base, e.g., DIEA. In some embodiments, the reaction is in the presence of copper salt. In some embodiments, a copper salt is a Cu(I) salt. In some embodiments, a copper salt is CuI.

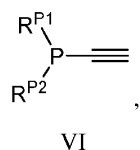
[0315] In some embodiments, the present disclosure provides a method for preparing a compound having the structure of $\equiv\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ or a salt thereof, comprising oxidizing a compound having the structure of $\equiv\text{P}(\text{OR}^{\text{PG}})_2$ or a salt thereof, wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula IV or a salt thereof, comprising oxidizing a compound of formula VI or a salt thereof:



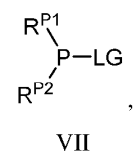
wherein each variable is independently as described herein.

[0316] In some embodiments, R^{P1} and R^{P2} are $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$. In some embodiments, an oxidant is mCPBA.

[0317] In some embodiments, the present disclosure provides a method for preparing a compound having the structure of $\equiv\text{P}(\text{OR}^{\text{PG}})_2$ or a salt thereof, comprising reacting $\equiv\text{P}-\text{MgBr}$ with a compound having the structure of $\text{LG}-\text{P}(\text{OR}^{\text{PG}})_2$ or a salt thereof, wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula VI or a salt thereof:



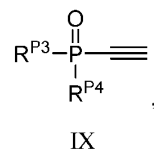
comprising reacting $\equiv\text{P}-\text{MgBr}$ with a compound of formula VII or a salt thereof:



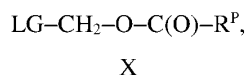
wherein each variable is independently as described herein. In some embodiments, LG is $-\text{Cl}$. In some embodiments, each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.

[0318] In some embodiments, the present disclosure provides a method for preparing a compound having the structure of $\text{LG}-\text{P}(\text{OR}^{\text{PG}})_2$ or a salt thereof, comprising reacting a compound having the structure of $\text{HO}-\text{R}^{\text{PG}}$ or a salt thereof with PCl_3 , wherein each variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula VII or a salt thereof, comprising reacting $\text{HOCH}_2\text{CH}_2\text{CN}$ or a salt thereof with PCl_3 , wherein each variable is independently as described herein. In some embodiments, a reaction is performed in the presence of a base. In some embodiments, a base is TEA.

[0319] In some embodiments, the present disclosure provides a method for preparing a compound having the structure of $\equiv-\text{P}(\text{O})(\text{OR}^{\text{PG}})_2$ or a salt thereof, comprising reacting a compound having the structure of $\text{LG}-\text{R}^{\text{PG}}$ or a salt thereof with a compound of formula IX or a salt thereof:



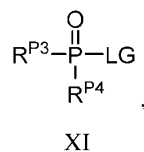
wherein each of R^{P3} and R^{P4} is independently $-\text{O}-\text{R}^{\text{SP}}$, wherein R^{SP} is an optionally substituted optionally substituted C_{1-6} aliphatic, and each other variable is independently as described herein. In some embodiments, the present disclosure provides a method for preparing a compound of formula IV or a salt thereof, comprising reacting a compound of formula IX or a salt thereof with a compound having the structure of formula X or a salt thereof:



wherein each variable is independently as described herein.

[0320] In some embodiments, each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl. In some embodiments, each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is tert-butyl. In some embodiments, R^{SP} is optionally substituted C_{1-6} alkyl. In some embodiments, R^{SP} is methyl. In some embodiments, R^{P3} is $-\text{OMe}$. In some embodiments, R^{P4} is $-\text{OMe}$. In some embodiments, R^{P3} and R^{P4} are the same. In some embodiments, R^{P3} and R^{P4} are different. In some embodiments, LG is $-\text{I}$. In some embodiments, the reaction is performed in the presence of molecular sieves, e.g., 4A MS.

[0321] In some embodiments, the present disclosure provides a method for preparing a compound of formula IX or a salt thereof, comprising reacting $\equiv-\text{MgBr}$ with a compound of formula XI or a salt thereof:



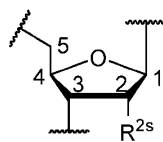
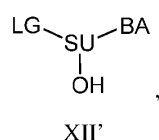
wherein each variable is independently as described herein.

LG is a leaving group

each of $\text{R}^{\text{P}3}$ and $\text{R}^{\text{P}4}$ is independently $-\text{O}-\text{R}^{\text{SP}}$, wherein each R^{SP} is independently optionally substituted C_{1-6} aliphatic.

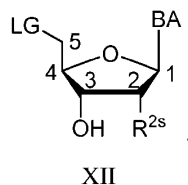
[0322] In some embodiments, each of $\text{R}^{\text{P}3}$ and $\text{R}^{\text{P}4}$ is $-\text{OMe}$. In some embodiments, LG is $-\text{Cl}$.

[0323] In some embodiments, the present disclosure provides a method for preparing a compound of formula III' or a salt thereof, comprising reacting a compound of formula XII' or a salt thereof:



wherein LG is a leaving group and SU is a sugar (e.g., with an azide), wherein R^{2s} is as described herein), with an azide.

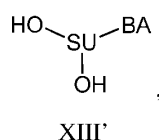
[0324] In some embodiments, the present disclosure provides a method for preparing a compound of formula III or a salt thereof, comprising reacting a compound of formula XII or a salt thereof:

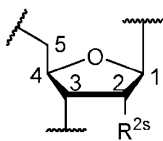


wherein LG is a leaving group, with an azide.

[0325] In some embodiments, LG is $-\text{I}$. In some embodiments, an azide is a salt. In some embodiments, an azide is sodium azide.

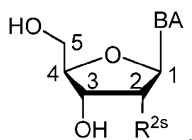
[0326] In some embodiments, the present disclosure provides a method for preparing a compound of formula XII' or a salt thereof, comprising reacting a compound of formula XIII' or a salt thereof:





with I_2 , wherein SU is a sugar (e.g., R^{2s} , wherein R^{2s} is as described herein). In some embodiments, the reaction is performed in the presence of a phosphine. In some embodiments, the reaction is performed in the presence of a PPh_3 . In some embodiments, the reaction is performed in the presence of a base. In some embodiments, the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-N=$ or $-N(R)-$. In some embodiments, the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-N=$ or $-NH-$. In some embodiments, the reaction is performed in the presence of imidazole.

[0327] In some embodiments, the present disclosure provides a method for preparing a compound of formula XII or a salt thereof, comprising reacting a compound of formula XIII or a salt thereof:



XIII

with I_2 . In some embodiments, the reaction is performed in the presence of a phosphine. In some embodiments, the reaction is performed in the presence of a PPh_3 . In some embodiments, the reaction is performed in the presence of a base. In some embodiments, the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-N=$ or $-N(R)-$. In some embodiments, the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-N=$ or $-NH-$. In some embodiments, the reaction is performed in the presence of imidazole.

[0328] Those skilled in the art reading the present disclosure appreciate that various technologies, e.g., solvents, temperatures, reaction times, etc., can be utilized to perform methods described herein. In some embodiments, provided methods may comprise use of a temperature higher and/or lower than room temperature. In some embodiments, methods, e.g., in a reaction forming a phosphoramidite, comprise lowered temperatures, such as temperatures equal to or lower than about -78 , -75 , -70 , -65 , -60 , -55 , -50 , -45 , -40 , -35 , -30 , -25 , -20 , -15 , -10 , -5 , 0 , 5 , 10 , 15 , or 20 °C. In some embodiments, provided methods comprise elevated temperature, such as temperatures equal to or more than about 30 , 35 , 40 , 45 , 50 , 55 , 60 , 65 , 70 , 75 , 80 , 85 , 90 , 95 , 100 , 110 , 120 , 130 , 140 or 150 °C. In some embodiments, provided methods comprise a temperature increase from a lowered temperature to another lowered temperature. In some embodiments, provided methods comprise a temperature increase from a lowered temperature to room temperature. In some embodiments, provided methods comprise a temperature increase from room temperature to an elevated temperature. In some embodiments, provided methods comprise a temperature

increase from a lowered temperature to an elevated temperature. In some embodiments, provided methods comprise a temperature decrease from an elevated temperature to another elevated temperature. In some embodiments, provided methods comprise a temperature decrease from an elevated temperature to room temperature. In some embodiments, provided methods comprise a temperature decrease from room temperature to a lowered temperature. In some embodiments, provided methods comprise a temperature decrease from an elevated temperature to a lowered temperature.

[0329] Various solvents are suitable for use in provided technologies in accordance with the present disclosure. In some embodiments, a method is performed using a single solvent. In some embodiments, a method is performed using a mixture of two or more solvents.

[0330] In some embodiments, one or more reactions or steps are performed under an inert gas. In some embodiments, an inert gas is argon. In some embodiments, an inert gas is nitrogen.

[0331] Various oligonucleotide manufacturing technologies, e.g., cycles, reagents, conditions, supports, chiral auxiliaries for preparing chirally controlled oligonucleotide compositions, etc., are reported and can be utilized in accordance with the present disclosure. Certain useful technologies are described in US 10167309, US 11643657, US 11718638, US 11608355, US 20230089442, and US 20230203087.

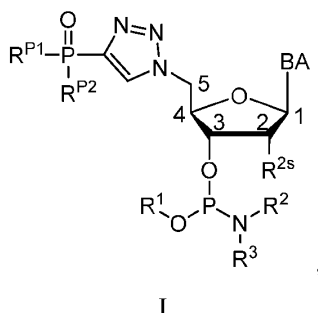
[0332] In some embodiments, a compound, e.g., phosphoramidite, nucleoside, oligonucleotide, etc., has a purity which is about or more than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, a purity is about 50% or more. In some embodiments, a purity is about 55% or more. In some embodiments, a purity is about 60% or more. In some embodiments, a purity is about 65% or more. In some embodiments, a purity is about 70% or more. In some embodiments, a purity is about 75% or more. In some embodiments, a purity is about 80% or more. In some embodiments, a purity is about 85% or more. In some embodiments, a purity is about 90% or more. In some embodiments, a purity is about 91% or more. In some embodiments, a purity is about 92% or more. In some embodiments, a purity is about 93% or more. In some embodiments, a purity is about 94% or more. In some embodiments, a purity is about 95% or more. In some embodiments, a purity is about 96% or more. In some embodiments, a purity is about 97% or more. In some embodiments, a purity is about 98% or more. In some embodiments, a purity is about 99% or more. In some embodiments, a purity is about 99.5% or more. In some embodiments, purity is weight %. In some embodiments, purity is mole %. In some embodiments, purity is based on chromatography peak area %, e.g., HPLC UV peak area % at a wave length (e.g., 220 nM, 260 nM, etc.), mass spectrometry peak area %, etc.

[0333] In some embodiments, a compound has a stereochemical purity of about or more than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some

embodiments, a compound has an enantiomeric purity of about or more than 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, a compound has a diastereomeric purity of about or more than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. In some embodiments, for each chiral center, enantiomeric and/or diastereomeric excess is independently about or at least about 10%, 20%, 30%, 40%, 50%, 60%, 50%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

[0334] Among other things, the present disclosure provides the following Example Embodiments:

1. A compound, wherein the compound is a compound of formula I or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $\text{---}(\text{A})\text{---}(\text{R}^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{CN}$, $-\text{N}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{L}^s\text{---R}^{s11}$, $-\text{L}^s\text{---OR}^{s11}$, $-\text{L}^s\text{---SR}^{s11}$, $-\text{L}^s\text{---N}(\text{R}^{s11})_2$, $-\text{O---L}^s\text{---OR}^{s11}$, $-\text{O---L}^s\text{---SR}^{s11}$, $-\text{O---L}^s\text{---N}(\text{R}^{s11})_2$, $-\text{C}(\text{R}^{s11})_3$ or $-\text{L}^s\text{---Si}(\text{R}^{s11})_3$;

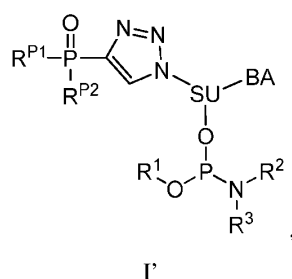
each R^{s11} is independently R' ;

each L^s is independently L;

each R' is independently $-\text{R}$, $-\text{C}(\text{O})\text{R}$, $-\text{CO}_2\text{R}$, or $-\text{S}(\text{O})_2\text{R}$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

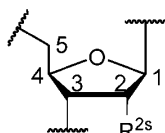
each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

2. A compound, wherein the compound is a compound of formula I' or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-\text{O---CH}_2\text{CH}_2\text{CN}$ or $-\text{O---CH}_2\text{---O---C}(\text{O})\text{---R}^P$, wherein R^P is optionally substituted tert-butyl;

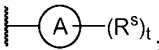


SU is a sugar (e.g., R^{2s}), wherein R^{2s} is as described herein);

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms

independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form ;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently -F, -Cl, -Br, -I, -CN, -N₃, -NO, -NO₂, -L^s-R^{s11}, -L^s-OR^{s11}, -L^s-SR^{s11}, -L^s-N(R^{s11})₂, -O-L^s-OR^{s11}, -O-L^s-SR^{s11}, -O-L^s-N(R^{s11})₂, -C(R^{s11})₃ or -L^s-Si(R^{s11})₃;

each R^{s11} is independently R' ;

each L^s is independently L;

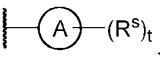
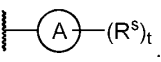
each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C₁₋₁₀ aliphatic and C₁₋₁₀ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by -Cy-, -O-, -S-, -N(R')-, -C(O)-, -C(S)-, -C(NR')-, -C(O)N(R')-, -N(R')C(O)N(R')-, -N(R')C(O)O-, -S(O)-, -S(O)₂-, -S(O)₂N(R')-, -SC(O)-, or -C(O)O-;

-Cy- is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently -R, -C(O)R, -CO₂R, or -S(O)₂R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C₁₋₁₀ aliphatic, C₁₋₁₀ heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C₆₋₁₄ aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

3. The compound of any one of the preceding Embodiments, wherein R^1 is -CH₂CH₂CN.
4. The compound of any one of the preceding Embodiments, wherein R^2 is optionally substituted C₁₋₆ aliphatic.
5. The compound of any one of the preceding Embodiments, wherein R^2 is isopropyl.

6. The compound of any one of the preceding Embodiments, wherein R^3 is optionally substituted C_{1-6} aliphatic.
7. The compound of any one of the preceding Embodiments, wherein R^3 is isopropyl.
8. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is $-P(OCH_2CH_2CN)N[CH(CH_3)_2]_2$.
9. The compound of Embodiment 1 or 2, wherein or two or three of R^1 , R^2 , and R^3 are taken together with their intervening atoms to form .
10. The compound of Embodiment 1 or 2, wherein R^1 , R^2 , and R^3 are taken together with their intervening atoms to form .
11. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted monocyclic ring.
12. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic ring.
13. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted polycyclic ring.
14. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 7-12 membered ring.
15. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 8-10 membered ring.
16. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 7-membered ring.
17. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 8-membered ring.
18. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 9-membered ring.
19. The compound of any one of Embodiments 9-10, wherein Ring A is an optionally substituted bicyclic 10-membered ring.
20. The compound of any one of Embodiments 9-19, wherein Ring A comprises an optionally substituted 5-membered ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$.
21. The compound of any one of Embodiments 9-19, wherein Ring A comprises an optionally substituted 6-membered ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$.

22. The compound of any one of Embodiments 20-21, wherein the ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$ does not have any other ring heteroatoms.
23. The compound of any one of Embodiments 20-22, wherein the ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$ is saturated.
24. The compound of any one of Embodiments 9-23, wherein each monocyclic ring unit of Ring A is independently an optionally substituted 3-7 membered ring.
25. The compound of any one of Embodiments 9-23, wherein each monocyclic ring unit of Ring A is independently an optionally substituted 4-6 membered ring.
26. The compound of any one of Embodiments 9-23, wherein each monocyclic ring unit of Ring A is independently an optionally substituted 5-6 membered ring.
27. The compound of any one of Embodiments 9-23, wherein each monocyclic ring unit of Ring A is independently an optionally substituted 5-membered ring.
28. The compound of any one of Embodiments 9-27, wherein Ring A is or comprises optionally

substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH$.

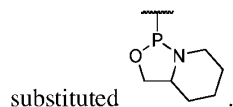
29. The compound of any one of Embodiments 9-27, wherein Ring A is or comprises optionally

substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group, wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH$.

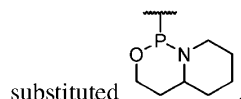
30. The compound of any one of Embodiments 9-29, wherein Ring A comprises no additional heteroatoms.
31. The compound of any one of Embodiments 9-30, wherein Ring A is saturated.
32. The compound of any one of Embodiments 9-30, wherein Ring A is partially unsaturated.
33. The compound of any one of Embodiments 9-30, wherein Ring A is or comprises optionally

substituted .

34. The compound of any one of Embodiments 9-30, wherein Ring A is or comprises optionally



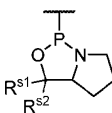
35. The compound of any one of Embodiments 9-30, wherein Ring A is or comprises optionally

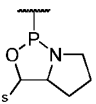


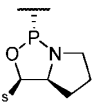
36. The compound of any one of Embodiments 9-35, wherein t is 1-5.

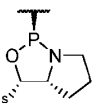
37. The compound of any one of Embodiments 9-35, wherein t is 1.

38. The compound of any one of Embodiments 9-35, wherein an occurrence of R^s is bonded to a carbon atom bonded to the oxygen of $-P(OR^1)N(R^2)(R^3)$.

39. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is , wherein each of R^{s1} and R^{s2} is independently R^s .

40. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is .

41. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is .

42. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is .

43. The compound of any one of Embodiments 9-42, wherein an occurrence of L^s is optionally substituted $-CH_2-$.

44. The compound of any one of Embodiments 9-42, wherein an occurrence of L^s is $-CH_2-$.

45. The compound of any one of Embodiments 9-42, wherein an occurrence of L^s is a covalent bond.

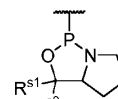
46. The compound of any one of Embodiments 9-45, wherein an occurrence of R^s is $-L^s-Si(R^{s11})_3$.

47. The compound of Embodiment 46, wherein each R^{s11} is not hydrogen.

48. The compound of Embodiment 46, wherein each R^{s11} is independently an optionally substituted group selected from C_{1-6} aliphatic and phenyl.

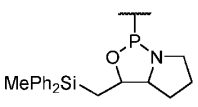
49. The compound of Embodiment 46, wherein each R^{s11} is independently optionally substituted C_{1-6} aliphatic.

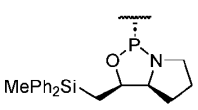
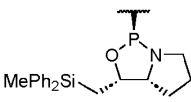
50. The compound of Embodiment 47 or 48, wherein an occurrence of R^{s11} is optionally substituted C_{1-6} aliphatic.
51. The compound of any one of Embodiments 47-48 and 50, wherein an occurrence of R^{s11} is optionally substituted phenyl.
52. The compound of Embodiment 46, wherein $-Si(R^{s11})_3$ is $-Si(CH_3)(Ph)_2$.
53. The compound of any one of Embodiments 9-45, wherein R^s is $-L^s-R^{s11}$.
54. The compound of Embodiment 53, wherein R^{s11} is $-S(O)_2R$ wherein R is optionally substituted C_{1-6} aliphatic.
55. The compound of Embodiment 53, wherein R^{s11} is $-S(O)_2R$ wherein R is t-Bu.
56. The compound of Embodiment 53, wherein R^{s11} is $-S(O)_2R$ wherein R is methyl.
57. The compound of Embodiment 53, wherein R^{s11} is $-S(O)_2R$ wherein R is optionally substituted phenyl.
58. The compound of Embodiment 53, wherein R^{s11} is $-S(O)_2R$ wherein R is phenyl.
59. The compound of Embodiment 53, wherein R^{s11} is optionally substituted phenyl.
60. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is optionally substituted C_{1-6} aliphatic.
61. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is methyl.
62. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is optionally substituted phenyl.
63. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is phenyl.
64. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is $-C(R^{s11})_3$.
65. The compound of any one of Embodiments 9-42, wherein an occurrence of R^s is $-CH(R^{s11})_2$.
66. The compound of Embodiment 65, wherein the two R^{s11} are taken together with the carbon atom to which they are attached to form an optionally substituted 3-20 membered ring having 0-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

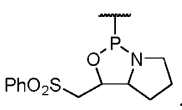


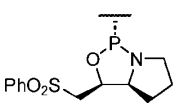
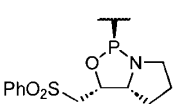
67. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is $R^{s1}R^{s2}$, wherein each of R^{s1} and R^{s2} is independently R^s .
68. The compound of Embodiment 67, wherein R^{s1} is hydrogen.
69. The compound of Embodiment 67, wherein R^{s1} is optionally substituted C_{1-6} aliphatic.
70. The compound of Embodiment 67, wherein R^{s1} is methyl.
71. The compound of Embodiment 67, wherein R^{s1} is optionally substituted phenyl.
72. The compound of Embodiment 67, wherein R^{s1} is phenyl.

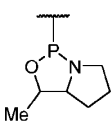
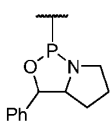
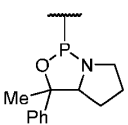
73. The compound of any one of Embodiments 67-72, wherein R^{s2} is hydrogen.
74. The compound of any one of Embodiments 67-72, wherein R^{s2} is optionally substituted C_{1-6} aliphatic.
75. The compound of any one of Embodiments 67-72, wherein R^{s2} is methyl.
76. The compound of any one of Embodiments 67-72, wherein R^{s2} is optionally substituted phenyl.
77. The compound of any one of Embodiments 67-72, wherein R^{s2} is phenyl.

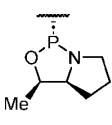
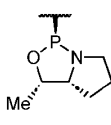
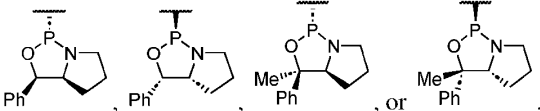
78. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is .

79. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is  or .

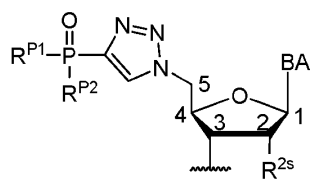
80. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is .

81. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is  or .

82. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is , , or .

83. The compound of Embodiment 1 or 2, wherein $-P(OR^1)N(R^2)(R^3)$ is , , or .

84. The compound of any one of Embodiments 9-35, wherein t is 0.
85. A compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I or a salt thereof:



O-I

wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

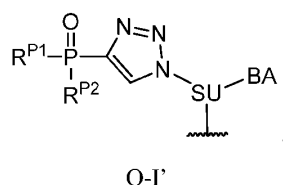
$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered

heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

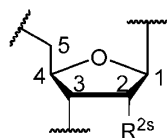
86. A compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I' or a salt thereof:



wherein:

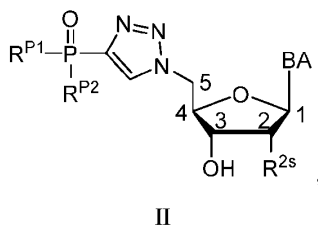
each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase; and



SU is a sugar (c.g., R^{2s}), wherein R^{2s} is as described herein).

87. A compound, wherein the compound is a compound of formula II or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5; each L^{2s} is independently L;

each R^{2a} is independently R';

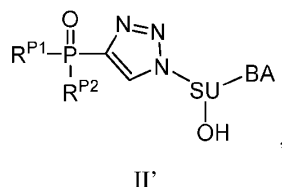
each L is independently a bivalent, optionally substituted group selected from C₁₋₁₀ aliphatic and C₁₋₁₀ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by -Cy-, -O-, -S-, -N(R')-, -C(O)-, -C(S)-, -C(NR')-, -C(O)N(R')-, -N(R')C(O)N(R')-, -N(R')C(O)O-, -S(O)-, -S(O)₂-, -S(O)₂N(R')-, -SC(O)-, or -C(O)O-;

-Cy- is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently -R-, -C(O)R-, -CO₂R-, or -S(O)₂R-, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C₁₋₁₀ aliphatic, C₁₋₁₀ heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C₆₋₁₄ aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

88. A compound, wherein the compound is a compound of formula II' or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is -O-CH₂CH₂CN or -O-CH₂-O-C(O)-R^P, wherein R^P is optionally substituted tert-butyl;

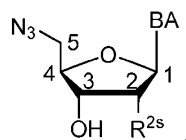
SU is a sugar; and

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase.

89. The compound of any one of the preceding Embodiments, wherein R^{P1} and R^{P2} are -O-CH₂CH₂CN.

90. The compound of any one of the preceding Embodiments, wherein R^{P1} and R^{P2} are $-O-CH_2-O-C(O)-tBu$.

91. A compound, wherein the compound is a compound of formula III or a salt thereof:



III

wherein:

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

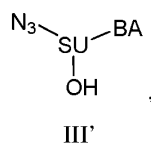
each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')-$, $-C(O)-$, $-C(S)-$, $-C(NR')-$, $-C(O)N(R')-$, $-N(R')C(O)N(R')-$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')-$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

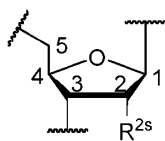
each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

92. A compound, wherein the compound is a compound of formula III' or a salt thereof:



wherein:

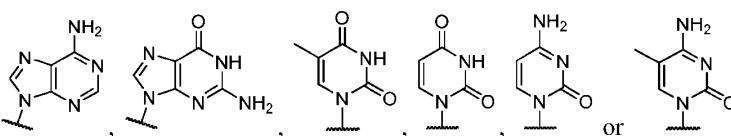
BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase; and



SU is a sugar (e.g., R^{2s}), wherein R^{2s} is as described herein).

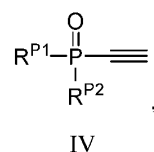
93. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.
94. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 3-20 membered ring having 0-5 heteroatoms independently selected from oxygen, nitrogen and sulfur.
95. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 3-20 membered ring having 0-5 heteroatoms independently selected from oxygen and nitrogen.
96. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 3-20 membered ring having at least one nitrogen atom.
97. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 5-15 membered ring.
98. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 5-10 membered ring.
99. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 5-9 membered ring.
100. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 9-membered ring.
101. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted 10-membered ring.
102. The compound of any one of Embodiments 1-99, wherein BA is an optionally substituted 5-membered ring.

103. The compound of any one of Embodiments 1-99, wherein BA is an optionally substituted 6-membered ring.
104. The compound of any one of the preceding Embodiments, wherein BA is an optionally substituted monocyclic ring.
105. The compound of any one of Embodiments 1-101, wherein BA is an optionally substituted bicyclic ring.
106. The compound of any one of the preceding Embodiments, wherein BA is bonded to C1 at a nitrogen atom.
107. The compound of any one of Embodiments 1-99, wherein BA is an optionally substituted group,

wherein the group is , or a tautomer thereof.

108. The compound of any one of Embodiments 1-99, wherein BA is an optionally protected nucleobase.
109. The compound of any one of Embodiments 1-99, wherein BA is a protected nucleobase.
110. The compound of any one of Embodiments 108-109, wherein the nucleobase is selected from A, T, C, G, U and 5mU.
111. The compound of any one of Embodiments 108-110, wherein the protection is suitable for oligonucleotide synthesis.
112. The compound of any one of the preceding Embodiments, wherein R^{2s} is $-H$.
113. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-F$.
114. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-OR^{2a}$.
115. The compound of Embodiment 114, wherein R^{2a} is optionally substituted C_{1-6} alkyl.
116. The compound of any one of Embodiments 1-111, wherein L^{2s} is not a covalent bond.
117. The compound of any one of Embodiments 1-111, wherein L^{2s} is optionally substituted C_{1-10} alkylene.
118. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-L^{2s}-R^{2a}$.
119. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-O-L^{2s'}-R^{2a}$, wherein $L^{2s'}$ is a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-4 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of $L^{2s'}$ are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$.

120. The compound of Embodiment 119, wherein $L^{2s'}$ is a covalent bond.
121. The compound of Embodiment 119, wherein $L^{2s'}$ is an optionally substituted bivalent C_{1-10} aliphatic group.
122. The compound of Embodiment 119, wherein $L^{2s'}$ is an optionally substituted C_{1-10} alkylene group.
123. The compound of Embodiment 119, wherein $L^{2s'}$ is optionally substituted $-(CH_2)_{1-10}-$.
124. The compound of Embodiment 119, wherein $L^{2s'}$ is $-(CH_2)_{1-10}-$.
125. The compound of Embodiment 119, wherein $L^{2s'}$ is $-(CH_2)_{10}-$.
126. The compound of any one of Embodiments 119-125, wherein R^{2a} is optionally substituted C_{1-10} aliphatic.
127. The compound of any one of Embodiments 119-125, wherein R^{2a} is optionally substituted C_{1-10} alkyl.
128. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-O(CH_2)_{0-15}CH_3$.
129. The compound of any one of Embodiments 1-111, wherein R^{2s} is $-O(CH_2)_{15}CH_3$.
130. The compound of any one of Embodiments 1-, wherein R^{2s} is $-O-L^{2s}-OR^{2a}$.
131. The compound of Embodiment 130, wherein R^{2a} is $-OMe$.
132. The compound of Embodiment 130, wherein R^{2s} is $-OCH_2CH_2OMe$.
133. The compound of any one of Embodiments 1-111, wherein R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5.
134. The compound of any one of Embodiments 1-111, wherein R^{2s} is L connecting C2 with C4.
135. The compound of any one of Embodiments 1-111 and 133-134, wherein L is $(C2)-O-(unsubstituted\ methylene)-$.
136. The compound of any one of Embodiments 1-111 and 133-134, wherein L is $(C2)-O-(substituted\ methylene)-$.
137. The compound of any one of Embodiments 1-111 and 133-134, wherein L is $(C2)-O-(substituted\ methylene)-$, wherein the methylene group is substituted with methyl.
138. The compound of any one of Embodiments 1-111 and 133-134, wherein L is $(C2)-O-(substituted\ methylene)-$, wherein the methylene group is substituted with ethyl.
139. The compound of any one of Embodiments 136-138, wherein the carbon atom of the methylene group is *R*.
140. The compound of any one of Embodiments 136-138, wherein the carbon atom of the methylene group is *S*.
141. A compound, wherein the compound is a compound of formula IV or a salt thereof:



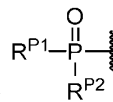
wherein:

each of $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl.

142. The compound of Embodiment 141, wherein $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ are $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.

143. The compound of Embodiment 141, wherein $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ are $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{tBu}$.

144. The compound of any one of the preceding Embodiments, wherein the purity of the compound is about or more than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.



145. A method for preparing an oligonucleotide, comprising $\text{---P}(\text{O})(\text{OH})_2$ (e.g., a salt form thereof) of an oligonucleotide into $-\text{P}(\text{O})(\text{OH})_2$ (e.g., a salt form thereof).

146. A method for preparing an oligonucleotide, comprising coupling a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I or a salt thereof, to an oligonucleotide.

147. A method for preparing an oligonucleotide, comprising coupling a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I' or a salt thereof, to an oligonucleotide.

148. A method for preparing an oligonucleotide, comprising reacting a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I or a salt thereof, with a compound comprising a $-\text{OH}$ group.

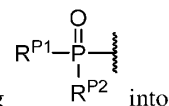
149. A method for preparing an oligonucleotide, comprising reacting a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I' or a salt thereof, with a compound comprising a $-\text{OH}$ group.

150. The method of Embodiment 148 or 149, wherein the $-\text{OH}$ group is 5'-OH of an oligonucleotide.

151. The method of any one of Embodiments 146-150, wherein the oligonucleotide preparation comprises utilized a solid support.

152. The method of any one of Embodiments 146-151, comprising reacting a compound of any one of Embodiments 1-84, 87-90, and 93-140 with an oligonucleotide on a solid support.

153. The method of any one of Embodiments 146-151, comprising preparing a compound of any one of Embodiments 85, 89-90, and 93-140, wherein the compound is a compound of formula O-I or a salt thereof.



154. The method of any one of Embodiments 146-151, comprising converting $-\text{P}(\text{O})(\text{OH})_2$ (e.g., a salt form thereof).

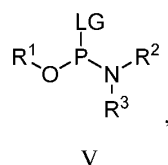
155. The method of Embodiment 154, wherein R^{P1} and R^{P2} are $-\text{OCH}_2\text{CH}_2\text{CN}$, and the conversion is performed in the presence of a base.

156. The method of Embodiment 154, wherein R^{P1} and R^{P2} are $-\text{OCH}_2\text{OC}(\text{O})\text{tBu}$.

157. The method of any one of Embodiments 146-156, wherein the conversion occurs during cleavage and deprotection that removes base protection groups and/or chiral auxiliaries and/or cleaves the oligonucleotide from a solid support.

158. The method of any one of Embodiments 146-157, wherein the oligonucleotide prepared is a compound of any one of Embodiments 85, 89-90, and 93-140, wherein the compound is a compound of formula O-I or a salt thereof.

159. A method for preparing a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I or a salt thereof, comprising reacting a compound of any one of Embodiments 87-90 and 93-140, wherein the compound is a compound of formula II or a salt thereof, with a compound of formula V or a salt thereof:



wherein:

LG is a leaving group;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $-(\text{A})-(\text{R}^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{CN}$, $-\text{N}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{L}^s-\text{R}^{\text{s11}}$, $-\text{L}^s-\text{OR}^{\text{s11}}$, $-\text{L}^s-\text{SR}^{\text{s11}}$, $-\text{L}^s-\text{N}(\text{R}^{\text{s11}})_2$, $-\text{O}-\text{L}^s-\text{OR}^{\text{s11}}$, $-\text{O}-\text{L}^s-\text{SR}^{\text{s11}}$, $-\text{O}-\text{L}^s-\text{N}(\text{R}^{\text{s11}})_2$, $-\text{C}(\text{R}^{\text{s11}})_3$ or $-\text{L}^s-\text{Si}(\text{R}^{\text{s11}})_3$;

each R^{s11} is independently R' ;

each L^s is independently L ;

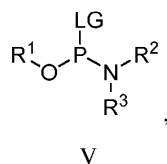
each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

160. A method for preparing a compound of any one of Embodiments 1-84, 87-90, and 93-140, wherein the compound is a compound of formula I' or a salt thereof, comprising reacting a compound of any one of Embodiments 87-90 and 93-140, wherein the compound is a compound of formula II' or a salt thereof, with a compound of formula V or a salt thereof:



wherein:

LG is a leaving group;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $-(A)-(R^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R^{s11}$, $-L^s-OR^{s11}$, $-L^s-SR^{s11}$, $-L^s-N(R^{s11})_2$, $-O-L^s-OR^{s11}$, $-O-L^s-SR^{s11}$, $-O-L^s-N(R^{s11})_2$, $-C(R^{s11})_3$ or $-L^s-Si(R^{s11})_3$;

each R^{s11} is independently R' ;

each L^s is independently L ;

each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

161. The method of Embodiment 159 or 160, wherein LG is $-Cl$.

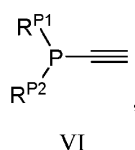
162. The method of any one of Embodiments 159-161, wherein the reaction is performed in the presence of a base.

163. The method of any one of Embodiments 159-162, wherein R^1 , R^2 and R^3 of formula V are as described in any one of Embodiments 3-84.

164. The method of any one of Embodiments 146-158, comprising a method of any one of Embodiments 159-163.

165. A method for preparing a compound of any one of Embodiments 87-90 and 93-140, wherein the compound is a compound of formula II or a salt thereof, comprising reacting a compound of any one of Embodiments 91-140, wherein the compound is a compound of formula III or a salt thereof, with a compound of any one of Embodiments 141-144.

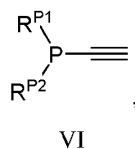
166. A method for preparing a compound of any one of Embodiments 87-90 and 93-140, wherein the compound is a compound of formula II' or a salt thereof, comprising reacting a compound of any one of Embodiments 91-140, wherein the compound is a compound of formula III' or a salt thereof, with a compound of any one of Embodiments 141-144.
167. The method of Embodiment 165, wherein the reaction is in the presence of a base.
168. The method of Embodiment 167, wherein the base is DIEA.
169. The method of any one of Embodiments 165-168, wherein the reaction is in the presence of copper salt.
170. The method of any one of Embodiments 165-168, wherein the reaction is in the presence of a Cu(I) salt.
171. The method of any one of Embodiments 165-168, wherein the reaction is in the presence of CuI.
172. The method of any one of Embodiments 146-164, comprising a method of any one of Embodiments 165-171.
173. A method for preparing a compound of any one of Embodiments 141-144, comprising oxidizing a compound of formula VI or a salt thereof:



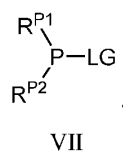
wherein:

each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl.

174. The method of Embodiment 173, wherein the oxidant is mCPBA.
175. The method of Embodiment 173 or 174, wherein R^{P1} and R^{P2} are $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.
176. The method of any one of Embodiments 146-172, comprising a method of any one of Embodiments 173-175.
177. A method for preparing a compound of formula VI or a salt thereof:



comprising reacting $\equiv-\text{MgBr}$ with a compound of formula VII or a salt thereof:



wherein:

LG is a leaving group; and

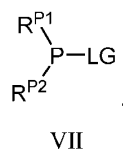
each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl.

178. The method of Embodiment 177, wherein LG is $-Cl$.

179. The method of any one of Embodiments 177-178, wherein each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$.

180. The method of any one of Embodiments 146-176, comprising a method of any one of Embodiments 177-179.

181. A method for preparing a compound of formula VII or a salt thereof:

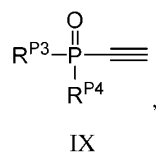


wherein LG is $-Cl$, comprising reacting $HO-CH_2CH_2-CN$ with PCl_3 in the presence of a base.

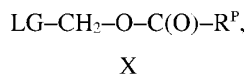
182. The method of Embodiment 181, wherein the base is TEA.

183. The method of any one of Embodiments 146-180, comprising a method of any one of Embodiments 181-182.

184. A method for preparing a compound of any one of Embodiments 141-144, wherein each of R^{P1} and R^{P2} is $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl, comprising reacting a compound of formula IX or a salt thereof:



with a compound having the structure of formula X or a salt thereof:



wherein:

R^P is optionally substituted tert-butyl;

LG is a leaving group;

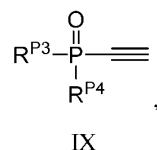
each of R^{P3} and R^{P4} is independently $-O-R^{SP}$, wherein R^{SP} is an optionally substituted optionally substituted C_{1-6} aliphatic.

185. The method of Embodiment 184, wherein each of R^{P3} and R^{P4} is $-OMe$.

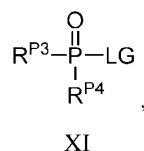
186. The method of any one of Embodiments 184-185, wherein LG is $-I$.

187. The method of any one of Embodiments 146-172, comprising a method of any one of Embodiments 184-186.

188. A method for preparing a compound of formula IX or a salt thereof:



comprising reacting $\equiv-MgBr$ with a compound of formula XI or a salt thereof:



wherein:

LG is a leaving group

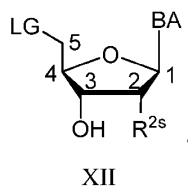
each of R^{P3} and R^{P4} is independently $-O-R^{SP}$, wherein each R^{SP} is independently optionally substituted C_{1-6} aliphatic.

189. The method of Embodiment 188, wherein each of R^{P3} and R^{P4} is $-OMe$.

190. The method of any one of Embodiments 188-189, wherein LG is $-Cl$.

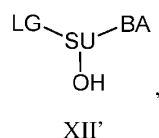
191. The method of any one of Embodiments 146-172 and 184-187, comprising a method of any one of Embodiments 188-190.

192. A method for preparing a compound of any one of Embodiments 91-140, wherein the compound is a compound of formula III or a salt thereof, comprising reacting a compound of formula XII or a salt thereof:



wherein LG is a leaving group, with an azide.

193. A method for preparing a compound of any one of Embodiments 91-140, wherein the compound is a compound of formula III' or a salt thereof, comprising reacting a compound of formula XII' or a salt thereof:



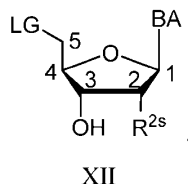
wherein LG is a leaving group, with an azide.

194. The method of Embodiment 192 or 193, wherein LG is -I.

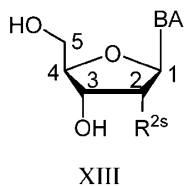
195. The method of any one of Embodiments 192-194, wherein the azide is sodium azide.

196. The method of any one of Embodiments 146-191, comprising a method of any one of Embodiments 192-195.

197. A method for preparing a compound of formula XII or a salt thereof:

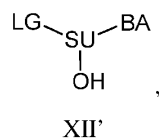


wherein LG is -I, comprising reacting a compound of formula XIII or a salt thereof:

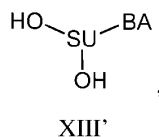


with I₂.

198. A method for preparing a compound of formula XII' or a salt thereof:



wherein LG is -I, comprising reacting a compound of formula XIII' or a salt thereof:



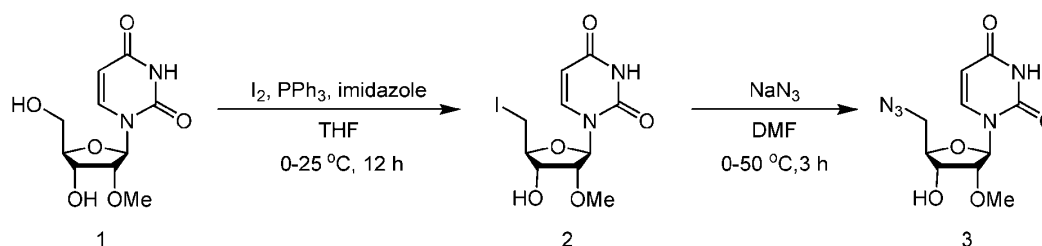
with I₂.

199. The method of Embodiment 197 or 198, wherein the reaction is performed in the presence of a phosphine.
200. The method of Embodiment 197 or 198, wherein the reaction is performed in the presence of a PPh_3 .
201. The method of any one of Embodiments 197-200, wherein the reaction is performed in the presence of a base.
202. The method of any one of Embodiments 197-200, wherein the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-\text{N}=-$ or $-\text{N(R)}-$.
203. The method of any one of Embodiments 197-200, wherein the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises $-\text{N}=-$ or $-\text{NH}-$.
204. The method of any one of Embodiments 197-200, wherein the reaction is performed in the presence of imidazole.
205. The method of any one of Embodiments 146-196, comprising a method of any one of Embodiments 197-204.

EXEMPLIFICATION

[0335] Certain examples of provided technologies (compounds (oligonucleotides, reagents, etc.), compositions, methods (e.g., for preparing nucleosides, phosphoramidites, oligonucleotides, etc.) are presented herein. Those skilled in the art appreciate that many technologies, e.g., those described in US 9982257, US 20170037399, US 20180216108, US 20180216107, US 9598458, WO 2017/062862, WO 2018/067973, WO 2017/160741, WO 2017/192679, WO 2017/210647, WO 2018/098264, WO 2018/223056, WO 2018/237194, WO 2019/032607, WO 2019/055951, WO 2019/075357, WO 2019/200185, WO 2019/217784, WO 2019/032612, WO 2020/191252, WO 2021/071858, WO 2022/099159, etc., can be utilized to prepare and/or assess properties and/or activities of provided technologies in accordance with the present disclosure.

[0336] Example 1. Preparation of compound 3.

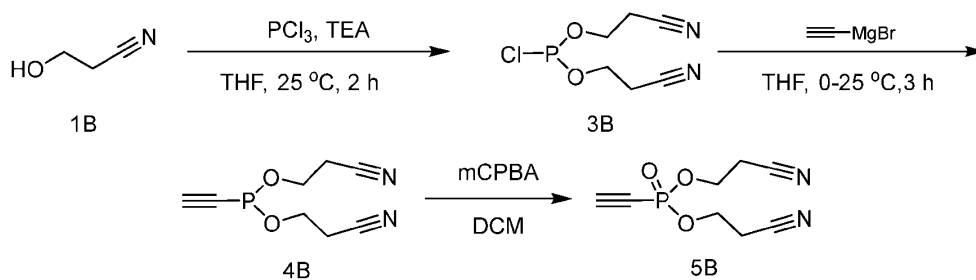


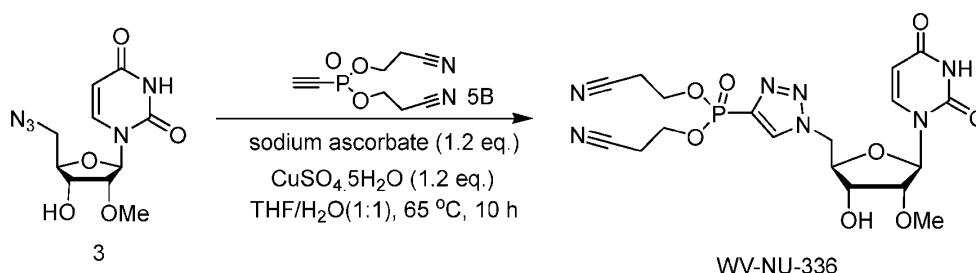
[0337] Step 1. To a solution of compound 1 (50 g, 193.63 mmol) in THF (700 mL) was added imidazole (34.27 g, 503.43 mmol), I_2 (78.63 g, 309.80 mmol) and PPh_3 (81.26 g, 309.80 mmol) at $0\text{ }^\circ\text{C}$. The mixture was stirred at $25\text{ }^\circ\text{C}$ for 12 hr. LCMS showed compound 1 was consumed completely and

the desired mass was detected. Four batches were combined together. The reaction was quenched by 10% aqueous sodium thiosulfate solution (500 ml). After removing the solvent and volatiles under reduced pressure, the residue was extracted into EtOAc (200 mL*3) and washed with saturated aqueous NaHCO₃ solution. The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1: 0 to 0: 1). Compound 2 (67.5 g, 94.73% yield) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.42 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 5.86 (d, J = 5.4 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 5.45 (d, J = 6.0 Hz, 1H), 4.06 - 4.01 (m, 1H), 4.00 - 3.96 (m, 1H), 3.85 (td, J = 5.0, 6.2 Hz, 1H), 3.55 (dd, J = 5.4, 10.6 Hz, 1H), 3.40 (dd, J = 6.9, 10.6 Hz, 1H), 3.34 (s, 3H) LCMS (M+H⁺): 368.9. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.5.

[0338] Step 2. To a solution of compound 2 (10 g, 27.16 mmol) in DMF (100 mL) was added NaN₃ (1.86 g, 28.66 mmol) at 0 °C. The mixture was stirred at 0-50 °C for 3 hr. LCMS showed compound 2 was consumed completely and the desired mass was detected. Six batches were combined together. The reaction was quenched by H₂O (1500 mL), and extracted with Ethyl acetate (500 mL*3). The combined organic layers were washed with saturated aqueous NaCl 100 mL, dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1:0 to 0:1). The crude product was purified by re-crystallization from DCM (200 mL) at 25 °C. Compound 3 (9.5 g, 93.44% yield) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.41 (br s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 5.83 (d, J = 4.9 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 5.35 (br d, J = 6.0 Hz, 1H), 4.07 (q, J = 5.3 Hz, 1H), 3.95 - 3.89 (m, 2H), 3.61 (d, J = 4.9 Hz, 2H), 3.36 (s, 3H). LCMS: (M+H⁺): 284.0, LCMS purity: 100%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.45.

[0339] Example 2. Synthesis of 2'-OMe-5'-Bis(2-cyanoethyl)-Triazolyl Phosphonate Uridine (WV-NU-336).





[0340] Step 1A. To a solution of PCl_3 (35 g, 254.86 mmol) in THF (1000 mL) was added TEA (51.58 g, 509.71 mmol, 70.95 mL) and 3-hydroxypropanenitrile (36.23 g, 509.71 mmol, 34.67 mL). The mixture was stirred at 25 °C for 2 hr. TLC indicated compound 1B was consumed completely and one new spot formed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. Without purification. Compound 3B (44 g, 83.58% yield) was obtained as a colorless oil. TLC: Petroleum ether : Ethyl acetate = 0:1, R_f = 0.12.

[0341] Step 2A. To a solution of compound 3B (10 g, 48.41 mmol) in THF (100 mL) was added bromo(ethynyl)magnesium (0.5 M, 116.19 mL) at 0 °C. The mixture was stirred at 0 - 25 °C for 5 hr. TLC indicated compound 3B was consumed completely and two new spots formed. The reaction mixture was quenched by addition of NH_4Cl 50 mL at 0 °C, and then diluted with water 100 mL and extracted with EtOAc (100 mL * 3). The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give a residue. Compound 4B (6 g, 63.19% yield) was obtained as a yellow oil. ^1H NMR (400 MHz, CHLOROFORM-d) δ = 4.17 - 4.09 (m, 4H), 3.19 (d, J = 2.1 Hz, 1H), 2.67 (t, J = 6.1 Hz, 4H). ^{31}P NMR (162 MHz, CHLOROFORM-d) δ = 132.04 (s, 1P). For a larger-scale batch, to a solution of compound 3B (44 g, 213.01 mmol) in THF (1000 mL) was added bromo(ethynyl)magnesium (0.5 M, 511.22 mL) at 0 °C. The mixture was stirred at 0-25 °C for 3hr. TLC indicated compound 3B was consumed completely and two new spots formed. The reaction mixture was quenched by sat. NH_4Cl (200 mL) at 0°C, then extracted with DCM (500 mL*3). The combined organic layers were dried over Na_2SO_4 , filtered to get the crude. No purification. Compound 4B (40 g, crude) was obtained as a yellow oil. TLC : Petroleum ether : Ethyl acetate = 1:1, R_f = 0.39.

[0342] Step 3A. To a solution of compound 4B (40 g, 203.93 mmol) in DCM (1000 mL) was added mCPBA (62.10 g, 305.90 mmol, 85% purity). The mixture was stirred at 0 - 25 °C for 2 hr. TLC indicated compound 4B was consumed completely and one new spot formed. The reaction mixture was quenched by sat. Na_2SO_3 (2000 mL) and NaHCO_3 (2000mL), then extracted with DCM (1000 mL * 2). The combined organic layers were washed with brine (500ml), dried over Na_2SO_4 , filtered, and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 10:1 to 0:1). Compound 5 B (13 g, 30.05%

yield) was obtained as a yellow oil. ^1H NMR (400 MHz, CHLOROFORM- d) δ = 4.37 - 4.29 (m, 4H), 3.16 (dd, J = 1.3, 13.9 Hz, 1H), 2.81 (t, J = 6.1 Hz, 4H). ^{31}P NMR (162 MHz, CHLOROFORM- d) δ = -8.61 (s, 1P). TLC: Petroleum ether : Ethyl acetate = 1:1, R_f = 0.23

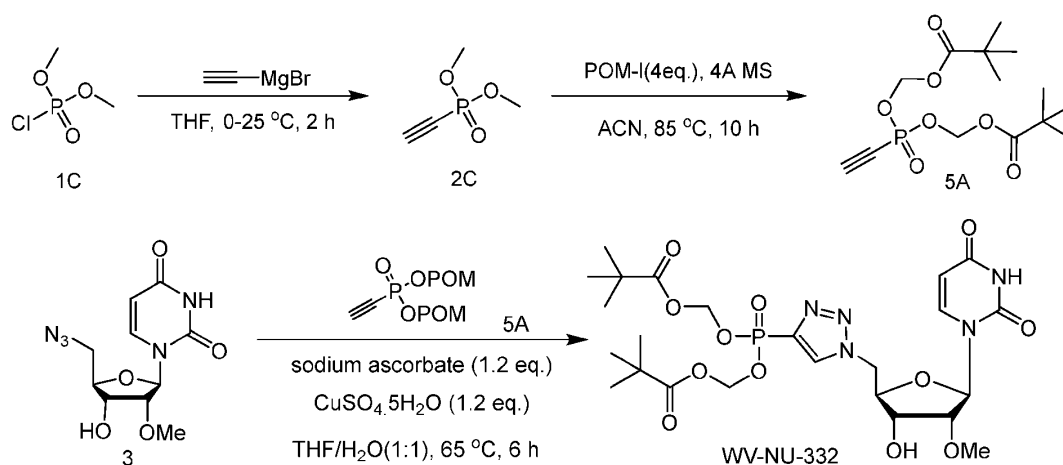
[0343] Step 1B. To a solution of compound 1 (50 g, 193.63 mmol) in THF (700 mL) was added imidazole (34.27 g, 503.43 mmol), I_2 (78.63 g, 309.80 mmol) and PPh_3 (81.26 g, 309.80 mmol) at 0 °C. The mixture was stirred at 25 °C for 12 hr. LCMS showed compound 1 was consumed completely and the desired mass was detected. Four batches were combined together. The reaction was quenched by 10% aqueous sodium thiosulfate solution (500 mL). After removing the solvent and volatiles under reduced pressure, the residue was extracted into EtOAc (200 mL*3) and washed with saturated aqueous NaHCO_3 solution. The organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and concentrated. The residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 1: 0 to 0: 1). Compound 2 (270 g, 94.73% yield, together with four batches) was obtained as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ = 11.42 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 5.86 (d, J = 5.4 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 5.45 (d, J = 6.0 Hz, 1H), 4.06 - 4.01 (m, 1H), 4.00 - 3.96 (m, 1H), 3.85 (td, J = 5.0, 6.2 Hz, 1H), 3.55 (dd, J = 5.4, 10.6 Hz, 1H), 3.40 (dd, J = 6.9, 10.6 Hz, 1H), 3.34 (s, 3H). LCMS (M+H $^+$): 368.9. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.5.

[0344] Step 2B. To a solution of compound 2 (10 g, 27.16 mmol) in DMF (100 mL) was added NaN_3 (1.86 g, 28.66 mmol) at 0 °C. The mixture was stirred at 0-50 °C for 3 hr. LCMS showed compound 2 was consumed completely and the desired mass was detected. Six batches were combined together. The reaction was quenched by H_2O (1500 mL), and extracted with Ethyl acetate (500 mL*3). The combined organic layers were washed with saturated aqueous NaCl 100 mL, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 1:0 to 0:1). The crude product was purified by re-crystallization from DCM (200 mL) at 25 °C. Compound 3 (57 g, 93.44% yield) was obtained as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ = 11.41 (br s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 5.83 (d, J = 4.9 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 5.35 (br d, J = 6.0 Hz, 1H), 4.07 (q, J = 5.3 Hz, 1H), 3.95 - 3.89 (m, 2H), 3.61 (d, J = 4.9 Hz, 2H), 3.36 (s, 3H). LCMS: (M+H $^+$): 284.0, LCMS purity: 100%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.45.

[0345] Step 4. To a solution of compound 3 (5 g, 17.65 mmol) and compound 5B (4.49 g, 21.18 mmol) in THF (10 mL) and H_2O (10 mL) was degassed and purged with N_2 for 3 times, then $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (5.29 g, 21.18 mmol), sodium ascorbate (4.20 g, 21.18 mmol) was added. The mixture was stirred at 65 °C for 10 hr under N_2 atmosphere. LCMS showed compound 3 was consumed completely and desired mass was detected. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2 , Petroleum ether:

Ethyl acetate = 10:1 to 0:1, Ethyl acetate MeCN = 8:1 to 0:1). Compound WV-NU-336 (5.3 g, 60.61% yield) was obtained as a white solid. ^1H NMR (400 MHz, DMSO- d_6) δ = 11.40 (d, J = 1.6 Hz, 1H), 8.72 (s, 1H), 7.60 (d, J = 8.0 Hz, 1H), 5.79 (d, J = 4.8 Hz, 1H), 5.66 (dd, J = 2.1, 8.0 Hz, 1H), 5.50 (d, J = 6.1 Hz, 1H), 4.87 - 4.73 (m, 2H), 4.32 - 4.17 (m, 5H), 4.14 (q, J = 5.5 Hz, 1H), 3.93 (t, J = 5.0 Hz, 1H), 3.36 (s, 3H), 2.95 (t, J = 5.9 Hz, 4H). ^{31}P NMR (162 MHz, DMSO- d_6) δ = 7.72 (s, 1P). LCMS (M+H $^{+}$): 496.1, purity: 93.49%. TLC: Dichloromethane: Methanol = 8:1, R_f = 0.13.

[0346] Example 3. Synthesis of 2'-OMe-5'-Bis(Pivaloyloxymethyl)-Triazolyl Phosphonate Uridine (WV-NU-332).



[0347] Step 1A. To a solution of compound 1C (10 g, 69.21 mmol, 7.46 mL) in THF (100 mL) was added bromo(ethynyl)magnesium (0.5 M, 166.10 mL) under N₂. The mixture was stirred at 0 - 25 °C for 2 hr. TLC indicated compound 1C was consumed completely and one new spot formed. The reaction mixture was quenched by addition NH₄Cl 50 mL at 0 °C, and then diluted with water 50 mL and extracted with EtOAc (100 mL * 3). The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give a residue. Without purification. Compound 2C (9 g, crude) was obtained as a yellow oil. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.34.

[0348] Step 2A. To a solution of compound 2C (9 g, 67.13 mmol) in ACN (200 mL) was added 4A MS (2 g, 67.13 mmol), iodomethyl 2,2-dimethylpropanoate (48.75 g, 201.39 mmol). The mixture was stirred at 82 °C for 10 hr. TLC indicated compound 2C was consumed completely and two new spots formed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. Compound 5A (5 g, 22.28% yield) was obtained as a colorless oil. ^1H NMR (400 MHz, CHLOROFORM- d) δ = 5.72 (d, J = 1.6 Hz, 2H), 5.69 (d, J = 0.9 Hz, 2H), 3.03 (d, J = 14.3 Hz, 1H), 1.22 (s, 18H). ^{31}P NMR (162 MHz, CHLOROFORM- d) δ = 10.31 (s, 1P). TLC: Petroleum ether: Ethyl acetate = 3:1, R_f = 0.38.

[0349] Step 1B. To a solution of compound 1 (50 g, 193.63 mmol) in THF (700 mL) was added imidazole (34.27 g, 503.43 mmol), I2 (78.63 g, 309.80 mmol) and PPh3 (81.26 g, 309.80 mmol) at 0 °C. The mixture was stirred at 25 °C for 12 hr. LCMS showed compound 1 was consumed completely and the desired mass was detected. Four batches were combined together. The reaction was quenched by 10% aqueous sodium thiosulfate solution (500 mL). After removing the solvent and volatiles under reduced pressure, the residue was extracted into EtOAc (200 mL*3) and washed with saturated aqueous NaHCO₃ solution. The organic layer was separated, dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1:0 to 0:1). Compound 2 (270 g, 94.73% yield, together with four batches) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.42 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 5.86 (d, J = 5.4 Hz, 1H), 5.69 (d, J = 8.0 Hz, 1H), 5.45 (d, J = 6.0 Hz, 1H), 4.06 - 4.01 (m, 1H), 4.00 - 3.96 (m, 1H), 3.85 (td, J = 5.0, 6.2 Hz, 1H), 3.55 (dd, J = 5.4, 10.6 Hz, 1H), 3.40 (dd, J = 6.9, 10.6 Hz, 1H), 3.34 (s, 3H). LCMS (M+H⁺): 368.9. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.5.

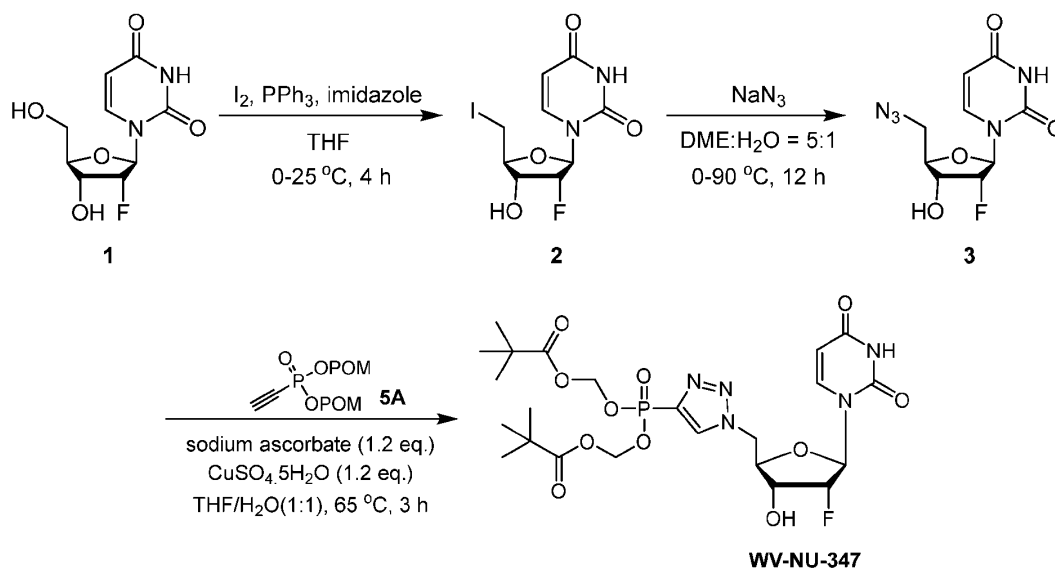
[0350] Step 2B. To a solution of compound 2 (10 g, 27.16 mmol) in DMF (100 mL) was added NaN₃ (1.86 g, 28.66 mmol) at 0 °C. The mixture was stirred at 0-50 °C for 3 hr. LCMS showed compound 2 was consumed completely and the desired mass was detected. Six batches were combined together. The reaction was quenched by H₂O (1500 mL), and extracted with Ethyl acetate (500 mL*3). The combined organic layers were washed with saturated aqueous NaCl 100 mL, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1:0 to 0:1). The crude product was purified by re-crystallization from DCM (200 mL) at 25 °C. Compound 3 (57 g, 93.44% yield, together with six batches) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.41 (br s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 5.83 (d, J = 4.9 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 5.35 (br d, J = 6.0 Hz, 1H), 4.07 (q, J = 5.3 Hz, 1H), 3.95 - 3.89 (m, 2H), 3.61 (d, J = 4.9 Hz, 2H), 3.36 (s, 3H). LCMS: (M+H⁺): 284.0, LCMS purity: 100%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.45.

[0351] Step 3. To a solution of compound 3 (3 g, 10.59 mmol) and compound 5A (4.25 g, 12.71 mmol, 1.2 eq) in H₂O (10 mL) was degassed and purged with nitrogen for 3 times, sodium ascorbate (2.52 g, 12.71 mmol, 1.2 eq), diacetylcopper (2.31 g, 12.71 mmol) was added. The mixture was stirred at 65 °C for 6 hr under N₂ atmosphere. TLC indicated compound 5A was consumed completely and one new spot formed. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 15:1 to 0:1 to Ethyl acetate: MeCN = 10:1 to 0:1 to Ethyl acetate: Methanol = 8:1). Compound WV-NU-332 (2 g, 46.67% yield, 70% purity) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.47 - 11.35 (m, 2H), 8.69 (s, 1H), 7.62 (d, J = 8.1 Hz, 1H), 5.80 (d, J = 5.0 Hz, 1H), 5.70 (s, 2H), 5.67

(s, 2H), 5.51 (d, J = 5.8 Hz, 1H), 4.80 (d, J = 3.8 Hz, 1H), 4.18 - 4.15 (m, 1H), 3.96 - 3.89 (m, 2H), 3.61 (d, J = 4.8 Hz, 1H), 3.36 (s, 3H), 1.06 (s, 18H). ^{31}P NMR (162 MHz, DMSO- d_6) δ = 7.08 (s, 1P).

LCMS:(M+H $^{+}$):618.2, purity:70.8%. TLC: Petroleum ether: Ethyl acetate = 0:1, R $_f$ = 0.06.

[0352] Example 4. Synthesis of 2'-Fluoro-5'-PO(POM)2-Triazolyl Phosphonate Uridine (WV-NU-347).

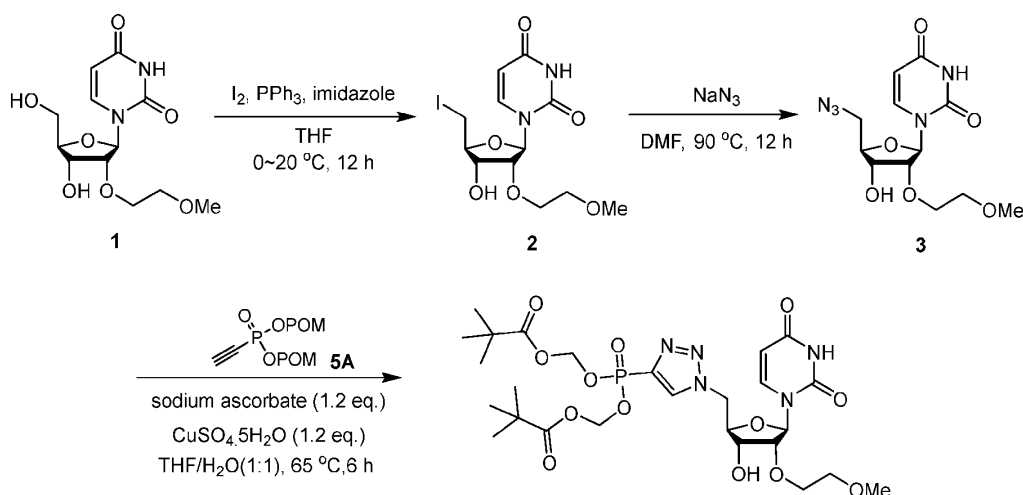


[0353] Step 1. To a solution of compound 1 (15 g, 60.93 mmol) in THF (210 mL) was added imidazole (10.78 g, 158.41 mmol), I $_2$ (24.74 g, 97.48 mmol) and PPh $_3$ (25.57 g, 97.48 mmol) at 0 °C. Mixture was stirred at 25 °C for 4 hr. LCMS showed compound 1 was consumed completely and desired mass was detected. Three batches were combined together. Reaction was quenched by 10% aqueous sodium thiosulfate solution (100 ml). After removing solvent and volatiles under reduced pressure, residue was extracted into EtOAc (50 mL * 5) and washed with saturated aqueous NaHCO $_3$ solution. Organic layer was separated, dried over anhydrous Na $_2$ SO $_4$, filtered and concentrated. Residue was purified by column chromatography (SiO $_2$, Petroleum ether: Ethyl acetate = 1: 0 to 0: 1 to Dichloromethane: Methanol =1: 0 to 3: 1). Crude product was purified by re-crystallization from Ethyl acetate (100 mL) and Methanol (10 mL) at 15 °C. Compound 2 (43 g (total three batches), 66.15% yield,) was obtained as a yellow solid. ^1H NMR (400 MHz, DMSO- d_6) δ = 11.46 (br s, 1H), 7.66 (d, J = 8.0 Hz, 1H), 5.94 - 5.79 (m, 2H), 5.66 (d, J = 8.0 Hz, 1H), 5.33 - 5.13 (m, 1H), 4.10 - 3.98 (m, 1H), 3.77 (dt, J = 3.4, 7.2 Hz, 1H), 3.60 (dd, J = 3.6, 11.0 Hz, 1H), 3.41 (dd, J = 6.8, 11.0 Hz, 1H). ^{19}F NMR (376 MHz, DMSO- d_6) δ = -199.11 (s, 1F). LCMS (M+H $^{+}$): 356.9, purity: 95.16%. TLC: Dichloromethane: Methanol = 10:1 R $_f$ =0.45.

[0354] Step 2. For two batches. To a solution of compound 2 (10 g, 28.08 mmol) in 1, 2-dimethoxyethane (100 mL) and H₂O (20 mL) was added NaN₃ (1.83 g, 28.08 mmol) at 0 °C under N₂. Mixture was stirred at 90 °C for 12 hr. LCMS showed compound 2 was remained and the desired mass was detected. Two batches were combined together. Reaction was quenched by H₂O (50 mL), and extracted with Ethyl acetate (100 mL*3). Combined organic layers were washed with saturated aqueous NaCl 50 mL, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1: 0 to 0: 1). Crude product was purified by re-crystallization from Ethyl acetate (100 mL) at 20 °C. Compound 3 (28 g, 85.63% yield) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.45 (br s, 1H), 7.67 (d, J = 8.0 Hz, 1H), 5.90 - 5.81 (m, 1H), 5.75 (br s, 1H), 5.66 (d, J = 8.0 Hz, 1H), 5.29 - 5.10 (m, 1H), 4.31 - 4.17 (m, 1H), 3.94-3.93 (m, 1H), 3.79 - 3.48 (m, 2H). ¹⁹F NMR (376 MHz, DMSO-d₆) δ = -198.74 (s, 1F). LCMS: (M+H⁺): 272.0, purity: 92.29%. TLC: Dichloromethane: Methanol = 10:1, R_f = 0.45.

[0355] Step 3. For two batches. To a solution of compound 3 (5 g, 18.44 mmol) and compound 5A (7.40 g, 22.12 mmol) in H₂O (25 mL) and THF (25 mL) was degassed and purged with N₂ for 3 times, CuSO₄·5H₂O (5.52 g, 22.12 mmol), sodium ascorbate (4.38 g, 22.12 mmol) was added. Mixture was stirred at 65 °C for 3 hr under N₂ atmosphere. LCMS showed compound 3 was consumed completely and the desired mass was detected. Two batches were combined together. Reaction mixture was extracted with Ethyl acetate (30mL*3). Combined organic layers were washed with saturated aqueous NaCl 20 mL, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate=1:0 to 0:1 to Ethyl acetate: Methanol=1:0 to 2:1). Compound WV-NU-347 (14.5 g, 65.91% yield) was obtained as a yellow solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.42 (d, J = 1.6 Hz, 1H), 8.71 (s, 1H), 7.56 (d, J = 8.2 Hz, 1H), 5.91 (d, J = 6.0 Hz, 1H), 5.87 - 5.78 (m, 1H), 5.69 (d, J = 13.8 Hz, 4H), 5.62 (dd, J = 2.0, 8.1 Hz, 1H), 5.31 - 5.09 (m, 1H), 4.91 - 4.71 (m, 2H), 4.30 - 4.16 (m, 2H), 1.08 (s, 18H). ³¹P NMR (162 MHz, DMSO-d₆) δ = 7.04 (s, 1P). ¹⁹F NMR (376 MHz, DMSO-d₆) δ = -199.16 (s, 1F). LCMS (M+H⁺): 606.2, purity: 98.32%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.55.

[0356] Example 5. Synthesis of 2'-OMOE-5'-PO(POM)2-Triazolyl Phosphonate Uridine (WV-NU-348).



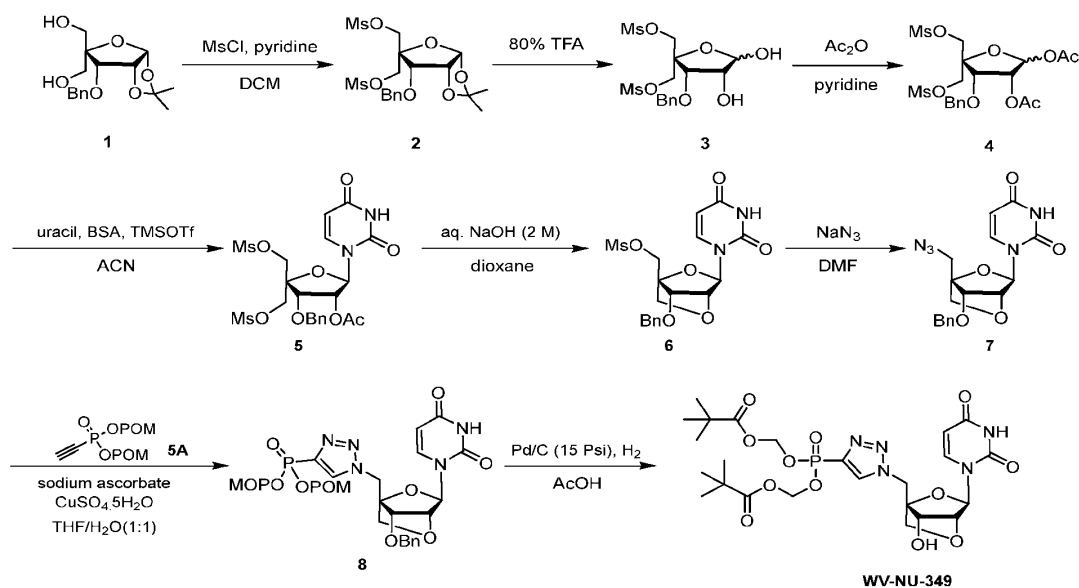
[0357] Step 1. Two batches were carried out in parallel: To a solution of compound 1 (17.5 g, 57.89 mmol) in THF (400 mL) was added imidazole (10.25 g, 150.52 mmol), I₂ (23.51 g, 92.63 mmol, 18.66 mL) and PPh₃ (24.30 g, 92.63 mmol) at 0 °C. Mixture was stirred at 20 °C for 12 h. LCMS showed compound 1 was consumed completely and one main peak with desired mass was detected. Two reactions were combined for work up. Reaction mixture was quenched by addition 10% Na₂S₂O₃ aq. 100 mL at 25 °C, and then concentrated under reduced pressure to remove THF, diluted with H₂O (700 mL) and extracted with EtOAc (500 mL * 2). The combined organic layers were washed with brine (600 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The crude product was used directly for the next step without purification. Compound 2 (48 g, crude) was obtained as a yellow oil. LCMS: (M+H⁺): 412.9.

[0358] Step 2: For two batches: To a solution of compound 2 (22 g, 53.38 mmol) in DMF (250 mL) was added NaN₃ (3.09 g, 47.53 mmol) at 0 °C under N₂. Mixture was stirred at 90 °C for 12 h. LCMS showed compound 2 was consumed completely and desired mass was detected. Reaction was quenched by H₂O (500 mL), and extracted with Ethyl acetate (500mL * 3). Combined organic dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1: 0 to 0: 1). Compound 3 (34 g, crude) was obtained as a yellow oil. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.40 (s, 1H), 7.70(d, J = 8.2 Hz, 1H), 5.82 (d, J = 4.5 Hz, 1H), 5.68 (d, J = 7.8 Hz, 1H), 5.26 (d, J = 5.6 Hz, 1H), 4.10 - 4.02 (m, 2H), 3.92 (q, J = 4.8 Hz, 1H), 3.74 - 3.67 (m, 1H), 3.66 - 3.57 (m, 3H), 3.47 - 3.42 (m, 2H), 3.25 - 3.20 (m, 3H). LCMS: (M+H⁺): 328.2. TLC: Petroleum ether: Ethyl acetate = 0: 1, R_f = 0.34.

[0359] To a solution of compound 3 (11.5 g, 35.14 mmol) and compound 5A (15.27 g, 45.68 mmol)

in THF (50 mL) and H₂O (50 mL) was added sodium ascorbate (8.35 g, 42.16 mmol) and CuSO₄·5H₂O (10.53 g, 42.16 mmol). The mixture was stirred at 65 °C for 6 h. LCMS showed compound 3 was consumed completely and desired mass was detected. The mixture was concentrated, the reaction mixture was extracted with EtOAc (50 mL * 3) and H₂O 50 mL. Then the combined organic layers were dried over Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1: 0 to 0: 1). Compound WV-NU-348 (10.7 g, 45.48% yield, 97.77% purity) was obtained as a yellow solid. ¹HNMR (400 MHz, DMSO-d₆) δ = 11.45 - 11.36 (m, 1H), 8.70 (s, 1H), 7.62 (d, J = 8.2 Hz, 1H), 5.80 (d, J = 4.6 Hz, 1H), 5.70 (s, 2H), 5.68 - 5.64 (m, 3H), 5.40 (d, J = 5.6 Hz, 1H), 4.85 - 4.71 (m, 2H), 4.21 - 4.17 (m, 1H), 4.14 - 4.08 (m, 2H), 3.75 - 3.67 (m, 1H), 3.67 - 3.59 (m, 1H), 3.46 (t, J = 4.8 Hz, 2H), 3.22 - 3.22 (m, 1H), 3.22 (s, 2H), 1.08 (s, 18H). ³¹PNMR (162 MHz, DMSO-d₆) δ = 7.08 (s, 1P). LCMS: (M+H⁺): 662.2, LCMS purity: 97.77%. TLC: Dichloromethane: Methanol = 10: 1, R_f = 0.32.

[0360] Example 6. Synthesis of 2'-LNA-5'-PO(POM)2-Triazolyl Phosphonate Uridine (WV-NU-349).



[0361] Step 1. For two batches: to a solution of compound 1 (50 g, 161.11 mmol) in DCM (800 mL) was added pyridine (82.84 g, 1.05 mol) and the mixture was cooled to 0 °C, then MsCl (46.18 g, 403.14 mmol) was slowly added the mixture. Mixture was stirred at 0 - 20 °C for 12 hr. LCMS showed compound 1 was consumed completely and desired mass was detected. Mixture was cooled to 0 °C, a mixture of 100 mL ice water and aqueous NaHCO₃ 100 mL was dropped to the mixture under N₂, and stirred for 5 min. Then two batches were combined together, organic layer was separated and washed

with saturated aqueous NaHCO_3 (300 mL*2) and with water. Combined organic phases were dried over MgSO_4 and solvent was removed under reduced pressure. Crude product was used into next step without further purification. Compound 2 (150 g, crude) was obtained as a yellow oil. ^1H NMR (400 MHz, CHCl_3 -d) δ = 7.41 - 7.35 (m, 5H), 5.79 (d, J = 3.8 Hz, 1H), 4.88 (d, J = 12.0 Hz, 1H), 4.77 (d, J = 11.6 Hz, 1H), 4.68 - 4.63 (m, 1H), 4.57 (d, J = 11.6 Hz, 1H), 4.41 (d, J = 12.0 Hz, 1H), 4.32 (d, J = 11.0 Hz, 1H), 4.22 - 4.12 (m, 2H), 3.08 (s, 3H), 2.98 (s, 3H), 1.68 (s, 3H), 1.34 (s, 3H). LCMS ($\text{M}+\text{Na}^+$): 489.

[0362] Step 2. For three batches: to a solution of compound 2 (50 g, 107.18 mmol) was added TFA (250 mL) and H_2O (62.5 mL). Mixture was stirred at 20 °C for 12 hr. LCMS showed compound 2 was consumed completely and desired mass was detected. Three batches were combined together. Reaction mixture was concentrated under reduced pressure to remove TFA. Residue was diluted with NaHCO_3 (500 mL) and extracted with DCM (300 mL * 2). Combined organic layers were washed with brine 500 mL, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. Crude product was used into the next step without further purification. Compound 3 (137 g, crude) was obtained as a yellow oil. ^1H NMR (400 MHz, CHCl_3 -d) δ = 8.64 (br d, J = 4.6 Hz, 1H), 7.48 - 7.45 (m, 1H), 7.41 - 7.30 (m, 5H), 5.39 - 5.34 (m, 1H), 4.66 - 4.59 (m, 2H), 4.35 - 4.33 (m, 1H), 4.31 - 4.20 (m, 1H), 4.19 - 4.14 (m, 1H), 4.11 (d, J = 5.0 Hz, 1H), 3.05 - 2.97 (m, 6H). LCMS ($\text{M}+\text{Na}^+$): 449.1.

[0363] Step 3. For two batches: to a solution of compound 3 (65 g, 152.42 mmol) in pyridine (600 mL) was added Ac_2O (57.57 g, 563.95 mmol). Mixture was stirred at 20 °C for 12 hr. LCMS showed compound 3 was consumed completely and desired mass was detected. Two batches were combined together. Reaction mixture was diluted with NaHCO_3 (1000 mL) and extracted with EtOAc (500 mL *2). Combined organic layers were washed with brine 800 mL, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 0:1 to 1:1). Compound 4 (155 g, crude) was obtained as a yellow oil. ^1H NMR (400 MHz, CHCl_3 -d) δ = 7.42 - 7.33 (m, 5H), 6.18 (s, 1H), 5.38 (d, J = 4.8 Hz, 1H), 4.65 - 4.60 (m, 1H), 4.55 - 4.48 (m, 2H), 4.43 (d, J = 4.8 Hz, 1H), 4.31 - 4.28 (m, 1H), 4.23 - 4.17 (m, 2H), 3.02 (d, J = 2.0 Hz, 6H), 2.16 (s, 3H), 2.11 (s, 3H). LCMS ($\text{M}+\text{Na}^+$): 533.1. TLC: Petroleum ether: Ethyl acetate = 1:1, R_f = 0.5.

[0364] Step 4. For three batches: to a solution of compound 4 (50 g, 97.94 mmol) and uracil (21.95 g, 195.87 mmol) in ACN (700 mL) was added BSA (69.73 g, 342.78 mmol) at 80 °C for 1 hr. Then TMSOTf (54.42 g, 244.84 mmol) was added to the mixture. Mixture was stirred at 60 °C for 12 hr. LCMS showed compound 4 was consumed completely and desired mass was detected. Reaction mixture was cooled to 0 °C, and added to NaHCO_3 (800 mL). Three batches were combined together. The residue was extracted with EtOAc (500 mL * 3). Combined organic layers were washed with brine 500 mL, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. Residue was

purified by column chromatography (SiO₂, Petroleum ether: Ethyl acetate = 1:0 to 0:1). Compound 5 (78 g, 47.27% yield) and 78 g (crude) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.46 (br s, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.39 - 7.29 (m, 5H), 5.98 (d, J = 4.6 Hz, 1H), 5.68 (d, J = 8.0 Hz, 1H), 5.55 - 5.50 (m, 1H), 4.65 - 4.53 (m, 3H), 4.45 - 4.37 (m, 3H), 4.36 - 4.30 (m, 1H), 3.26 (s, 3H), 3.19 (s, 3H), 2.05 (s, 3H). LCMS (M+Na⁺): 585.1, purity: 92.5%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.5.

[0365] Step 5. For two batches: To a solution of compound 5 (41.5 g, 73.77 mmol) in dioxane (100 mL) and H₂O (100 mL) was added NaOH (2 M, 221.31 mL). Mixture was stirred at 20 °C for 12 hr. LCMS showed compound 5 was consumed completely and one main peak with desired mass was detected. Two batches were combined together. Reaction mixture was washed with 10% AcOH (300 mL). The residue was diluted with H₂O (500 mL) and extracted with DCM (300 mL * 3). Combined organic layers were washed with NaHCO₃ (200 mL) and brine (200 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Crude product was triturated with EtOAc at 20 °C for 5 min. The mixture was filtered and the cake was concentrated under reduced pressure to give product. Compound 6 (50 g, 79.85% yield) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.42 (br s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.37 - 7.25 (m, 5H), 5.60 (d, J = 8.0 Hz, 1H), 5.56 (s, 1H), 4.83 (d, J = 12.0 Hz, 1H), 4.63 (d, J = 3.0 Hz, 2H), 4.61 - 4.56 (m, 2H), 4.03 - 3.97 (m, 2H), 3.84 (d, J = 8.0 Hz, 1H), 3.27 (s, 3H). LCMS (M+H⁺): 425.0, purity: 92.4%. TLC: Petroleum ether: Ethyl acetate = 0:1 R_f = 0.4.

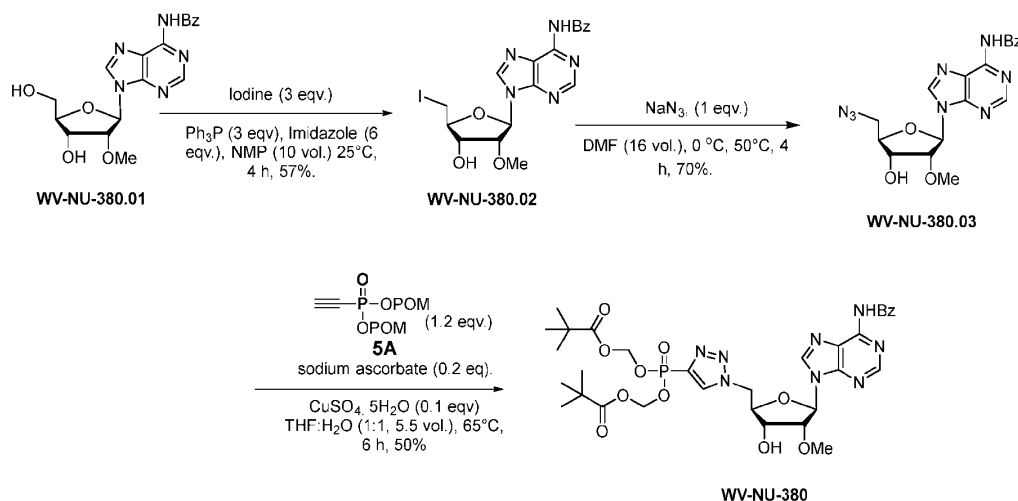
[0366] Step 6. To a solution of compound 6 (20 g, 47.12 mmol) in DMF (200 mL) was added NaN₃ (3.17 g, 48.76 mmol). Mixture was stirred at 60 °C for 12 hr. LCMS showed compound 6 was consumed completely and desired mass was detected. Reaction was quenched by H₂O (100 mL), and extracted with Ethyl acetate (200 mL*3). Combined organic was dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. Crude product was triturated with Petroleum ether: Ethyl acetate = 10:1, at 20 °C for 5 min. Then mixture was filtered, cake was washed with Petroleum ether, concentrated under reduced pressure to give product. Compound 7 (15.5 g, 88.58% yield) was obtained as a white solid. ¹H NMR (400 MHz, DMSO-d₆) δ = 11.42 (s, 1H), 7.60 (d, J = 8.2 Hz, 1H), 7.37 - 7.27 (m, 5H), 5.65 (d, J = 8.2 Hz, 1H), 5.54 (s, 1H), 4.66 - 4.57 (m, 2H), 4.54 (s, 1H), 4.01 (d, J = 14.0 Hz, 1H), 3.94 - 3.89 (m, 2H), 3.87 - 3.77 (m, 2H). LCMS (M+H⁺): 372.0.

[0367] Step 7. To a solution of compound 7 (10 g, 26.93 mmol) and compound 5A (10.80 g, 32.31 mmol) in THF (100 mL) and H₂O (100 mL) was added copper sulfate pentahydrate (8.07 g, 32.31 mmol) and sodium ascorbate. Mixture was stirred at 65 °C for 6 hr. TLC indicated compound 7 was consumed completely and one new spot formed. Reaction mixture was concentrated under reduced pressure to remove THF. Residue was diluted with H₂O (50 mL) and extracted with EtOAc (100 mL * 2).

Combined organic layers were dried over Na_2SO_4 , filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 1:0 to 0:1). Compound 8 (18 g, 94.72% yield) was obtained as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.39 (s, 1H), 8.78 (s, 1H), 7.36 (d, J = 4.2 Hz, 4H), 7.33 - 7.26 (m, 2H), 5.73 (s, 2H), 5.69 (s, 2H), 5.52 (br d, J = 8.0 Hz, 1H), 5.47 (s, 1H), 5.28 (br d, J = 15.0 Hz, 1H), 5.02 (br d, J = 15.0 Hz, 1H), 4.66 (s, 2H), 4.57 (s, 1H), 4.11 (d, J = 8.0 Hz, 1H), 3.58 (br d, J = 8.2 Hz, 2H), 1.10 (s, 18H). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 6.73 (s, 1P). LCMS ($\text{M}+\text{H}^+$): 706.3, purity: 94.7%. TLC: Petroleum ether: Ethyl acetate = 0:1, R_f = 0.3.

[0368] Step 8. For two batches: to a solution of compound 8 (9 g, 12.75 mmol) in AcOH (100 mL) was added Pd/C (1.36 g, 1.28 mmol, 10% purity). Mixture was stirred at 30 °C for 12 hr under H_2 (15 Psi). LCMS showed compound 8 was consumed completely and desired mass was detected. Reaction mixture was filtered and concentrated under reduced pressure to give a residue. Residue was purified by column chromatography (SiO_2 , Petroleum ether: Ethyl acetate = 1:0 to 0:1, Ethyl acetate: Methanol = 1:0 to 10:1). Compound WV-NU-349 (7.49 g, 49.93% yield) was obtained as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ = 11.38 (s, 1H), 8.78 (s, 1H), 7.29 (d, J = 8.0 Hz, 1H), 6.11 (br d, J = 4.0 Hz, 1H), 5.72 (br d, J = 13.8 Hz, 3H), 5.51 (d, J = 8.0 Hz, 1H), 5.39 (s, 1H), 5.22 (d, J = 15.0 Hz, 1H), 4.96 (d, J = 15.0 Hz, 1H), 4.25 (s, 1H), 4.07 (d, J = 8.0 Hz, 1H), 3.86 (d, J = 3.6 Hz, 1H), 3.50 (d, J = 8.0 Hz, 1H), 1.11 (s, 18H). ^{31}P NMR (162 MHz, DMSO-d_6) δ = 6.75 (s, 1P). LCMS ($\text{M}+\text{H}^+$): 616.2, purity: 98.88%. TLC: Ethyl acetate: Methanol = 10:1, R_f = 0.3.

[0369] Example 7. Synthesis of WV-NU-380.



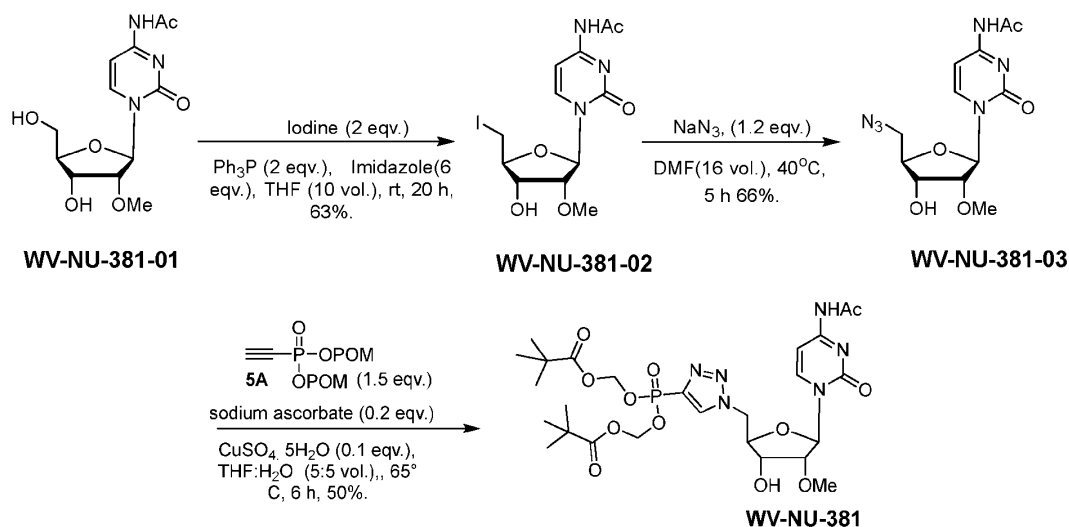
[0370] Step 1. To a stirred solution of WV-NU-380.01 (10 g, 0.0259 mol) in N-Methyl-2-pyrrolidone (100 mL, 10 vol.), was added iodine (19.7 g, 0.07793 mol), PPh₃ (20 g, 0.07792 mol.) and

imidazole (10.5 g 0.1558 mol.) at 25°C. Then resulting mixture was stirred at rt for 4 h. Progress of reaction was monitored by TLC. After completion, reaction was quenched with saturated sodium thiosulfate (100 mL), extracted with EtOAc (2 x 100 mL), washed with cold brine (2x80 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude material was purified by column chromatography over silica-gel (230-400 mesh) eluted in 4% MeOH in DCM to get an off white solid (WV-NU-380.02) (7.3 g, 57%), TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 11.26 (s, 1H), 8.79 (s, 1H), 8.75 (s, 1H), 8.05 (m, 2H), 7.66 (t, 1H, J1 = 7.4 Hz), 7.56 (m, 2H), 6.19 (d, 1H, J1 = 5.8 Hz), 5.64 (d, 1H, J1 = 5.6 Hz), 4.68 (t, 1H, J1 = 5.4 Hz), 4.43 (dd, 1H, J1 = 8.9 Hz, J2 = 5.2 Hz), 4.07 (m, 1H), 3.65 (dd, 1H, J1 = 10.5 Hz, J2 = 6.0 Hz), 3.52 (dd, 1H, J1 = 10.5 Hz, J2 = 6.9 Hz), 3.36 (s, 3H). MS: m/z calcd for C₁₈H₁₈IN₅O₄, 495.3; found 496.5, [M+H]⁺.

[0371] Step 2. To a stirred solution of (WV-NU-380.02) (18 g, 0.0363 mol) in dry DMF (288 mL, 16 vol.) was added sodium azide (2.36 g, 0.03634 mol) at 0°C. Resulting mixture was stirred at 50°C and kept for 4 h. Progress of the reaction was monitored by TLC. After that reaction was quenched with ice water (100 mL), extracted with EtOAc (3 x 80 mL), washed with cold brine (2 x 70 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude product was purified by column chromatography over silica-gel (230-400 mesh) eluted in 2% MeOH in DCM to get off white solid (WV-NU-380.03) (10.5 g, 70%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.24 (s, 1H), 8.79 (s, 1H), 8.74 (s, 1H), 8.05 (m, 2H, J1 = 7.6 Hz), 7.65 (t, 1H, J1 = 7.6 Hz), 7.56 (t, 2H, J1 = 7.6 Hz), 6.19 (d, 1H, J1 = 5.5 Hz), 5.53 (d, 1H, J1 = 5.5 Hz), 4.60 (t, 1H, J1 = 5.2 Hz), 4.44 (q, 1H, J1 = 5.0 Hz), 4.12 (m, 1H), 3.72 (q, 1H, J1 = 6.7 Hz), 3.61 (dd, 1H, J1 = 13.4 Hz, J2 = 3.8 Hz), 3.38 (s, 3H). MS: m/z calcd for C₁₈H₁₈N₈O₄, 410.4; found 411.6 [M+H]⁺.

[0372] Step 3. Compound (WV-NU-380.03) (10 g, 0.0243 mol) and compound 5A (12.2 g, 0.0243 mol) were dissolved in a mixture of solvent (THF: Water) (1:1) (50 mL, 5 vol.) and water (50 mL, 5 vol.). Then solution was degassed with nitrogen for 30 minutes at room temperature. After that sodium ascorbate (0.965 g, 0.00487 mol), CuSO₄·5H₂O (0.64 g, 0.00256 mol) was added to solution. Reaction mixture was allowed to 65°C for 6 h. Progress of the reaction was monitored by TLC. The reaction mass was diluted with EtOAc (80 mL), washed with water (2 x 30 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 2% MeOH in EtOAc to get light green solid (WV-NU-380) (9.2 g, 50%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.24 (s, 1H), 8.76 (d, 2H, J1 = 19.3 Hz), 8.67 (s, 1H), 8.05 (d, 2H, J1 = 7.6 Hz), 7.66 (t, 1H, J1 = 7.2 Hz), 7.56 (t, 2H, J1 = 7.6 Hz), 6.18 (d, 1H, J1 = 4.8 Hz), 5.66 (m, 5H), 4.92 (m, 2H), 4.55 (td, 2H, J1 = 9.6 Hz, J1 = 4.6 Hz), 4.39 (m, 1H), 3.39 (s, 3H), 1.02 (s, 18H). MS: m/z calcd for C₃₂H₄₁N₈O₁₁P, 744.7; found 745.9 [M+H]⁺.

[0373] Example 8. Synthesis of WV-NU-381.



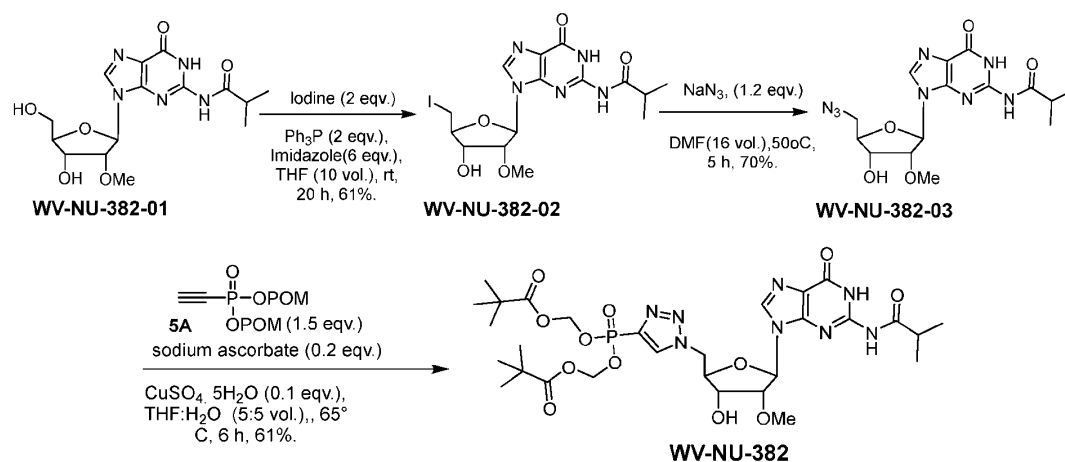
[0374] Step 1. To a stirred solution of WV-NU-380-01 (20 g, 0.0668 mol) in dry THF (300 mL, 15 vol.), was added iodine (33.8 g, 0.1337 mol), PPh₃ (35 g, 0.1337 mol.) and imidazole (27.2 g 0.4016 mol.) at 20°C. Then resulting mixture was stirred at rt for 20 h. Progress of the reaction was monitored by TLC. After completion, reaction was quenched with saturated sodium thiosulfate (200 mL), extracted with EtOAc (2 x 150 mL), washed with brine (1 x 100 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in DCM to get an off white solid (WV-NU-381-02) (17 g, 63%), TLC Mobile phase details: 5% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 10.96 (s, 1H), 8.11 (d, 1H, J1 = 7.6 Hz), 7.25 (s, 1H), 5.90 (d, 1H, J1 = 2.8 Hz), 3.91 (t, 3H, J1 = 3.1 Hz), 3.59 (dd, 1H, J1 = 10.3 Hz, J2 = 3.4 Hz), 3.49 (m, 1H), 3.11 (s, 3H), 2.11 (s, 3H). MS: m/z calcd for C₁₂H₁₆IN₃O₅, 409.18; found 410.1, [M+H]⁺.

[0375] Step 2. To a stirred solution of (WV-NU-381-02) (17 g, 0.0415 mol) in dry DMF (272 mL, 16 vol.) was added sodium azide (3.2 g, 0.0498 mol) at 0°C. Then resulting mixture was stirred at 40°C for 5 h. Progress of the reaction was monitored by TLC. Then reaction was quenched with ice water (150 mL), extracted with EtOAc (2 x 150 mL), washed with cold brine (2 X 100 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in DCM to get off white solid (WV-NU-381-03) (9 g, 66%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.94 (s, 1H), 8.11 (t, 1H, J1 = 7.2 Hz), 7.25 (q, 1H, J1 = 4.1 Hz), 5.88 (dd, 1H, J1 = 15.1 Hz, J2 = 2.8 Hz), 5.39 (dd, 1H, J1 = 41.0 Hz, J2 = 5.9 Hz), 3.98 (d, 2H, J1 = 2.8 Hz), 3.91 (t, 1H, J1 = 2.8 Hz), 3.83 (q, 1H, J1 = 2.5 Hz), 3.66 (m, 2H), 3.43 (d, 3H, J1 = 13.1 Hz), 2.11 (s, 3H). MS: m/z calcd for C₁₂H₁₆N₆O₅, 324.3;

found 325.36 [M+H]⁺.

[0376] Step 3. To a stirred solution of (WV-NU-381-03) (9 g, 0.0227 mol) and (WV-NU-380-B) (13.9 g 0.0416 mol) in mixture of solvent (1:1) THF (45 mL, 5 vol.), water (45 mL, 5 vol.) was degassed with nitrogen atmosphere for 25 minutes. Then added sodium ascorbate (0.1.09 g, 0.0055 mol), CuSO₄·5H₂O (0.69 g, 0.00277 mol) at room temperature. The reaction mixture was stirred at 65°C for 6 h. Progress of the reaction was monitored by TLC. Reaction mixture was cooled to room temperature and concentrated under reduced pressure. Crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in EtOAc to get light green solid (WV-NU-381) (9.2 g, 50%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 10.95 (s, 1H), 8.74 (s, 1H), 8.04 (d, 1H, J1 = 7.6 Hz), 7.26 (d, 1H, J1 = 6.9 Hz), 5.80 (d, 1H, J1 = 2.8 Hz), 5.70 (s, 2H), 5.67 (s, 2H), 5.51 (d, 1H, J1 = 6.9 Hz), 4.85 (m, 2H), 4.24 (m, 1H), 4.07 (d, 1H, J1 = 6.2 Hz), 3.88 (m, 1H, J1 = 3.4 Hz), 3.44 (s, 3H), 2.11 (s, 3H), 1.06 (s, 18H). MS: m/z calcd for C₂₆H₃₉N₆O₁₂P, 658.6; found 660.0 [M+H]⁺.

[0377] Example 9. Synthesis of WV-NU-382.



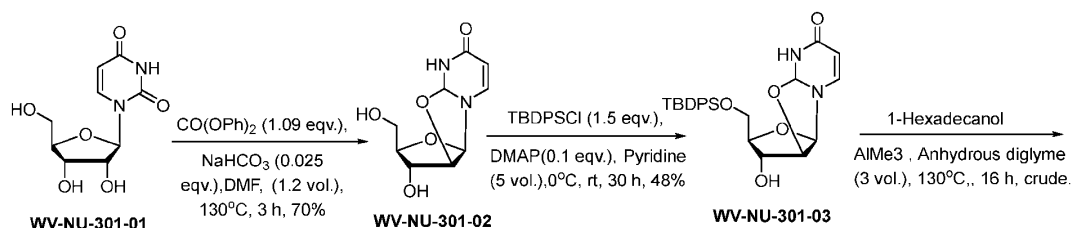
[0378] Step 1. To a stirred solution of WV-NU-380-01 (25 g, 0.0681 mol) in dry THF (375 mL, 15 vol.), was added iodine (34.4 g, 0.1362 mol), PPh₃ (35.6 g, 0.1362 mol.) and imidazole (27.8 g 0.4087 mol.) at 25°C. Then resulting mixture was stirred at rt for 20 h. Progress of the reaction was monitored by TLC. After completion, the reaction was quenched with saturated sodium thiosulfate (150 mL), extracted with EtOAc (2 x 150 mL), washed with brine (1 x 100 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 5% MeOH in DCM to get off white solid (WV-NU-382-02) (20 g, 61%), TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 12.08 (s, 1H), 11.64 (s, 1H), 8.29 (s, 1H), 5.93 (d, 1H, J1 = 6.2 Hz), 5.57 (d, 1H, J1 = 5.5 Hz), 4.47 (t, 1H, J1 = 5.9 Hz), 4.32 (q,

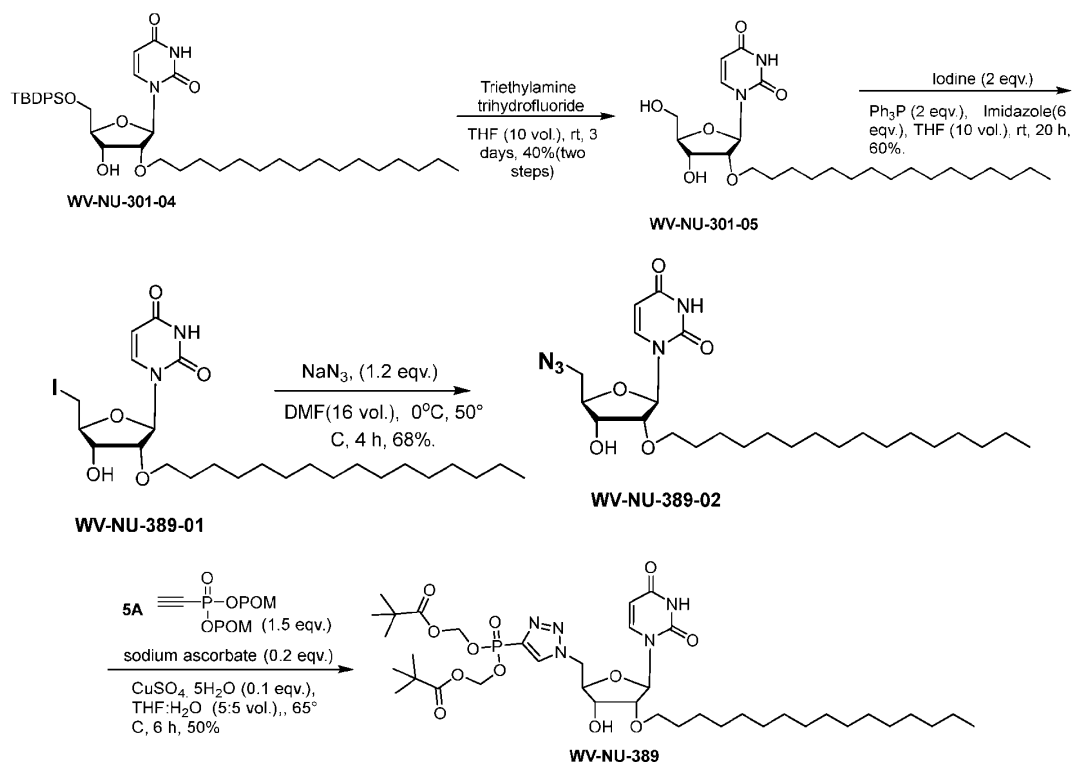
1H, J1 = 3.9 Hz), 4.02 (td, 1H, J1 = 6.7 Hz, J2 = 3.0 Hz), 3.58 (dd, 1H, J1 = 10.3 Hz, J2 = 6.9 Hz), 3.44 (dd, 1H, J1 = 10.3 Hz, J2 = 6.9 Hz), 3.34 (s, 3H), 2.76 (m, 1H), 1.13 (d, 6H, J1 = 6.2 Hz), MS: m/z calcd for C₁₅H₂₀N₅O₅, 477.26; found 478.48, [M+H]⁺.

[0379] Step 2. To a stirred solution of (WV-NU-380-02) (20 g, 0.0417 mol) in dry DMF (320 mL, 16 vol.) was added sodium azide (3.28 g, 0.0503 mol) at 0°C. Resulting mixture was stirred 50°C and kept for 5 h. Progress of the reaction was monitored by TLC. Then reaction was quenched with ice water (200 mL), extracted with EtOAc (2 x 100 mL), washed with cold brine (2 X 100 mL), dried over Na₂SO₄ and concentrated under reduced pressure. The crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in DCM to get off white solid (WV-NU-382-03) (11.5 g, 70%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 12.10 (s, 1H), 11.63 (s, 1H), 8.30 (s, 1H), 5.93 (d, 1H, J1 = 6.2 Hz), 5.47 (d, 1H, J1 = 5.1 Hz), 4.39 (dd, 1H, J1 = 6.0 Hz, J2 = 5.2 Hz), 4.30 (dd, 1H, J1 = 8.6 Hz, J2 = 4.9 Hz), 4.05 (m, 1H), 3.67 (q, 1H, J1 = 6.6 Hz), 3.58 (dd, 1H, J1 = 13.2 Hz, J2 = 4.1 Hz), 3.35 (s, 3H), 2.77 (m, 1H), 1.13 (dd, 6H, J1 = 6.9 Hz, J1 = 0.8 Hz), MS: m/z calcd for C₁₅H₂₀N₅O₅, 392.38; found 393.5, [M+H]⁺.

[0380] Step 3. To a stirred solution of (WV-NU-380-03) (11.5 g, 0.0293 mol) and (WV-NU-380-B) (14.6 g, 0.044 mol.) in mixture of solvent (1:1) THF (57.5 mL, 5 vol.), water (57.5 mL, 5 vol.) was degassed with nitrogen atmosphere for 35 minutes. Then added sodium ascorbate (1.11 g, 0.00561 mol), CuSO₄·5H₂O (0.73 g, 0.00293 mol) at rt. Reaction mixture was stirred at 65°C for 6 h. Progress of the reaction was monitored by TLC. Reaction mixture was cooled to rt and concentrated under reduced pressure. The crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in EtOAc to get light green solid (WV-NU-382) (13.1 g, 61%) TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 12.10 (s, 1H), 11.60 (s, 1H), 8.66 (s, 1H), 8.29 (s, 1H), 5.93 (d, 1H, J1 = 5.5 Hz), 5.65 (m, 5H), 4.84 (m, 2H), 4.46 (q, 1H, J1 = 4.1 Hz), 4.36 (t, 1H, J1 = 5.5 Hz), 4.32 (m, 1H), 3.36 (s, 3H), 2.75 (m, 1H), 1.11 (d, 6H, J1 = 6.9 Hz), 1.02 (s, 18H). MS: m/z calcd for C₂₉H₄₃N₈O₁₂P, 726.68; found 725.8 [M-H]⁺.

[0381] Example 10. Synthesis of WV-NU-389.





[0382] Step 1. To a stirred solution of Uridine (30 g, 0.122 mol) and diphenyl carbonate (26 g, 0.1229 mol.) in dry DMF (36 mL, 1.2 vol.) was added sodium bicarbonate (258 mg, 0.00307 mol) stirred at 130°C for 3 h. Progress of reaction was monitored by TLC. Then reaction mixture was cooled to rt, precipitated product was observed, filtered and washed with cold methanol (2 x 15 mL), dried under vacuum to get as off white solid (WV-NU-301-02) (21 g, 70%). TLC Mobile phase details: 15% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 7.83 (d, 1H, J1 = 6.9 Hz), 6.30 (d, 1H, J1 = 6.2 Hz), 5.88(s, 1H), 5.84 (d, 1H, J1 = 6.9 Hz), 5.19 (d, 1H, J1 = 5.5 Hz), 4.98 (t, 1H, J1 = 5.2 Hz), 4.38 (s, 1H), 4.07 (t, 1H, J1 = 4.5 Hz), 3.28 (m, 1H) 3.18 (m, 1H).

[0383] Step 2. To a stirred solution of (WV-NU-301-02) (40 g, 0.165 mol) and DMAP (2 g, 0.0165 mol.) in anhydrous pyridine (200 mL, 5 vol.) was added TBDPSCl (63 mL, 0.247 mol.) dropwise over a period of 30 mins, at 0°C. Above reaction mixture was stirred at rt for 30 h. Progress of the reaction was monitored by TLC. The reaction was diluted with cold sat. NaHCO₃ (150 mL) and extracted with DCM (2 x 200 mL), washed with brine (1 x 150 mL) solution, dried over Na₂SO₄ and concentrated under reduced pressure. The crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 4% MeOH in DCM to afford an off-white solid. (WV-NU-301-03) (38 g, 48%). TLC Mobile phase details: 10% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 7.92 (d, 1H, J1 = 7.6

Hz), 7.53 (m, 4H), 7.43 (m, 7H), 6.32 (d, 1H, J1 = 5.5 Hz), 6.02 (d, 1H, J1 = 4.8 Hz), 5.87 (d, 1H, J1 = 7.6 Hz), 5.26 (m, 1H), 4.45 (q, 1H, J1 = 2.8 Hz), 4.17 (m, 1H, Hz), 3.59 (q, 1H, J1 = 5.3 Hz), 3.47 (q, 1H, J1 = 6.0 Hz), 3.08 (d, 1H, J1 = 6.9 Hz), 0.92 (s, 9H).

[0384] Step 3. To a stirred solution of 1-Hexadecanol (130.6 g, 0.541 mol) in anhydrous diglyme (100 mL, 2.8 vol.) was added Trimethylaluminum (112.4 mL, 0.225 mol, 2M) dropwise over a period of 40 min. Resulting mixture was heated to 120°C and stirred for 2 h. Then mass was allowed to cool to room temperature and (WV-NU-301-03) (36 g, 0.075 mol) was added, stirred at 130°C for 16 h. Progress of the reaction was monitored by TLC. Reaction mixture was diluted 10% H₃PO₄ (500 mL) and EtOAc (300 mL), organic layer was separated washed with 5% NaCl (100 mL), dried over Na₂SO₄ and concentrated under vacuum to afford as a gummy syrup (64 g, crude). Syrup was dissolved in THF (360 mL, 10 vol.), was added triethylamine hydrofluoride (48 mL, 0.30 mol.), stirred rt for 3 days. Progress of reaction was monitored by TLC. Reaction was diluted 5% NaCl (250 mL) and EtOAc (300 mL), organic layer was separated washed with sat. NaCl solution (1 x 150 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in DCM to afford an off-white solid. (WV-NU-301-05) (14.3 g, 40%). TLC Mobile phase details: 10% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.33 (d, 1H, J1 = 1.4 Hz), 7.94 (d, 1H, J1 = 7.6 Hz), 5.84 (d, 1H, J1 = 4.8 Hz), 5.64 (dd, 1H, J1 = 7.9 Hz, J2 = 1.7 Hz), 5.13 (t, 1H, J1 = 5.2 Hz), 5.04 (d, 1H, J1 = 6.2 Hz), 4.09 (dt, 2H, J1 = 10.0 Hz, J1 = 4.8 Hz), 3.85 (t, 2H, J1 = 4.8 Hz), 3.63 (dq, 1H, J1 = 12.2 Hz, J2 = 2.8 Hz), 3.56 (m, 2H), 3.44 (m, 1H), 3.17 (d, 1H, J1 = 5.5 Hz), 1.47 (q, 2H, J1 = 6.7 Hz), 1.23 (s, 34H), 0.85 (t, 3H, J1 = 6.5 Hz), MS: m/z calcd for C₂₅H₄₄N₂O₆, 468.6; found 467.3 (M-H⁺).

[0385] Step 4. To a stirred solution of Uridine (30 g, 0.122 mol) and diphenyl carbonate (26 g, 0.1229 mol) in dry DMF (36 mL, 1.2 vol.) was added sodium bicarbonate (258 mg, 0.00307 mol) stirred at 130°C for 3 h. Progress of the reaction was monitored by TLC. Then reaction mixture was cooled to rt, precipitated product was observed, filtered and washed with cold methanol (2 x 15 mL), dried under vacuum to get as off white solid (WV-NU-301-02) (21 g, 70%). TLC Mobile phase details: 15% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 7.83 (d, 1H, J1 = 6.9 Hz), 6.30 (d, 1H, J1 = 6.2 Hz), 5.88 (s, 1H), 5.84 (d, 1H, J1 = 6.9 Hz), 5.19 (d, 1H, J1 = 5.5 Hz), 4.98 (t, 1H, J1 = 5.2 Hz), 4.38 (s, 1H), 4.07 (t, 1H, J1 = 4.5 Hz), 3.28 (m, 1H), 3.18 (m, 1H).

[0386] Step 5. To a stirred solution of (WV-NU-301-02) (40 g, 0.165 mol) and DMAP (2 g, 0.0165 mol) in anhydrous pyridine (200 mL, 5 vol.) was added TBDPSCI (63 mL, 0.247 mol) dropwise over a period of 30 mins, at 0°C. Above reaction mixture was stirred at rt for 30 h. Progress of the reaction was monitored by TLC. Reaction was diluted with cold sat. NaHCO₃ (150 mL) and extracted with DCM (2 x 200 mL), washed with brine (1 x 150 mL) solution, dried over Na₂SO₄ and concentrated under reduced

pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 4% MeOH in DCM to afford an off-white solid. (WV-NU-301-03) (38 g, 48%). TLC Mobile phase details: 10% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 7.92 (d, 1H, J₁ = 7.6 Hz), 7.53 (m, 4H), 7.43 (m, 7H), 6.32 (d, 1H, J₁ = 5.5 Hz), 6.02 (d, 1H, J₁ = 4.8 Hz), 5.87 (d, 1H, J₁ = 7.6 Hz), 5.26 (m, 1H), 4.45 (q, 1H, J₁ = 2.8 Hz), 4.17 (m, 1H, Hz), 3.59 (q, 1H, J₁ = 5.3 Hz), 3.47 (q, 1H, J₁ = 6.0 Hz), 3.08 (d, 1H, J₁ = 6.9 Hz), 0.92 (s, 9H).

[0387] Step 6. To a stirred solution of 1-Hexadecanol (130.6 g, 0.541 mol) in anhydrous diglyme (100 mL, 2.8 vol.) was added Trimethylaluminum (112.4 mL, 0.225 mol, 2M) dropwise over a period of 40 min. Resulting mixture was heated to 120°C and stirred for 2 h. Then mass was allowed to cool to room temperature and (WV-NU-301-03) (36 g, 0.075 mol) was added, stirred at 130°C for 16 h. Progress of the reaction was monitored by TLC. The reaction mixture was diluted 10% H₃PO₄ (500 mL) and EtOAc (300 mL), organic layer was separated washed with 5% NaCl (100 mL), dried over Na₂SO₄ and concentrated under vacuum to afford as a gummy syrup (64 g, crude). Syrup was dissolved in THF (360 mL, 10 vol.), was added triethylamine hydrofluoride (48 mL, 0.30 mol.), stirred rt for 3 days. Progress of the reaction was monitored by TLC. Reaction was diluted 5% NaCl (250 mL) and EtOAc (300 mL), organic layer was separated washed with sat. NaCl solution (1 x 150 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 3% MeOH in DCM to afford an off-white solid. (WV-NU-301-05) (14.3 g, 40%). TLC Mobile phase details: 10% MeOH in DCM. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.33 (d, 1H, J₁ = 1.4 Hz), 7.94 (d, 1H, J₁ = 7.6 Hz), 5.84 (d, 1H, J₁ = 4.8 Hz), 5.64 (dd, 1H, J₁ = 7.9 Hz, J₂ = 1.7 Hz), 5.13 (t, 1H, J₁ = 5.2 Hz), 5.04 (d, 1H, J₁ = 6.2 Hz), 4.09 (dt, 2H, J₁ = 10.0 Hz, J₁ = 4.8 Hz), 3.85 (t, 2H, J₁ = 4.8 Hz), 3.63 (dq, 1H, J₁ = 12.2 Hz, J₂ = 2.8 Hz), 3.56 (m, 2H), 3.44 (m, 1H), 3.17 (d, 1H, J₁ = 5.5 Hz), 1.47 (q, 2H, J₁ = 6.7 Hz), 1.23 (s, 34H), 0.85 (t, 3H, J₁ = 6.5 Hz), MS: m/z calcd for C₂₅H₄₄N₂O₆, 468.6; found 467.3 (M-H⁺).

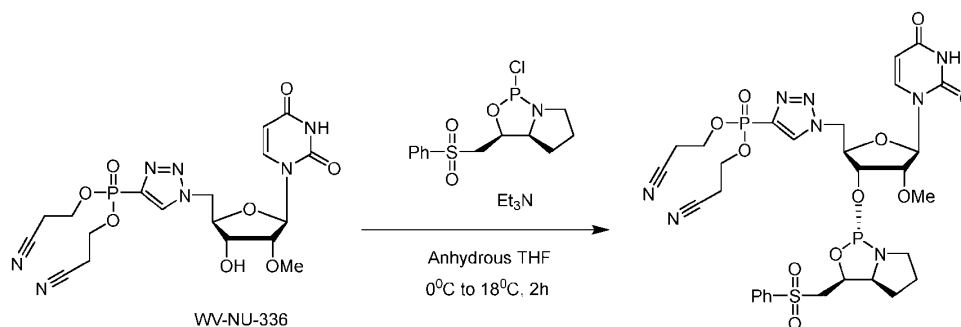
[0388] Step 7. To a stirred solution of WV-NU-301.05 (14 g, 0.0299 mol) in dry THF (140 mL, 10 vol.), was added iodine (15 g, 0.0598 mol), PPh₃ (15.6 g, 0.0598 mol.) and imidazole (12.2 g 0.178 mol.) at rt. Then resulting mixture was stirred at rt for 20 h. Progress of the reaction was monitored by TLC. After completion, the reaction was quenched with saturated sodium thiosulfate (120 mL), extracted with EtOAc (2 x 100 mL), washed with cold brine (1 x 100 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 4% MeOH in DCM to get off white solid (WV-NU-389-01) (10.5 g, 61%), TLC Mobile phase details: 7% MeOH in DCM. ¹H NMR (400 MHz, DMSO-d₆): δ in ppm = 11.39 (s, 1H), 7.69 (d, 1H, J₁ = 8.3 Hz), 5.86 (d, 1H, J₁ = 5.5 Hz), 5.68 (dd, 1H, J₁ = 5.3 Hz, J₁ = 2.1 Hz), 5.34 (d, 1H, J₁ = 6.2 Hz), 4.07 (m, 1H), 4.05 (t, 1H, J₁ = 5.5 Hz), 3.99 (q, 1H, J₁ = 5.5 Hz), 3.86 (q, 1H, J₁ = 5.5 Hz),

3.56 (m, 2H), 1.46 (q, 1H, J1 = 6.4 Hz), 1.26 (s, 28H), 0.85 (t, 3H, J1 = 6.9 Hz), . MS: m/z calcd for $C_{25}H_{43}IN_2O_5$, 578.53; found 577.53, [M-H]⁺.

[0389] Step 8. To a stirred solution of (WV-NU-389-01) (13 g, 0.0224 mol) in dry DMF (208 mL, 16 vol.) was added sodium azide (1.75 g, 0.0269 mol) at 0°C. Resulting mixture was stirred 50°C and kept for 4 h. Progress of the reaction was monitored by TLC. Then reaction was quenched with ice water (150 mL), extracted with EtOAc (2 x 150 mL), washed with cold brine (2 X 100 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Crude mass was purified by column chromatography over silica-gel (230-400 mesh) eluted in 40% EtOAc in Hexane to get off white solid (WV-NU-389-02) (7.5 g, 68%) TLC Mobile phase details: 60% EtOAc in Hexane. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.39 (s, 1H), 7.70 (d, 1H, J1 = 8.3 Hz), 5.82 (d, 1H, J1 = 4.8 Hz), 5.67 (d, 1H, J1 = 8.3 Hz), 5.25 (d, 1H, J1 = 6.2 Hz), 4.02 (q, 1H, J1 = 5.3 Hz), 3.98 (t, 1H, J1 = 5.2 Hz), 3.93 (q, 1H, J1 = 5.0 Hz), 3.58 (m, 1H), 3.44 (m, 1H), 1.48 (t, 2H, J1 = 6.5 Hz) 1.23 (s, 28H), 0.85 (t, 3H, J1 = 6.9 Hz). MS: m/z calcd for $C_{25}H_{43}N_5O_5$, 493.65; found 494.56 [M+H]⁺.

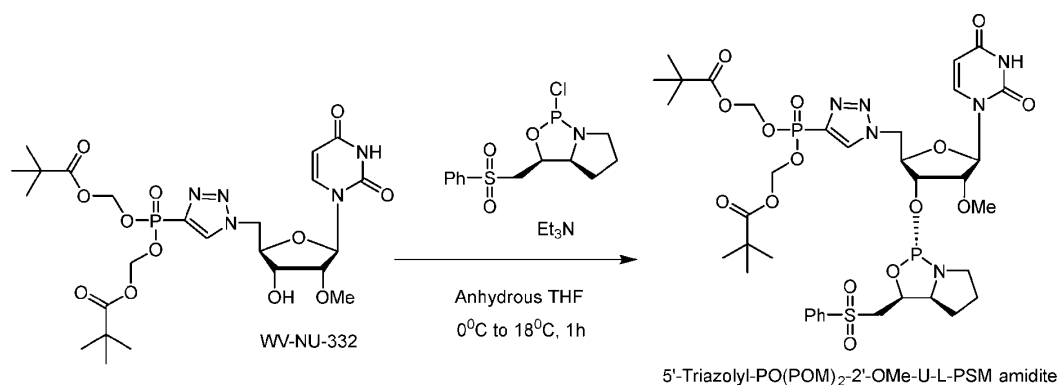
[0390] Step 9. To a stirred solution of (WV-NU-389-02) (11 g, 0.0223 mol) and (WV-NU-380-B) (11.17, 0.033 mol) in mixture of solvent (1:1) THF (55 mL, 5 vol.), water (55 mL, 5 vol.) was degassed with nitrogen atmosphere for 30 minutes. Then added sodium ascorbate (0.88 g, 0.00446 mol), $CuSO_4 \cdot 5H_2O$ (0.55 g, 0.00223 mol) at rt. Reaction mixture was stirred at 65°C for 6 h. Progress of the reaction was monitored by TLC. The reaction mass was diluted EtOAc (100 mL), washed with water (2 x 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Crude compound was purified by column chromatography over silica-gel (230-400 mesh) eluted in 60% EtOAc in Hexane to get gummy syrup (WV-NU-389) (10.5 g, 56%) TLC Mobile phase details: 70% EtOAc in Hexane. ¹H NMR (500 MHz, DMSO-d₆): δ in ppm = 11.40 (d, 1H, J1 = 2.1 Hz), 8.69 (s, 1H), 7.64 (d, 1H, J1 = 8.3 Hz), 5.79 (d, 1H, J1 = 4.8 Hz), 5.67 (m, 4H), 5.40 (d, 1H, J1 = 5.5 Hz), 4.79 (m, 2H), 4.19 (m, 1H), 4.00 (t, 1H, J1 = 5.2 Hz), 3.58 (m, 1H), 3.45 (m, 1H), 1.48 (t, 2H, J1 = 6.5 Hz), 1.23 (s, 28H), 1.07 (s, 18H), 0.85 (t, 3H, J1 = 6.9 Hz). MS: m/z calcd for $C_{39}H_{66}N_5O_{12}P$, 827.95; found 829.00 [M+H]⁺.

[0391] Example 11. Synthesis of 5'-Triazolyl-PO(CNE)₂-2'OMe-U-L-PSM amidite.



[0392] Step 1. WV-NU-336 (1.0 eq., 2.56 g) in an appropriate size three necked flask was azeotroped with anhydrous toluene (15 mL/g) and anhydrous acetonitrile (15 mL/g) and was dried for 24 hours on high vacuum. To the flask was added anhydrous THF (0.2 M solution) under argon and solution was cooled to 0°C. To the reaction mixture was added triethyl amine (2.2-3.0 eq.) followed by addition of L-PSM-Cl (0.86-0.94 M) solution (1.6-2.2 eq.) over the period of 5-10 min. The reaction mixture was warmed to room temperature and reaction progress was monitored by HPLC. After disappearance of starting material, the reaction mixture was filtered through fritted glass tube. Reaction flask and precipitate was washed with anhydrous THF. Obtained filtrate was collected and solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, 40-80% Ethyl acetate in Hexanes with 1.25% triethyl amine and 20-100% Acetonitrile in Ethyl acetate with 1.25% triethyl amine) to give 5'-Triazolyl-PO(CNE)₂-2'-OMe-U-L-PSM amidite as off-white solid (1.99 g, 50.4% yield). LCMS: C₃₀H₃₆N₈O₁₁P₂S (M-H⁻): 777.29. ¹H NMR (600 MHz, CDCl₃) δ 9.04 (s, 1H), 8.42 (s, 1H), 7.98 – 7.93 (m, 2H), 7.70 – 7.65 (m, 1H), 7.59 (t, J = 7.8 Hz, 2H), 7.07 (d, J = 8.1 Hz, 1H), 5.71 (d, J = 8.0 Hz, 1H), 5.38 (d, J = 3.1 Hz, 1H), 5.26 (ddd, J = 8.2, 5.8, 4.1 Hz, 1H), 4.85 (qd, J = 14.8, 4.2 Hz, 2H), 4.50 – 4.39 (m, 5H), 4.34 (ddd, J = 7.4, 5.0, 3.3 Hz, 1H), 4.17 (dd, J = 5.8, 3.2 Hz, 1H), 3.76 – 3.69 (m, 1H), 3.57 (dddd, J = 33.6, 15.3, 9.3, 5.1 Hz, 2H), 3.49 (s, 3H), 3.41 (dd, J = 14.4, 4.0 Hz, 1H), 3.33 – 3.26 (m, 1H), 2.88 – 2.77 (m, 4H), 1.97 – 1.82 (m, 2H), 1.68 – 1.61 (m, 1H), 1.20 – 1.10 (m, 1H). ³¹P NMR (243 MHz, CDCl₃) δ 154.25, 8.01. ¹³C NMR (151 MHz, CDCl₃) δ 163.07, 149.81, 142.90, 139.87, 134.31, 133.56, 133.33, 129.65, 128.20, 116.84, 116.76, 103.11, 94.59, 81.01, 80.99, 80.36, 74.58, 74.51, 70.48, 70.41, 66.58, 66.56, 61.83, 61.80, 61.69, 61.66, 59.34, 58.12, 58.10, 50.01, 46.66, 46.42, 27.51, 26.21, 26.19, 20.04, 20.03, 20.00, 19.98, 14.37.

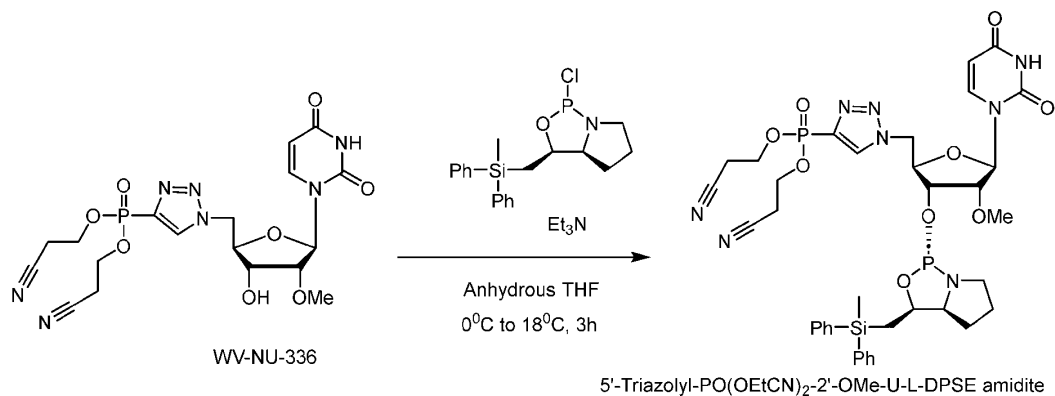
[0393] Example 12. Synthesis of 5'-Triazolyl-PO(POM)₂-2'-OMe-U-L-PSM amidite.



[0394] Step 1. WV-NU-332 (1.0 eq., 2.26 g) in an appropriate size three necked flask was azeotroped with anhydrous toluene (15 mL/g) and anhydrous acetonitrile (15 mL/g) and was dried for 24 hours on high vacuum. To the flask was added anhydrous THF (0.2 M solution) under argon and solution

was cooled to 0°C. To the reaction mixture was added triethyl amine (2.2-3.0 eq.) followed by addition of L-PSM-Cl (0.86-0.94 M) solution (1.6-2.2 eq.) over the period of 5-10 min. The reaction mixture was warmed to room temperature and reaction progress was monitored by HPLC. After disappearance of starting material, the reaction mixture was filtered through fritted glass tube. Reaction flask and precipitate was washed with anhydrous THF. Obtained filtrate was collected and solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, 40-80% Ethyl acetate in Hexanes with 1.25% triethyl amine and 20-100% Acetonitrile in Ethyl acetate with 1.25% triethyl amine) to give 5'-Triazolyl-PO(POM)₂-2'-OMe-U-L-PSM-amidite as off-white solid (2.03g, 61.9% yield). LCMS: C₃₆H₅₀N₆O₁₅P₂S (M-H⁻): 899.27. ¹H NMR (600 MHz, CDCl₃) δ 8.71 (s, 1H), 8.19 (s, 1H), 7.98 – 7.95 (m, 2H), 7.68 – 7.64 (m, 1H), 7.60 – 7.56 (m, 2H), 7.02 (d, J = 8.1 Hz, 1H), 5.82 – 5.76 (m, 4H), 5.73 (d, J = 8.0 Hz, 1H), 5.42 (d, J = 3.3 Hz, 1H), 5.24 (ddd, J = 8.2, 5.9, 4.1 Hz, 1H), 4.79 (qd, J = 14.8, 4.2 Hz, 2H), 4.51 (ddd, J = 9.3, 7.0, 5.9 Hz, 1H), 4.29 (ddd, J = 7.0, 4.9, 3.3 Hz, 1H), 4.06 (dd, J = 5.9, 3.3 Hz, 1H), 3.74 (dq, J = 9.8, 6.0 Hz, 1H), 3.61 – 3.51 (m, 2H), 3.48 (s, 3H), 3.39 (dd, J = 14.5, 4.1 Hz, 1H), 3.28 (tdd, J = 10.2, 8.7, 4.2 Hz, 1H), 1.96 – 1.81 (m, 2H), 1.66 (q, J = 6.7 Hz, 1H), 1.18 (d, J = 5.3 Hz, 18H). ³¹P NMR (243 MHz, CDCl₃) δ 153.73, 6.16. ¹³C NMR (151 MHz, CDCl₃) δ 162.62, 149.72, 142.32, 139.89, 134.25, 133.01, 132.77, 129.60, 128.23, 103.34, 93.99, 82.29, 82.27, 82.24, 81.23, 81.21, 80.43, 74.73, 74.66, 70.49, 70.43, 66.51, 66.49, 59.32, 58.19, 58.16, 50.05, 46.62, 46.39, 38.89, 27.50, 26.96, 26.94, 26.22, 26.20.

[0395] Example 13. Synthesis of 5'-Triazolyl-PO(CNE)₂-2'-OMe-U-L-DPSE amidite.



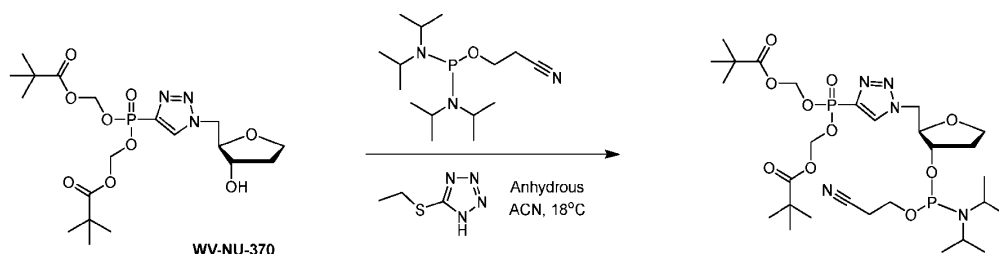
[0396] WV-NU-336 (1.0 eq., 1.32 g) in an appropriate size three necked flask was azeotroped with anhydrous toluene (15 mL/g) and anhydrous acetonitrile (15 mL/g) and was dried for 24 hours on high vacuum. To the flask was added anhydrous THF (0.1-0.2 M solution) under argon and solution was cooled to 0°C. To the reaction mixture was added triethyl amine (2.2 eq.) followed by addition of L-DPSE-Cl (0.87 M) solution (1.6 eq.) over the period of 5-10 min. The reaction mixture was warmed to room temperature and reaction progress was monitored by HPLC. After disappearance of starting

material, the reaction mixture was filtered through fritted glass tube. Reaction flask and precipitate was washed with anhydrous THF. Obtained filtrate was collected and solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, 40-80% Ethyl acetate in Hexanes and 20-100% Acetonitrile in Ethyl acetate) to give 5'-Triazolyl-PO(CNE)₂-2'OMe-U-L-DPSE-amidite as off-white solid (795 mg, 38.4% yield). LCMS: C₃₇H₄₄N₈O₉P₂Si (M-H): 833.12. ¹H NMR (600 MHz, CDCl₃) δ 8.56 (s, 1H), 8.19 (s, 1H), 7.56 – 7.49 (m, 5H), 7.36 – 7.30 (m, 7H), 6.93 (d, J = 8.1 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 5.22 (d, J = 3.2 Hz, 1H), 4.92 (dt, J = 8.5, 6.0 Hz, 1H), 4.63 – 4.50 (m, 2H), 4.46 – 4.39 (m, 5H), 4.15 (dd, J = 6.9, 3.8 Hz, 1H), 3.91 (dd, J = 5.7, 3.2 Hz, 1H), 3.61 (ddt, J = 14.4, 10.6, 7.6 Hz, 1H), 3.50 (dq, J = 9.4, 5.9 Hz, 1H), 3.38 (s, 3H), 3.22 (dtd, J = 10.6, 8.8, 7.6, 4.5 Hz, 1H), 2.87 – 2.78 (m, 4H), 1.90 (qd, J = 8.9, 4.6 Hz, 1H), 1.76 (q, J = 10.9, 10.0 Hz, 1H), 1.66 (dd, J = 14.7, 8.4 Hz, 1H), 1.48 (td, J = 13.2, 11.5, 5.0 Hz, 2H), 0.68 (s, 3H). ³¹P NMR (243 MHz, CDCl₃) δ 152.57, 8.14. ¹³C NMR (151 MHz, CDCl₃) δ 143.16, 136.75, 136.11, 134.74, 134.72, 134.51, 133.11, 132.89, 129.62, 129.57, 128.12, 128.08, 116.68, 116.64, 102.94, 95.21, 80.78, 80.76, 80.55, 79.79, 79.72, 70.44, 68.06, 68.04, 61.71, 61.68, 61.63, 61.60, 59.17, 50.21, 46.88, 27.41, 26.09, 26.07, 20.04, 20.02, 19.99, 19.97, 17.90, - 3.20.

[0397] Example 14. Exemplary synthesis procedure for cyanoethyl (CNE) amidites.

[0398] Nucleosides (1.0 eq.) in an appropriate size three necked flask was azeotroped with anhydrous toluene (15 mL/g) and anhydrous acetonitrile (15 mL/g) and was dried for 24 hours on high vacuum. To the flask was added anhydrous acetonitrile (0.1 M solution) under argon at room temperature. To reaction mixture was added 5-ethylsulfanyl-1H-tetrazole (1.0 eq.) followed by dropwise addition of 3-bis(diisopropylamino)phosphanyloxypropanenitrile (1.2-1.5 eq.) over the period of 5-10 min. Reaction progress was monitored by HPLC. After disappearance of starting material, the reaction mixture was filtered through fritted glass tube. Reaction flask and precipitate was washed with anhydrous acetonitrile. Obtained filtrate was collected and solvent was removed under reduced pressure. The residue was purified by column chromatography (SiO₂, 0-100% Ethyl acetate in Hexanes with 5% triethyl amine) to give NU-CNE Amidite as off-white solid.

[0399] Example 15. Preparation of 5'-Triazolyl-PO(POM)₂-abasic-CNE amidite.



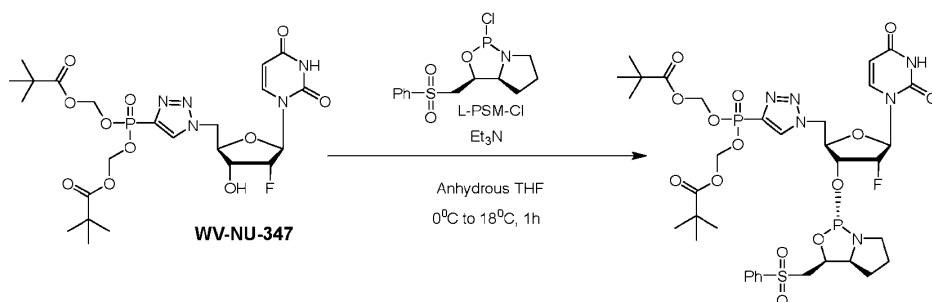
[0400] Nucleoside 5'-Triazolyl-PO(POM)₂-abasic (WV-NU-370, 1.82 g) was converted to 5'-

Triazolyl-PO(POM)₂-abasic-CNE-amidite (1.27 g, 49.2% yield) as an off-white solid as shown in the scheme above and the protocol in Example 14. LCMS: C₄₉H₅₆N₇O₈P (M+Na⁺): 700.24. ¹H NMR (600 MHz, CDCl₃) δ 8.19 (d, J = 5.4 Hz, 1H), 5.77 (tdd, J = 14.1, 7.5, 4.0 Hz, 4H), 4.68 (ddd, J = 21.0, 14.2, 3.3 Hz, 1H), 4.24 (dtt, J = 11.0, 7.4, 4.0 Hz, 1H), 4.10 (dtt, J = 15.0, 7.1, 3.7 Hz, 1H), 3.96 – 3.81 (m, 3H), 3.76 – 3.69 (m, 1H), 3.59 (dh, J = 10.3, 6.7 Hz, 2H), 2.69 (td, J = 6.2, 3.4 Hz, 1H), 2.63 (t, J = 6.3 Hz, 1H), 2.06 – 1.91 (m, 1H), 1.84 (dtd, J = 20.3, 7.8, 7.4, 6.0 Hz, 1H), 1.25 – 1.07 (m, 31H). ³¹P NMR (243 MHz, CDCl₃) δ 148.72, 148.53, 6.39 (d, J = 16.8 Hz). ¹³C NMR (151 MHz, CDCl₃) δ 176.77, 132.36, 132.12, 82.99, 82.97, 82.80, 82.14, 82.11, 75.30, 75.20, 74.97, 74.87, 67.68, 67.59, 58.33, 58.21, 58.09, 52.18, 51.94, 43.48, 43.43, 43.40, 43.35, 38.81, 34.27, 34.24, 26.91, 26.88, 24.76, 24.74, 24.71, 24.69, 24.62, 24.57, 20.57, 20.52.=.

[0401] Example 16. Exemplary synthesis procedure for L-PSM and D-PSM amidites.

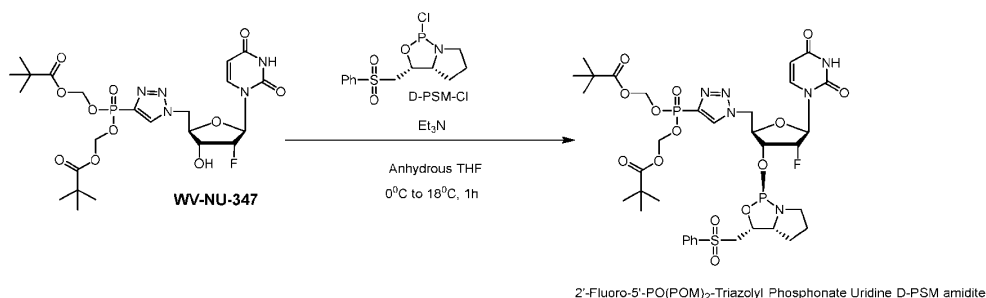
[0402] Nucleosides (1.0 eq.) in an appropriate size three necked flask were azeotroped with anhydrous toluene (15 mL/g) and anhydrous acetonitrile (15 mL/g) and were dried for 24 hours on high vacuum. To the flask was added anhydrous THF (0.2 M solution) under argon and solution were cooled to 0°C. To the reaction mixture was added triethyl amine (2.2 eq.) followed by addition of L-PSM-Cl or D-PSM-Cl (0.86-0.94 M) solution (1.6 eq.) over a period of 5-10 min. Reaction mixture was warmed to room temperature and reaction progress was monitored by HPLC. After disappearance of starting material, reaction mixture was filtered through fritted glass tube. Reaction flask and precipitate was washed with anhydrous THF. Obtained filtrate was collected and solvent was removed under reduced pressure. Residue was purified by column chromatography (SiO₂, 40-80% Ethyl acetate in Hexanes with 1.25% triethyl amine and 20-100% Acetonitrile in Ethyl acetate with 1.25% triethyl amine) to give to give 5'-Triazolyl-Phosphonate amidite as off-white solid.

[0403] Example 17. Preparation of 2'-Fluoro-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite.



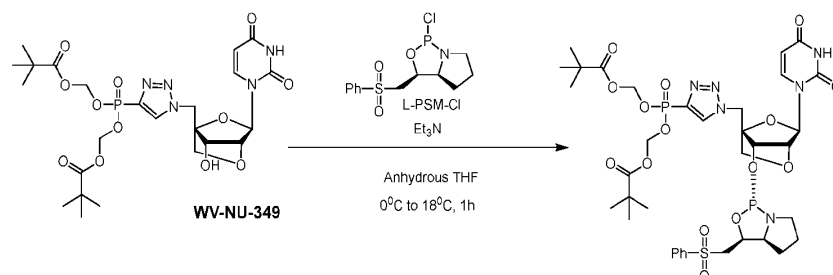
[0405] LCMS: $C_{35}H_{47}N_6O_{14}P_2S$ (M-H⁻): 887.05. ¹H-NMR (600 MHz, CDCl₃) δ 8.85 (s, 1H), 8.10 (s, 1H), 7.98 – 7.94 (m, 2H), 7.67 – 7.63 (m, 1H), 7.57 (dd, J = 8.4, 7.2 Hz, 2H), 7.10 (d, J = 8.1 Hz, 1H), 5.85 – 5.70 (m, 5H), 5.36 – 5.29 (m, 2H), 5.24 (dq, J = 6.0, 4.1 Hz, 1H), 4.88 (dd, J = 15.0, 3.9 Hz, 1H), 4.75 (dd, J = 15.0, 2.9 Hz, 1H), 4.66 (dtd, J = 19.1, 8.7, 5.2 Hz, 1H), 4.27 (dt, J = 8.7, 3.4 Hz, 1H), 3.75 (dq, J = 9.8, 6.0 Hz, 1H), 3.62 – 3.49 (m, 2H), 3.39 (dd, J = 14.6, 4.1 Hz, 1H), 3.31 (tdd, J = 10.3, 8.6, 4.6 Hz, 1H), 1.90 (pq, J = 13.1, 7.5, 5.8 Hz, 2H), 1.70 – 1.64 (m, 1H), 1.20 (d, J = 7.8 Hz, 18H). ³¹P-NMR (243 MHz, CDCl₃) δ 151.40 (d, J = 4.9 Hz), 6.18. ¹³C-NMR (151 MHz, CDCl₃) δ 177.31, 162.45, 149.46, 143.24, 139.90, 134.17, 133.01, 129.51, 128.42, 103.64, 95.55, 95.29, 91.40, 82.34, 82.31, 79.53, 75.11, 75.04, 66.50, 66.48, 58.34, 49.18, 46.53, 46.29, 38.93, 38.92, 27.58, 26.97, 26.95, 26.25. ¹⁹F NMR (565 MHz, CDCl₃) δ -190.96 (d, J = 4.9 Hz).

[0406] Example 18. Preparation of 2'-Fluoro-5'-PO(POM)₂-Triazolyl Phosphonate Uridine D-PSM amidite.



[0407] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-Fd-U (WV-NU-347, 4.92 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-Fd-U-D-PSM-amidite by general procedure (4.56 g, 64.8% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: $C_{35}H_{47}N_6O_{14}P_2S$ (M-H⁻): 887.14. ¹H-NMR (600 MHz, CDCl₃) δ 9.02 (s, 1H), 8.08 (s, 1H), 7.95 – 7.92 (m, 2H), 7.67 – 7.63 (m, 1H), 7.56 (t, J = 7.8 Hz, 2H), 7.09 (d, J = 8.0 Hz, 1H), 5.86 – 5.69 (m, 5H), 5.36 – 5.17 (m, 3H), 4.82 – 4.74 (m, 2H), 4.62 (dtd, J = 19.0, 8.4, 5.2 Hz, 1H), 4.29 (ddd, J = 8.2, 4.6, 3.1 Hz, 1H), 3.75 (dq, J = 9.9, 6.0 Hz, 1H), 3.59 (ddt, J = 14.9, 10.5, 7.7 Hz, 1H), 3.51 (dd, J = 14.6, 7.7 Hz, 1H), 3.40 (dd, J = 14.6, 4.7 Hz, 1H), 3.29 (tdd, J = 10.3, 8.9, 4.0 Hz, 1H), 1.96 – 1.78 (m, 2H), 1.66 (qd, J = 8.0, 2.6 Hz, 1H), 1.20 (s, 9H), 1.18 (s, 9H). ³¹P-NMR (243 MHz, CDCl₃) δ 155.86 (d, J = 5.7 Hz), 6.09. ¹³C-NMR (151 MHz, CDCl₃) δ 177.30, 177.16, 162.61, 149.47, 143.35, 139.72, 134.17, 132.91, 129.50, 128.35, 103.57, 95.77, 95.51, 92.80, 91.55, 82.36, 82.33, 82.31, 82.28, 79.58, 79.54, 74.30, 74.24, 69.42, 66.31, 66.29, 60.56, 58.21, 58.19, 49.65, 46.65, 38.92, 38.88, 27.56, 26.96, 26.93, 26.25, 26.23, 21.22, 14.37. ¹⁹F NMR (565 MHz, CDCl₃) -190.60 (d, J = 5.7 Hz).

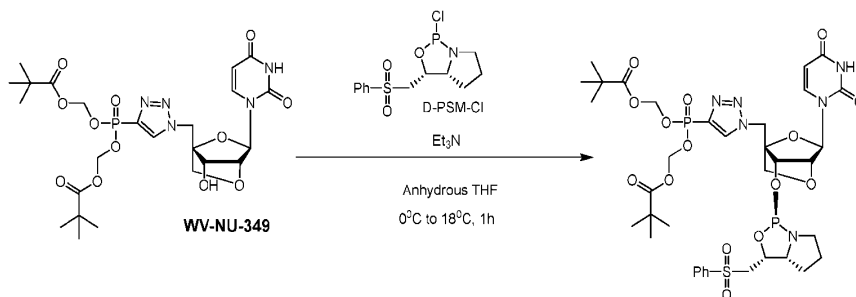
[0408] Example 19. Preparation of 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite.



[0409] Nucleoside 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine (WV-NU-349, 1.99 g) was converted to 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite (1.99 g, 72.5% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS:

C₃₆H₄₈N₆O₁₅P₂S (M-H⁻): 897.09. ¹H-NMR (600 MHz, CDCl₃) δ 8.46 (s, 1H), 8.38 (s, 1H), 7.95 – 7.92 (m, 2H), 7.70 – 7.66 (m, 1H), 7.62 – 7.56 (m, 2H), 7.10 (d, J = 8.2 Hz, 1H), 5.85 – 5.76 (m, 4H), 5.71 (d, J = 8.2 Hz, 1H), 5.62 (s, 1H), 5.30 – 5.24 (m, 1H), 5.13 (d, J = 15.3 Hz, 1H), 4.91 (d, J = 15.3 Hz, 1H), 4.59 (s, 1H), 4.17 (dd, J = 14.8, 7.8 Hz, 2H), 3.90 (d, J = 8.3 Hz, 1H), 3.73 – 3.65 (m, 1H), 3.54 (dddd, J = 21.7, 14.3, 8.3, 3.3 Hz, 2H), 3.36 (dd, J = 14.3, 3.1 Hz, 1H), 3.26 (tdd, J = 10.4, 8.9, 3.9 Hz, 1H), 1.96 – 1.78 (m, 2H), 1.59 (dddd, J = 9.4, 7.4, 5.4, 2.4 Hz, 1H), 1.18 (d, J = 6.2 Hz, 19H). ³¹P-NMR (243 MHz, CDCl₃) δ 158.14, 5.44. ¹³C-NMR (151 MHz, CDCl₃) δ 176.82, 176.80, 162.85, 149.77, 139.71, 138.38, 134.38, 133.33, 133.10, 129.71, 128.03, 102.53, 87.84, 86.20, 82.24, 82.22, 82.20, 79.50, 79.46, 74.00, 73.94, 72.36, 72.25, 72.17, 66.44, 57.66, 57.64, 46.76, 46.54, 38.84, 27.25, 26.94, 26.92, 26.11, 26.09.

[0410] Example 20. Preparation of 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine D-PSM amidite.

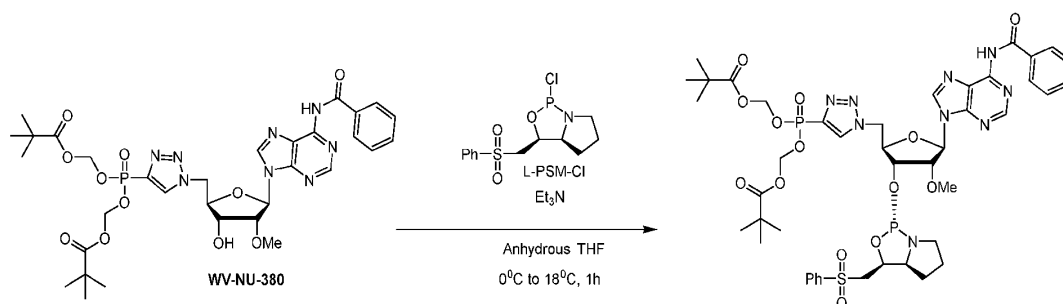


[0411] Nucleoside of 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine (WV-NU-349, 2.01 g) was converted to of 2'-LNA-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite (1.89 g, 68.1% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS:

C₃₆H₄₈N₆O₁₅P₂S (M-H⁻): 897.09. ¹H-NMR (600 MHz, CDCl₃) δ 8.45 (s, 1H), 8.24 (s, 1H), 7.97 – 7.94 (m, 2H), 7.70 – 7.66 (m, 1H), 7.59 (t, J = 7.8 Hz, 2H), 6.78 (d, J = 8.2 Hz, 1H), 5.85 – 5.75 (m, 4H), 5.69 (d, J = 8.2 Hz, 1H), 5.62 (s, 1H), 5.14 – 5.09 (m, 1H), 4.90 (s, 2H), 4.58 (s, 1H), 4.14 – 4.07 (m, 2H), 4.03

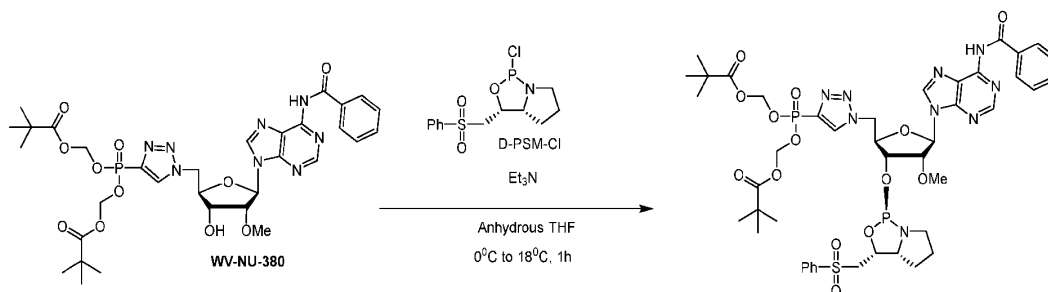
(d, $J = 5.8$ Hz, 1H), 3.77 – 3.68 (m, 2H), 3.58 – 3.49 (m, 2H), 3.39 (dd, $J = 14.6, 4.6$ Hz, 1H), 3.25 (tdd, $J = 10.2, 8.8, 3.9$ Hz, 1H), 1.96 – 1.79 (m, 2H), 1.72 – 1.64 (m, 1H), 1.18 (d, $J = 2.5$ Hz, 19H). ^{31}P -NMR (243 MHz, CDCl_3) δ 156.55, 5.24. ^{13}C -NMR (151 MHz, CDCl_3) δ 176.89, 162.40, 149.51, 139.47, 137.91, 134.33, 132.69, 132.46, 129.61, 128.37, 102.75, 88.26, 86.17, 82.25, 82.21, 82.18, 78.99, 74.56, 72.14, 72.09, 72.05, 66.38, 66.35, 58.01, 57.98, 47.13, 46.94, 46.71, 38.88, 27.50, 26.96, 26.20, 26.17, 14.37.

[0412] Example 21. Preparation of 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz)-L-PSM amidite.



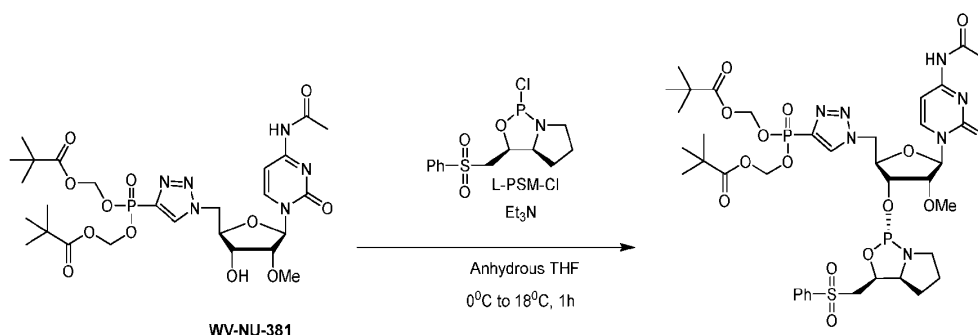
[0413] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz) (WV-NU-380, 5.1 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz)-L-PSM-amidite (5.73 g, 82.1% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: $\text{C}_{36}\text{H}_{48}\text{N}_6\text{O}_{15}\text{P}_2\text{S}$ (M-H⁻): 1026.46. ^1H -NMR (600 MHz, CDCl_3) δ 9.09 (s, 1H), 8.73 (s, 1H), 8.08 (s, 1H), 8.06 – 8.03 (m, 2H), 8.00 (s, 1H), 7.93 – 7.90 (m, 2H), 7.65 – 7.59 (m, 2H), 7.53 (dt, $J = 12.3, 7.7$ Hz, 4H), 5.97 (d, $J = 4.1$ Hz, 1H), 5.79 – 5.70 (m, 4H), 5.31 (ddd, $J = 8.0, 5.9, 4.2$ Hz, 1H), 5.01 (dt, $J = 9.2, 5.6$ Hz, 1H), 4.92 – 4.84 (m, 2H), 4.61 – 4.56 (m, 1H), 4.45 (td, $J = 5.8, 3.9$ Hz, 1H), 3.82 – 3.77 (m, 1H), 3.65 – 3.50 (m, 2H), 3.47 (s, 3H), 3.39 (dd, $J = 14.5, 4.2$ Hz, 1H), 3.28 (tdd, $J = 10.4, 8.8, 4.2$ Hz, 1H), 1.97 – 1.82 (m, 2H), 1.72 – 1.65 (m, 1H), 1.13 (d, $J = 1.9$ Hz, 19H). ^{31}P -NMR (243 MHz, CDCl_3) δ 153.92, 6.09. ^{13}C -NMR (151 MHz, CDCl_3) δ 176.82, 164.72, 152.84, 151.27, 150.16, 142.80, 139.72, 134.22, 132.98, 132.42, 129.55, 129.02, 128.16, 128.09, 124.44, 88.89, 82.18, 82.14, 82.10, 81.48, 81.26, 74.78, 74.72, 71.08, 66.62, 66.60, 60.52, 59.31, 58.14, 58.12, 50.82, 46.65, 46.42, 38.80, 27.52, 26.88, 26.18, 26.16, 14.34.

[0414] Example 22. Preparation of 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz)-D-PSM amidite.



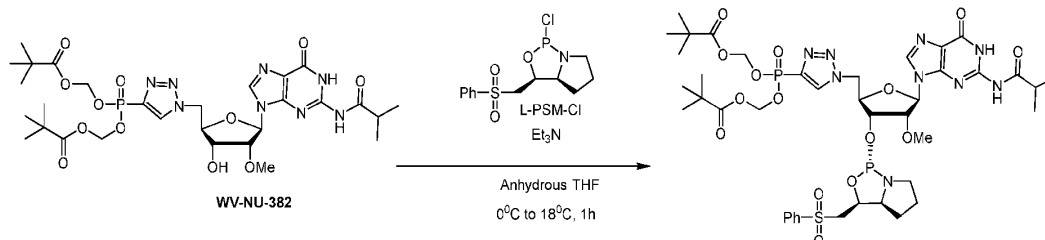
[0415] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz) (WV-NU-380, 1.05 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-OMe-A(Bz)-D-PSM-amidite (1.12 g, 78.0% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₆H₄₈N₆O₁₅P₂S (M-H⁻): 1026.37. ¹H-NMR (600 MHz, CDCl₃) δ 9.12 (s, 1H), 8.79 (s, 1H), 8.05 – 8.02 (m, 4H), 7.97 – 7.92 (m, 2H), 7.67 – 7.49 (m, 7H), 6.02 (d, J = 4.3 Hz, 1H), 5.79 – 5.71 (m, 4H), 5.29 – 5.23 (m, 1H), 5.04 (dt, J = 8.9, 5.2 Hz, 1H), 4.95 (dd, J = 14.5, 3.8 Hz, 1H), 4.88 (dd, J = 14.4, 7.0 Hz, 1H), 4.61 (t, J = 4.7 Hz, 1H), 4.50 (ddd, J = 6.9, 5.2, 3.7 Hz, 1H), 3.76 (dq, J = 9.8, 6.0 Hz, 1H), 3.64 – 3.47 (m, 2H), 3.45 – 3.39 (m, 4H), 3.27 – 3.18 (m, 1H), 1.94 – 1.77 (m, 2H), 1.67 (dtd, J = 12.2, 5.9, 2.8 Hz, 1H), 1.13 (d, J = 1.4 Hz, 18H). ³¹P-NMR (243 MHz, CDCl₃) δ 154.02, 6.02. ¹³C-NMR (151 MHz, CDCl₃) δ 176.79, 164.71, 152.85, 151.26, 150.15, 142.93, 139.83, 134.19, 133.60, 132.96, 129.47, 128.99, 128.25, 128.05, 124.47, 88.49, 82.14, 82.10, 82.07, 81.78, 81.75, 81.22, 74.80, 74.73, 71.37, 66.37, 66.35, 58.93, 58.23, 58.21, 51.23, 46.73, 46.50, 38.76, 27.54, 26.84, 26.17, 26.15.

[0416] Example 23. Preparation of 5'-Triazolyl-PO(POM)₂-2'-OMe-C(Ac)-L-PSM amidite.



[0417] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-OMe- C(Ac) (WV-NU-381, 5.1 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-OMe- C(Ac)-L-PSM-amidite (2.12 g, 73.0% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₈H₅₃N₇O₁₅P₂S (M-H⁻): 940.69. ¹H-NMR (600 MHz, CDCl₃) δ 9.41 (s, 1H), 8.68 (s, 1H), 7.98 – 7.94 (m, 2H), 7.67 – 7.62 (m, 1H), 7.57 (dd, J = 8.3, 7.2 Hz, 2H), 7.28 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 5.89 – 5.75 (m, 4H), 5.65 (d, J = 3.0 Hz, 1H), 5.22 (dt, J = 7.4, 5.1 Hz, 1H), 4.93 (dd, J = 14.7, 3.4 Hz, 1H), 4.78 (dd, J = 14.7, 4.9 Hz, 1H), 4.49 (dt, J = 9.2, 6.7 Hz, 1H), 4.33 (ddd, J = 7.7, 4.8, 3.4 Hz, 1H), 3.95 (dd, J = 6.1, 3.0 Hz, 1H), 3.77 (dq, J = 9.9, 5.9 Hz, 1H), 3.57 (ddd, J = 14.3, 7.5, 4.6 Hz, 2H), 3.48 (s, 3H), 3.41 (dd, J = 14.5, 4.7 Hz, 1H), 3.30 – 3.20 (m, 1H), 2.25 (s, 3H), 1.95 – 1.80 (m, 2H), 1.72 – 1.64 (m, 1H), 1.16 (d, J = 6.1 Hz, 19H). ³¹P-NMR (243 MHz, CDCl₃) δ 154.42, 6.19. ¹³C-NMR (151 MHz, CDCl₃) δ 176.98, 176.83, 170.84, 162.93, 154.60, 145.57, 139.72, 134.16, 129.51, 128.30, 97.04, 93.22, 82.36, 82.32, 82.28, 81.59, 81.57, 80.71, 80.69, 74.64, 74.57, 70.87, 70.80, 66.33, 66.31, 59.11, 58.09, 58.07, 49.88, 46.66, 46.42, 38.83, 38.81, 27.44, 26.92, 26.88, 26.20, 26.17, 25.03.

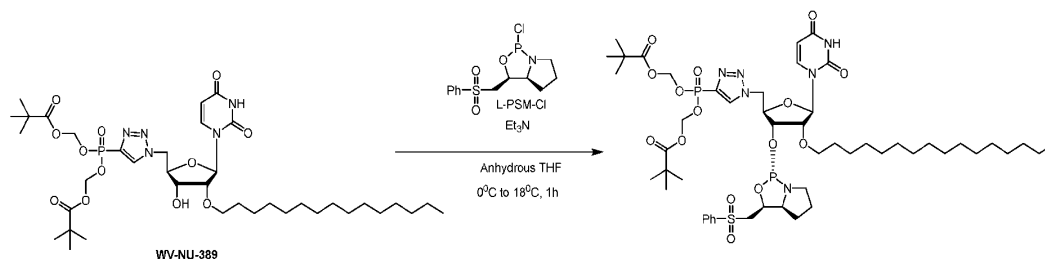
[0418] Example 24. Preparation of 5'-Triazolyl-PO(POM)₂-2'-OMe-G(iBu)-L-PSM amidite.



[0419] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-OMe- G(iBu) (WV-NU-382, 2.48 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-OMe- G(iBu)-L-PSM-amidite (1.92 g, 56.4% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₆H₄₈N₆O₁₅P₂S (M-H⁻):

1007.98. ¹H-NMR (600 MHz, CDCl₃) δ 9.77 (s, 1H), 8.40 (s, 1H), 7.99 – 7.94 (m, 2H), 7.70 – 7.65 (m, 1H), 7.64 (s, 1H), 7.59 (t, J = 7.8 Hz, 2H), 5.79 – 5.65 (m, 5H), 5.26 (ddd, J = 9.0, 5.8, 3.8 Hz, 1H), 5.14 (dd, J = 14.9, 5.2 Hz, 1H), 4.77 (dd, J = 14.9, 2.9 Hz, 1H), 4.54 (dt, J = 9.2, 5.6 Hz, 1H), 4.44 (td, J = 5.2, 2.9 Hz, 1H), 4.23 (t, J = 5.9 Hz, 1H), 3.73 (ddd, J = 12.3, 10.4, 5.9 Hz, 1H), 3.55 (ddd, J = 14.3, 9.5, 7.0 Hz, 2H), 3.42 (dd, J = 14.4, 3.8 Hz, 1H), 3.32 (s, 3H), 3.25 (tdd, J = 10.6, 8.9, 4.1 Hz, 1H), 2.91 (h, J = 6.9 Hz, 1H), 1.97 – 1.77 (m, 3H), 1.62 (tdd, J = 7.7, 5.2, 2.4 Hz, 1H), 1.24 (t, J = 6.8 Hz, 6H), 1.14 (d, J = 17.3 Hz, 20H). ³¹P-NMR (243 MHz, CDCl₃) δ 156.76, 5.85. ¹³C-NMR (151 MHz, CDCl₃) δ 180.07, 176.75, 155.68, 148.35, 148.13, 139.78, 139.10, 134.32, 132.74, 132.50, 129.64, 128.12, 122.89, 88.09, 82.13, 82.10, 82.07, 81.53, 80.48, 74.12, 74.06, 70.03, 69.94, 66.44, 66.42, 58.92, 58.91, 57.93, 57.91, 50.55, 46.75, 46.51, 38.79, 38.76, 36.05, 27.33, 26.89, 26.82, 26.13, 26.11, 19.28, 19.11.

[0420] Example 25. Preparation of 5'-Triazolyl-PO(POM)₂-2'-O-C16 lipid-U-L-PSM amidite.

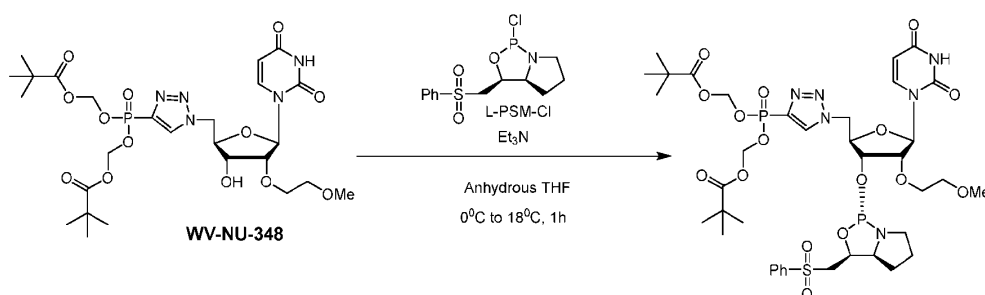


[0421] Nucleoside 5'-Triazolyl-PO(POM)₂-2'-O-C16 lipid-U (WV-NU-389, 6.57 g) was converted to 5'-Triazolyl-PO(POM)₂-2'-O-C16 lipid-U-L-PSM-amidite (5.42 g, 64.4% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₆H₄₈N₆O₁₅P₂S (M-H⁻):

1109.48. ¹H-NMR (600 MHz, CDCl₃) δ 8.84 (s, 1H), 8.19 (s, 1H), 7.98 – 7.94 (m, 2H), 7.67 – 7.63 (m, 1H), 7.58 (dd, J = 8.4, 7.2 Hz, 2H), 7.00 (d, J = 8.1 Hz, 1H), 5.79 (dd, J = 13.1, 3.5 Hz, 4H), 5.72 (d, J = 8.1 Hz, 1H), 5.42 (d, J = 3.4 Hz, 1H), 5.21 (ddd, J = 7.8, 5.8, 4.4 Hz, 1H), 4.76 (d, J = 4.4 Hz, 2H), 4.45 (dt, J = 9.4, 6.3 Hz, 1H), 4.29 (dt, J = 6.7, 4.3 Hz, 1H), 4.14 (dd, J = 5.8, 3.5 Hz, 1H), 3.73 (dq, J = 9.9,

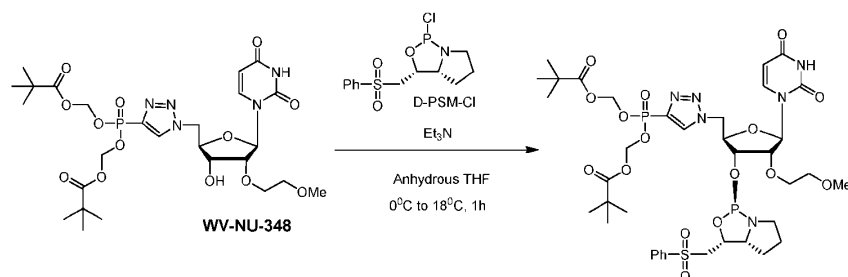
5.9 Hz, 1H), 3.65 – 3.47 (m, 5H), 3.40 (dd, $J = 14.5, 4.4$ Hz, 1H), 3.26 (tdd, $J = 10.3, 8.8, 4.1$ Hz, 1H), 1.93 – 1.82 (m, 2H), 1.69 – 1.63 (m, 1H), 1.58 – 1.51 (m, 2H), 1.27 – 1.21 (m, 25H), 1.18 (d, $J = 5.1$ Hz, 19H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{31}P -NMR (243 MHz, CDCl_3) δ 153.92, 6.18. ^{13}C -NMR (151 MHz, CDCl_3) δ 177.00, 162.73, 149.72, 142.34, 139.80, 134.18, 132.93, 129.55, 128.22, 103.20, 94.27, 82.24, 82.22, 82.20, 82.18, 80.59, 79.46, 79.44, 74.52, 74.46, 71.92, 70.58, 70.52, 66.42, 66.40, 58.08, 58.06, 50.22, 46.61, 46.38, 38.83, 32.04, 29.92, 29.83, 29.82, 29.78, 29.74, 29.61, 29.48, 27.44, 26.91, 26.90, 26.17, 22.81, 14.25.

[0422] Example 26. Preparation of 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite.



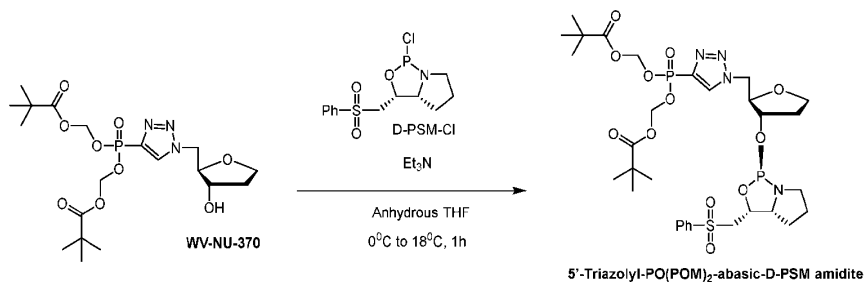
[0423] Nucleoside 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine (WV-NU-348, 4.72 g) was converted to 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine L-PSM amidite (4.86 g, 73.8% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: $\text{C}_{38}\text{H}_{54}\text{N}_6\text{O}_{16}\text{P}_2\text{S}$ (M-H⁻): 943.06. ^1H -NMR (600 MHz, CDCl_3) δ 8.70 (s, 1H), 8.16 (s, 1H), 7.97 – 7.93 (m, 2H), 7.67 – 7.62 (m, 1H), 7.58 (dd, $J = 8.4, 7.2$ Hz, 2H), 7.05 (d, $J = 8.1$ Hz, 1H), 5.79 (dq, $J = 12.0, 5.2, 1.5$ Hz, 4H), 5.71 (d, $J = 8.0$ Hz, 1H), 5.41 (d, $J = 3.0$ Hz, 1H), 5.24 – 5.19 (m, 1H), 4.74 (dd, $J = 3.9, 1.9$ Hz, 2H), 4.54 (ddd, $J = 9.4, 7.2, 5.9$ Hz, 1H), 4.28 (ddd, $J = 8.0, 6.5, 3.7$ Hz, 2H), 3.90 (ddd, $J = 11.6, 5.3, 2.9$ Hz, 1H), 3.75 – 3.68 (m, 2H), 3.62 – 3.47 (m, 4H), 3.38 (dd, $J = 14.5, 4.2$ Hz, 1H), 3.33 (s, 3H), 3.27 (tdd, $J = 10.2, 8.0, 4.4$ Hz, 1H), 1.96 – 1.82 (m, 2H), 1.66 (dtd, $J = 12.0, 6.4, 3.1$ Hz, 1H), 1.18 (d, $J = 4.6$ Hz, 18H). ^{31}P -NMR (243 MHz, CDCl_3) δ 152.23, 6.28. ^{13}C -NMR (151 MHz, CDCl_3) δ 177.09, 162.76, 149.69, 143.16, 139.91, 134.20, 132.93, 132.70, 129.58, 128.24, 102.98, 95.43, 82.30, 82.27, 82.24, 80.48, 80.46, 80.29, 74.86, 74.80, 72.38, 71.19, 70.25, 66.50, 66.47, 59.13, 58.26, 58.23, 50.07, 46.56, 46.33, 38.88, 27.55, 26.96, 26.94, 26.24, 26.22.

[0424] Example 27. Preparation of 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine D-PSM amidite.



[0425] Nucleoside 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine (WV-NU-348, 1.91 g) was converted to 2'-OMOE-5'-PO(POM)₂-Triazolyl Phosphonate Uridine D-PSM amidite (2.12 g, 79.5% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₈H₅₄N₆O₁₆P₂S (M-H⁻): 943.20. ¹H-NMR (600 MHz, CDCl₃) δ 8.94 (s, 1H), 8.14 (s, 1H), 7.96 – 7.93 (m, 2H), 7.69 – 7.65 (m, 1H), 7.58 (t, J = 7.8 Hz, 2H), 7.06 (d, J = 8.1 Hz, 1H), 5.83 – 5.75 (m, 4H), 5.71 (d, J = 8.0 Hz, 1H), 5.44 (d, J = 3.2 Hz, 1H), 5.25 (dt, J = 7.4, 5.3 Hz, 1H), 4.75 (qd, J = 14.6, 4.7 Hz, 2H), 4.56 (ddd, J = 9.7, 6.9, 5.8 Hz, 1H), 4.33 (dq, J = 7.4, 4.1, 3.5 Hz, 2H), 3.89 (ddd, J = 11.5, 4.9, 2.7 Hz, 1H), 3.75 (dq, J = 9.6, 6.0 Hz, 1H), 3.66 (ddd, J = 11.6, 7.3, 2.7 Hz, 1H), 3.60 – 3.45 (m, 4H), 3.39 (dd, J = 14.5, 5.0 Hz, 1H), 3.32 (s, 3H), 3.24 (tdd, J = 10.2, 8.7, 4.3 Hz, 1H), 1.95 – 1.76 (m, 2H), 1.65 (dddd, J = 12.1, 8.7, 5.5, 3.0 Hz, 1H), 1.18 (d, J = 6.5 Hz, 19H). ³¹P-NMR (243 MHz, CDCl₃) δ 154.99, 6.18. ¹³C-NMR (151 MHz, CDCl₃) δ 176.99, 162.93, 149.78, 143.31, 139.89, 134.25, 132.78, 132.54, 129.55, 128.33, 102.93, 95.38, 82.27, 82.26, 82.22, 80.59, 80.56, 80.31, 74.73, 74.67, 72.46, 70.99, 70.96, 70.88, 66.27, 59.17, 58.31, 50.55, 46.79, 46.56, 38.87, 38.86, 27.59, 26.94, 26.92, 26.24, 26.21.

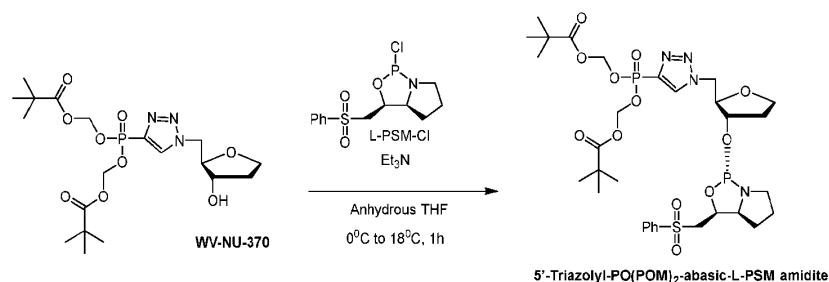
[0426] Example 28. Preparation of 5'-Triazolyl-PO(POM)₂-abasic-D-PSM amidite



[0427] Nucleoside 5'-Triazolyl-PO(POM)₂-abasic (WV-NU-370, 1.15 g) was converted to 5'-Triazolyl-PO(POM)₂-abasic-D-PSM-amidite (1.04 g, 56.8% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₆H₄₈N₆O₁₅P₂S (M-H⁺): 761.75. ¹H-NMR (600 MHz, CDCl₃) δ 8.18 (s, 1H), 7.94 – 7.91 (m, 2H), 7.70 – 7.65 (m, 1H), 7.60 – 7.55 (m, 2H), 5.82 – 5.74 (m, 4H), 5.10 (ddd, J = 7.7, 5.8, 4.5 Hz, 1H), 4.64 (dd, J = 14.3, 3.4 Hz, 1H), 4.55 (dd, J = 14.3, 5.7 Hz, 1H), 4.32 (ddt, J = 8.4, 6.9, 4.0 Hz, 1H), 4.09 – 4.05 (m, 1H), 3.93 – 3.84 (m, 2H), 3.70 (dq, J = 9.8, 5.9

Hz, 1H), 3.52 (ddd, J = 14.8, 7.7, 4.9 Hz, 2H), 3.40 (dd, J = 14.6, 4.4 Hz, 1H), 3.25 (tdd, J = 10.2, 8.7, 4.0 Hz, 1H), 1.91 – 1.60 (m, 6H), 1.18 (dd, J = 8.7, 0.6 Hz, 18H). ³¹P-NMR (243 MHz, CDCl₃) δ 155.92, 6.33. ¹³C-NMR (151 MHz, CDCl₃) δ 176.82, 139.73, 134.23, 132.43, 132.19, 129.50, 128.27, 82.63, 82.59, 82.18, 82.17, 82.15, 82.13, 74.48, 74.38, 74.07, 74.01, 67.65, 66.32, 66.30, 58.15, 58.13, 51.81, 47.05, 46.82, 38.86, 34.28, 27.50, 26.95, 26.92, 26.23, 26.21.

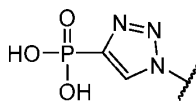
[0428] Example 29. Preparation of 5'-Triazolyl-PO(POM)₂-abasic-L-PSM amidite

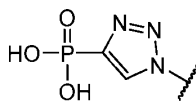


[0429] Nucleoside 5'-Triazolyl-PO(POM)₂-abasic (WV-NU-370, 4.77g) was converted to 5'-Triazolyl-PO(POM)₂-abasic-L-PSM-amidite (4.23 g, 55.7% yield) as an off-white solid according to the scheme above and the protocol in Example 16. LCMS: C₃₆H₄₈N₆O₁₅P₂S (M-H⁻): 759.00. ¹H-NMR (600 MHz, CDCl₃) δ 8.22 (s, 1H), 7.99 – 7.96 (m, 2H), 7.67 (td, J = 7.2, 1.3 Hz, 1H), 7.59 (t, J = 7.8 Hz, 2H), 5.83 – 5.74 (m, 4H), 5.13 – 5.08 (m, 1H), 4.68 – 4.59 (m, 2H), 4.38 – 4.31 (m, 1H), 4.01 – 3.97 (m, 1H), 3.88 (ddd, J = 9.2, 5.6, 3.8 Hz, 2H), 3.74 – 3.68 (m, 1H), 3.60 – 3.49 (m, 2H), 3.39 (dd, J = 14.4, 4.6 Hz, 1H), 3.24 (tdd, J = 10.4, 8.7, 3.9 Hz, 1H), 1.99 – 1.76 (m, 4H), 1.69 – 1.62 (m, 1H), 1.17 (dd, J = 9.0, 2.1 Hz, 19H). ³¹P-NMR (243 MHz, CDCl₃) δ 154.48, 6.45. ¹³C-NMR (151 MHz, CDCl₃) δ 176.78, 139.64, 134.22, 132.51, 132.27, 129.57, 128.27, 82.73, 82.72, 82.15, 82.11, 74.34, 74.26, 74.21, 74.15, 67.57, 66.51, 66.49, 58.06, 58.04, 51.43, 46.96, 46.72, 38.82, 34.30, 34.27, 27.44, 26.92, 26.89, 26.14, 26.11.

[0430] Example 30. Exemplary oligonucleotide synthesis.

[0431] Provided phosphoramidites are utilized for preparing oligonucleotides comprising



–P(O)(OH)₂ groups, e.g., those comprising , at 5'-ends. In some preparations, one or more technologies (e.g., reagents, conditions (e.g., cleavage, deprotection, chiral auxiliary removal, etc.), cycles, chiral auxiliaries, etc.) described in US 10167309, US 11643657, US 11718638, US 11608355, US 20230089442, US 20230203087, etc. are utilized.

[0432] For example, in some embodiments, automated solid-phase oligonucleotide synthesis was performed according to the cycles shown in Table 1 (regular amidite cycle, e.g., for natural phosphate linkages), Table 2 (regular amidite cycle, e.g., for stereorandom phosphorothioate internucleotidic

linkages), Table 3 (DPSE amidite cycle, e.g., for chirally controlled PS linkages (e.g., phosphorothioate internucleotidic linkages)), and/or Table 4 (PSM amidite cycle, e.g., for chiral PN linkages (e.g., chiral n001 internucleotidic linkages)).

Table 1. Regular Amidite Synthetic Cycle for PO linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	oxidation	50mM I ₂ / pyridine-H ₂ O (9:1, v/v)	2.0 mL	1 min
4	cap-2	20% Ac ₂ O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 2. Regular Amidite Synthetic Cycle for stereo-random PS linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	sulfurization	0.2M XH / pyridine	2.0 mL	6 min
4	cap-2	20% Ac ₂ O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 3. DPSE Amidite Synthetic Cycle for chiral PS linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min
3	cap-1	20% Ac ₂ O, 30% 2,6-lutidine / MeCN	2.0 mL	2 min
4	sulfurization	0.2M XH / pyridine	2.0 mL	6 min
5	cap-2	20% Ac ₂ O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

Table 4. PSM Amidite Synthetic Cycle for chiral PN linkages

step	operation	reagents and solvent	volume	waiting time
1	detritylation	3% TCA / DCM	10 mL	65 s
2	coupling	0.2M monomer / 20% IBN-MeCN 0.5M CMIMT / MeCN	0.5 mL 1.0 mL	8 min

3	cap-1	20% Ac ₂ O, 30% 2,6-lutidine / MeCN	2.0 mL	2 min
4	imidation	0.5M ADIH reagent / MeCN	2.0 mL	6 min
5	cap-2	20% Ac ₂ O, 30% 2,6-lutidine / MeCN 20% MeIm / MeCN	1.0 mL 1.0 mL	45 s

1X reagent: TEA-3HF : TEA : H₂O : DMSO = 5.0 : 1.8 : 15.5 : 77.7 (v/v/v/v)

ADIH: 2-azido-1,3-dimethylimidazolium hexafluorophosphate

CMIMT: *N*-cyanomethylimidazolium triflate

CPG: controlled pore glass

DCM: dichloromethane, CH₂Cl₂

DMSO: dimethylsulfoxide

GalNAc: *N*-acetylgalactosamine

HF: hydrogen fluoride

IBN: isobutyronitrile

MeCN: acetonitrile

MeIm: *N*-methylimidazole

TCA: trichloroacetic acid

TEA: triethylamine

XH: xanthane hydride

[0433] In some embodiments, preparations comprise one or more DPSE and/or PSM cycles.

[0434] In some embodiments, the following C&D conditions (25 umol scale) were utilized: After completion of synthesis, CPG solid support was dried and transferred into 50 mL plastic tube. The CPG was treated with 1X reagent (2.5 mL; 100 uL/umol) for 3 h at 28 °C, then added concentrated NH₃ (5.0 mL; 200 uL/umol) for 24 h at 37°C. Reaction mixture was cooled to room temperature and the CPG was separated by membrane filtration, washed with 15 mL of H₂O. Crude material (filtrate) was analyzed, e.g., by LTQ and RP-UPLC. Those skilled in the art appreciate other C&D conditions, and/or analytical and/or purification technologies may be utilized in accordance with the present disclosure.

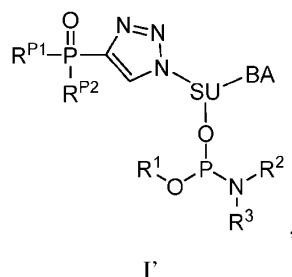
[0435] In some embodiments, oligonucleotide synthesis procedures are compatible with one or more compounds (e.g., phosphoramidites) as described herein. In some embodiments, oligonucleotide synthesis procedures, including those described in the present Example, are compatible with a 5'-Bis(2-cyanoethyl)-Triazolyl Phosphonate compound (e.g., WV-NU-336) as described herein. In some embodiments, one or more compounds (e.g., phosphoramidites) comprising one or more protected -P(O)(OH)₂ groups (e.g., -P(O)(OR^{PG})₂, wherein R^{PG} is as described herein) or -P(O)(OR^{P1})(OR^{P2}) that

can be deprotected under oligonucleotide synthesis conditions, including those described in the present Example. In some embodiments, provided technologies provide methods for incorporating $-P(O)(OH)_2$ into oligonucleotides using one or more compounds (e.g., phosphoramidites) comprising one or more protected $-P(O)(OH)_2$ groups (e.g., $-P(O)(OR^{PG})_2$, wherein R^{PG} is as described herein) or $-P(O)(OR^{P1})(OR^{P2})$ with higher yield, higher purity, higher efficiency, lower operation complexity, milder reaction condition, shorter preparation time, and/or lower cost, etc. when compared to using, e.g., $-P(O)(OMe)_2$, or $-P(O)(OEt)_2$.

[0436] While various embodiments of the present disclosure have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions, obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present disclosure. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be examples and that actual parameters, dimensions, materials, and/or configurations may depend upon specific application or applications for which the teachings of the present disclosure are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example and that, within the scope of the appended claims and equivalents thereto, the disclosure may be practiced otherwise than as specifically described and claimed. The present disclosure is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present disclosure.

CLAIMS

1. A compound, wherein the compound is a compound of formula I' or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

SU is a sugar;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $\text{---}(\text{A})\text{---}(\text{R}^s)_t$;

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R^{s11}$, $-L^s-OR^{s11}$, $-L^s-SR^{s11}$, $-L^s-N(R^{s11})_2$, $-O-L^s-OR^{s11}$, $-O-L^s-SR^{s11}$, $-O-L^s-N(R^{s11})_2$, $-C(R^{s11})_3$ or $-L^s-Si(R^{s11})_3$;

each R^{s11} is independently R' ;

each L^s is independently L;

each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

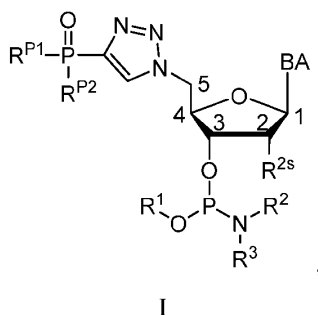
$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to

the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C₁₋₁₀ aliphatic, C₁₋₁₀ heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C₆₋₁₄ aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

2. A compound, wherein the compound is a compound of formula I or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is -O-CH₂CH₂CN or -O-CH₂-O-C(O)-R^P, wherein R^P is optionally substituted *tert*-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is -F, -Cl, -Br, -I, -CN, -N₃, -NO, -NO₂, -L^{2s}-R^{2a}, -L^{2s}-OR^{2a}, -L^{2s}-SR^{2a}, -L^{2s}-N(R^{2a})₂, -O-L^{2s}-OR^{2a}, -O-L^{2s}-SR^{2a}, or -O-L^{2s}-N(R^{2a})₂, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

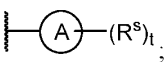
each L^{2s} is independently L ;

each R^{2a} is independently R' ;

each L is independently a covalent bond, or a bivalent, optionally substituted group selected from C₁₋₁₀ aliphatic and C₁₋₁₀ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by -Cy-, -O-, -S-, -N(R')-, -C(O)-, -C(S)-, -C(NR')-, -C(O)N(R')-, -N(R')C(O)N(R')-, -N(R')C(O)O-, -S(O)-, -S(O)₂-, -S(O)₂N(R')-, -SC(O)-, or -C(O)O-;

–Cy– is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form .

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; t is 0-5;

each R^s is independently $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^s-R^{s11}$, $-L^s-OR^{s11}$, $-L^s-SR^{s11}$, $-L^s-N(R^{s11})_2$, $-O-L^s-OR^{s11}$, $-O-L^s-SR^{s11}$, $-O-L^s-N(R^{s11})_2$, $-C(R^{s11})_3$ or $-L^s-Si(R^{s11})_3$;

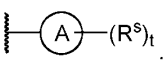
each R^{s11} is independently R' ;

each L^s is independently L ;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

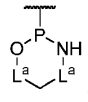
each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

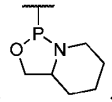
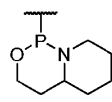
3. The compound of any one of the preceding claims, wherein R^1 is $-CH_2CH_2CN$.
4. The compound of any one of the preceding claims, wherein R^2 is optionally substituted C_{1-6} aliphatic (e.g., isopropyl).
5. The compound of any one of the preceding claims, wherein R^3 is optionally substituted C_{1-6} aliphatic (e.g., isopropyl).
6. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is $-P(OCH_2CH_2CN)N[CH(CH_3)_2]_2$.
7. The compound of claim 1, wherein two or three of R^1 , R^2 , and R^3 are taken together with

their intervening atoms to form .

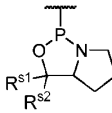
8. The compound of claim 7, wherein Ring A is an optionally substituted polycyclic ring.
9. The compound of claim 7, wherein Ring A is an optionally substituted bicyclic 7-12 membered ring.
10. The compound of claim 7, wherein Ring A comprises an optionally substituted 5- or 6-membered ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$.

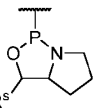
11. The compound of claim 10, wherein the ring comprising the phosphorus, oxygen and nitrogen atoms of $-P(OR^1)N(R^2)(R^3)$ does not have any other ring heteroatoms.
12. The compound of claim 7, wherein each monocyclic ring unit of Ring A is independently an optionally substituted 3-7 membered ring.

13. The compound of claim 7, wherein Ring A is or comprises optionally substituted , wherein each L^a is independently a covalent bond or a bivalent C_{1-5} aliphatic group wherein one or more methylene units of each L^a are optionally and independently replaced by $-O-$, $-S-$, or $-NH$.

14. The compound of claim 7, wherein Ring A is or comprises optionally substituted .
15. The compound of claim 7, wherein Ring A is or comprises optionally substituted .
16. The compound of claim 7, wherein Ring A is or comprises optionally substituted .

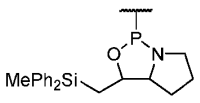
17. The compound of any one of claims 7-16, wherein t is 1-5.
18. The compound of any one of claims 7-16, wherein an occurrence of R^s is bonded to a carbon atom bonded to the oxygen of $-P(OR^1)N(R^2)(R^3)$.

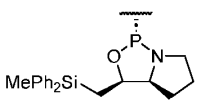
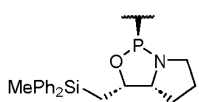
19. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is , wherein each of R^{s1} and R^{s2} is independently R^s .

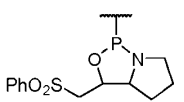
20. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is .

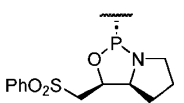
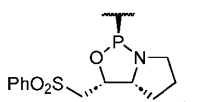
21. The compound of any one of claims 7-20, wherein an occurrence of L^s is optionally substituted $-CH_2-$.
22. The compound of any one of claims 7-20, wherein an occurrence of L^s is a covalent bond.
23. The compound of any one of claims 7-22, wherein an occurrence of R^s is $-L^s-Si(R^{s11})_3$.
24. The compound of claim 23, wherein each R^{s11} is not hydrogen.

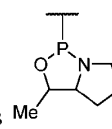
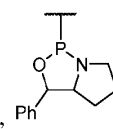
25. The compound of claim 23, wherein each R^{s11} is independently an optionally substituted group selected from C_{1-6} aliphatic and phenyl.
26. The compound of claim 23, wherein $-Si(R^{s11})_3$ is $-Si(CH_3)(Ph)_2$.
27. The compound of any one of claims 7-22, wherein R^s is $-L^s-R^{s11}$.
28. The compound of claim 27, wherein R^{s11} is $-S(O)_2R$ wherein R is optionally substituted C_{1-6} aliphatic.
29. The compound of claim 27, wherein R^{s11} is $-S(O)_2R$ wherein R is t-Bu.
30. The compound of claim 27, wherein R^{s11} is $-S(O)_2R$ wherein R is methyl.
31. The compound of claim 27, wherein R^{s11} is $-S(O)_2R$.
32. The compound of any one of claims 7-20, wherein an occurrence of R^s is $-C(R^{s11})_3$.
33. The compound of any one of claims 7-20, wherein an occurrence of R^s is $-CH(R^{s11})_2$.
34. The compound of claim 33, wherein the two R^{s11} are taken together with the carbon atom to which they are attached to form an optionally substituted 3-20 membered ring having 0-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.
35. The compound of claim 20, wherein R^{s2} is optionally substituted C_{1-6} aliphatic, or R^{s2} is optionally substituted phenyl.

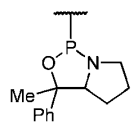
36. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is .

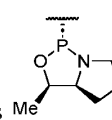
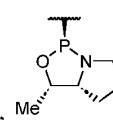
37. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is  or .

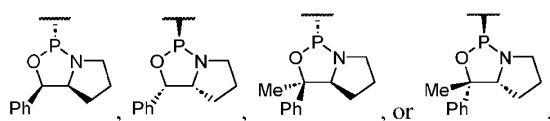
38. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is .

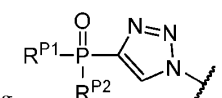
39. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is  or .

40. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is , , or

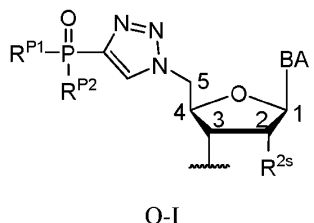


41. The compound of claim 1, wherein $-P(OR^1)N(R^2)(R^3)$ is , ,



42. A compound, wherein the compound is an oligonucleotide comprising , wherein each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl.

43. A compound, wherein the compound is an oligonucleotide comprising a moiety of formula O-I or a salt thereof:



wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L ;

each R^{2a} is independently R^* ;

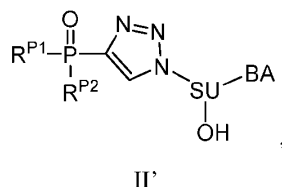
each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R^*)-$, $-C(O)-$, $-C(S)-$, $-C(NR^*)-$, $-C(O)N(R^*)-$, $-N(R^*)C(O)N(R^*)-$, $-N(R^*)C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R^*)-$, $-SC(O)-$, or $-C(O)O-$;

$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R^* is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R^* are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

44. A compound, wherein the compound is a compound of formula II' or a salt thereof:



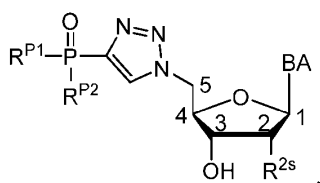
wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

SU is a sugar; and

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase.

45. A compound, wherein the compound is a compound of formula II or a salt thereof:



II

wherein:

each of R^{P1} and R^{P2} is $-O-CH_2CH_2CN$ or $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl;

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L ;

each R^{2a} is independently R' ;

each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

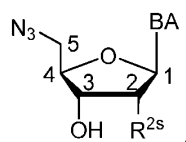
$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

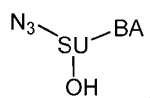
each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

46. The compound of any one of the preceding claims, wherein R^{P1} and R^{P2} are $-O-CH_2CH_2CN$.

47. The compound of any one of the preceding claims, wherein R^{P1} and R^{P2} are $-O-CH_2-O-C(O)-tBu$.
48. A compound, wherein the compound is a compound of formula III or formula III', or a salt thereof:



III



III'

wherein:

BA is hydrogen, or an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; or BA is an optionally substituted or protected nucleobase;

SU is a sugar;

R^{2s} is $-F$, $-Cl$, $-Br$, $-I$, $-CN$, $-N_3$, $-NO$, $-NO_2$, $-L^{2s}-R^{2a}$, $-L^{2s}-OR^{2a}$, $-L^{2s}-SR^{2a}$, $-L^{2s}-N(R^{2a})_2$, $-O-L^{2s}-OR^{2a}$, $-O-L^{2s}-SR^{2a}$, or $-O-L^{2s}-N(R^{2a})_2$, or R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5;

each L^{2s} is independently L;

each R^{2a} is independently R' ;

each L is independently a bivalent, optionally substituted group selected from C_{1-10} aliphatic and C_{1-10} heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by $-Cy-$, $-O-$, $-S-$, $-N(R')$, $-C(O)-$, $-C(S)-$, $-C(NR')$, $-C(O)N(R')$, $-N(R')C(O)N(R')$, $-N(R')C(O)O-$, $-S(O)-$, $-S(O)_2-$, $-S(O)_2N(R')$, $-SC(O)-$, or $-C(O)O-$;

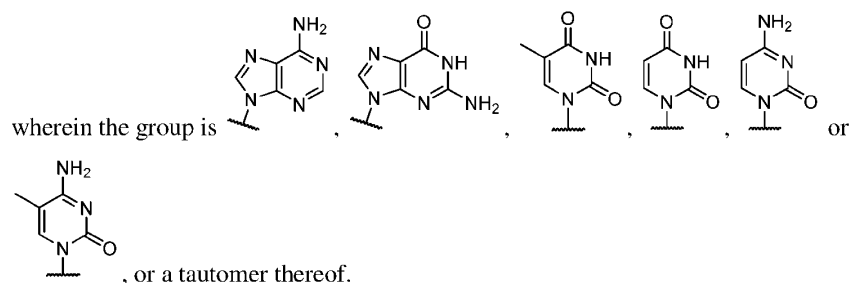
$-Cy-$ is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen,

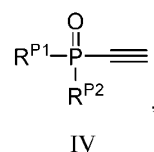
oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

49. The compound of any one of the preceding claims, wherein BA is an optionally substituted 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.
50. The compound of any one of the preceding claims, wherein BA is bonded to C1 at a nitrogen atom.
51. The compound of any one of claims 1-50, wherein BA is an optionally substituted group,



52. The compound of any one of claims 1-51, wherein BA is an optionally protected nucleobase.
53. The compound of claim 52, wherein the nucleobase is selected from A, T, C, G, U and 5mU.
54. The compound of any one of the preceding claims, wherein R^{2s} is $-H$.
55. The compound of any one of claims 1-53, wherein R^{2s} is $-F$.
56. The compound of any one of claims 1-53, wherein R^{2s} is $-OR^{2a}$.
57. The compound of claim 56, wherein R^{2a} is methyl.
58. The compound of any one of claims 1-53, wherein L^{2s} is not a covalent bond.
59. The compound of any one of claims 1-53, wherein L^{2s} is optionally substituted C_{1-10} alkylene.
60. The compound of any one of claims 1-53, wherein R^{2s} is $-L^{2s}-R^{2a}$.
61. The compound of claim 60, wherein L^{2s} is $-O-L^{2s'}-R^{2a}$, wherein $L^{2s'}$ is optionally substituted $-(CH_2)_{1-10}-$.
62. The compound of any one of claims 1-53, wherein R^{2s} is $-O-L^{2s}-OR^{2a}$.
63. The compound of claim 62, wherein R^{2a} is $-OMe$.
64. The compound of claim 62, wherein R^{2s} is $-OCH_2CH_2OMe$.
65. The compound of any one of claims 1-53, wherein R^{2s} is L^{2s} connecting C2 with C1, C2, C3, C4 or C5.

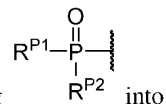
66. The compound of any one of claims 1-53 and 65, wherein L is (C2)–O–(unsubstituted methylene)–.
67. The compound of any one of claims 1-53 and 65, wherein L is (C2)–O–(substituted methylene)–
68. The compound of claim 67, wherein the carbon atom of the methylene group is *R*.
69. The compound of claim 67, wherein the carbon atom of the methylene group is *S*.
70. A compound, wherein the compound is a compound of formula IV or a salt thereof:



wherein:

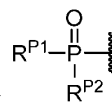
each of $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl.

71. The compound of claim 70, wherein $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ are $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.
72. The compound of claim 70, wherein $\text{R}^{\text{P}1}$ and $\text{R}^{\text{P}2}$ are $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{tBu}$.
73. The compound of any one of the preceding claims, wherein the purity of the compound is about or more than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

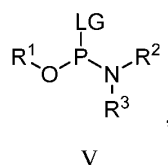


74. A method for preparing an oligonucleotide, comprising converting $-\text{P}(\text{O})(\text{OH})_2$ (e.g., a salt form thereof) into
75. A method for preparing an oligonucleotide, comprising coupling a compound of any one of claims 1-41, 45-47, and 49-69, wherein the compound is a compound of formula I or formula I', or a salt thereof, to an oligonucleotide.
76. A method for preparing an oligonucleotide, comprising reacting a compound of any one of claims 1-41, 45-47, and 49-69, wherein the compound is a compound of formula I or formula I', or a salt thereof, with a compound comprising a $-\text{OH}$ group.
77. The method of claim 76, wherein the $-\text{OH}$ group is 5'-OH of an oligonucleotide.
78. The method of any one of claims 75-77, wherein the oligonucleotide preparation comprises utilizing a solid support.

79. The method of any one of claims 75-78, comprising preparing a compound of any one of claims 42, 46-47, and 49-69, wherein the compound is a compound of formula O-I or a salt thereof.



80. The method of any one of claims 75-78, comprising converting $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^{\text{P1}}-\text{P}- \\ | \\ \text{R}^{\text{P2}} \end{array} \begin{array}{c} \text{---} \\ \text{---} \\ \text{---} \end{array}$ into $-\text{P}(\text{O})(\text{OH})_2$ (e.g., a salt form thereof).
81. The method of claim 80, wherein R^{P1} and R^{P2} are $-\text{OCH}_2\text{CH}_2\text{CN}$, and the conversion is performed in the presence of a base.
82. The method of claim 80, wherein R^{P1} and R^{P2} are $-\text{OCH}_2\text{OC}(\text{O})\text{tBu}$.
83. The method of any one of claims 75-82, wherein the conversion occurs during cleavage and deprotection that removes base protection groups and/or chiral auxiliaries and/or cleaves the oligonucleotide from a solid support.
84. The method of any one of claims 75-83, wherein the oligonucleotide prepared is a compound of any one of claims 42, 46-47, and 49-69, wherein the compound is a compound of formula O-I or a salt thereof.
85. A method for preparing a compound of any one of claims 1-41, 45-47, and 49-69, wherein the compound is a compound of formula I or formula I', or a salt thereof, comprising reacting a compound of any one of claims 45-47 and 49-69, wherein the compound is a compound of formula II or formula II', or a salt thereof, with a compound of formula V or a salt thereof:



wherein:

LG is a leaving group;

each of R^1 , R^2 , and R^3 is independently R' , or two or three of R^1 , R^2 , and R^3 are taken together

with their intervening atoms to form $\text{---}(\text{A})\text{---}(\text{R}^s)_t$,

Ring A is an optionally substituted 3-20 membered ring having, in addition to the intervening atoms, 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

t is 0-5;

each R^s is independently $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{CN}$, $-\text{N}_3$, $-\text{NO}$, $-\text{NO}_2$, $-\text{L}^s-\text{R}^{\text{s11}}$, $-\text{L}^s-\text{OR}^{\text{s11}}$, $-\text{L}^s-\text{SR}^{\text{s11}}$, $-\text{L}^s-\text{N}(\text{R}^{\text{s11}})_2$, $-\text{O}-\text{L}^s-\text{OR}^{\text{s11}}$, $-\text{O}-\text{L}^s-\text{SR}^{\text{s11}}$, $-\text{O}-\text{L}^s-\text{N}(\text{R}^{\text{s11}})_2$, $-\text{C}(\text{R}^{\text{s11}})_3$ or $-\text{L}^s-\text{Si}(\text{R}^{\text{s11}})_3$;

each R^{sl} is independently R²;

each L^s is independently L;

each L is independently a bivalent, optionally substituted group selected from C₁₋₁₀ aliphatic and C₁₋₁₀ heteroaliphatic group having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, wherein one or more methylene units of L are optionally and independently replaced by -Cy-, -O-, -S-, -N(R')-, -C(O)-, -C(S)-, -C(NR')-, -C(O)N(R')-, -N(R')C(O)N(R')-, -N(R')C(O)O-, -S(O)-, -S(O)₂-, -S(O)₂N(R')-, -SC(O)-, or -C(O)O-;

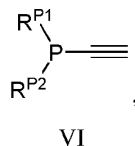
-Cy- is an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently -R, -C(O)R, -CO₂R, or -S(O)₂R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C₁₋₁₀ aliphatic, C₁₋₁₀ heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C₆₋₁₄ aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

86. The method of claim 85, wherein LG is -Cl.
87. The method of any one of claims 85-86, wherein the reaction is performed in the presence of a base.
88. The method of any one of claims 85-87, wherein R¹, R² and R³ of formula V are as described in any one of claims 3-41.
89. The method of any one of claims 75-84, comprising a method of any one of claims 85-88.
90. A method for preparing a compound of any one of claims 45-47 and 49-69, wherein the compound is a compound of formula II or formula II', or a salt thereof, comprising reacting a compound of any one of claims 48-69, wherein the compound is a compound of formula III or formula III', or a salt thereof, with a compound of any one of claims 70-73.
91. The method of claim 90, wherein the reaction is in the presence of a base.
92. The method of claim 91, wherein the base is DIEA.
93. The method of any one of claims 90-92, wherein the reaction is in the presence of copper salt.
94. The method of any one of claims 90-92, wherein the reaction is in the presence of CuI.
95. The method of any one of claims 75-89, comprising a method of any one of claims 90-94.

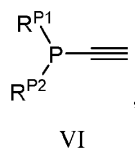
96. A method for preparing a compound of any one of claims 70-73, comprising oxidizing a compound of formula VI or a salt thereof:



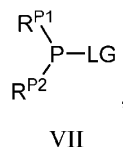
wherein:

each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl.

97. The method of claim 96, wherein the oxidant is mCPBA.
98. The method of claim 96 or 97, wherein R^{P1} and R^{P2} are $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.
99. The method of any one of claims 75-95, comprising a method of any one of claims 96-98.
100. A method for preparing a compound of formula VI or a salt thereof:



comprising reacting $\equiv\text{-MgBr}$ with a compound of formula VII or a salt thereof:

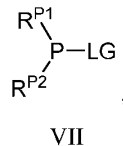


wherein:

LG is a leaving group; and

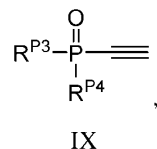
each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$ or $-\text{O}-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{R}^{\text{P}}$, wherein R^{P} is optionally substituted tert-butyl.

101. The method of claim 100, wherein LG is $-\text{Cl}$.
102. The method of any one of claims 100-101, wherein each of R^{P1} and R^{P2} is $-\text{O}-\text{CH}_2\text{CH}_2\text{CN}$.
103. The method of any one of claims 75-99, comprising a method of any one of claims 100-102.
104. A method for preparing a compound of formula VII or a salt thereof:

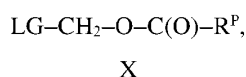


wherein LG is $-\text{Cl}$, comprising reacting $\text{HO}-\text{CH}_2\text{CH}_2-\text{CN}$ with PCl_3 in the presence of a base.

105. The method of claim 104, wherein the base is TEA.
106. The method of any one of claims 75-103, comprising a method of any one of claims 104-105.
107. A method for preparing a compound of any one of claims 70-73, wherein each of R^{P1} and R^{P2} is $-O-CH_2-O-C(O)-R^P$, wherein R^P is optionally substituted tert-butyl, comprising reacting a compound of formula IX or a salt thereof:



with a compound having the structure of formula X or a salt thereof:



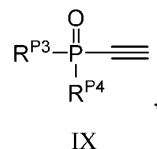
wherein:

R^P is optionally substituted tert-butyl;

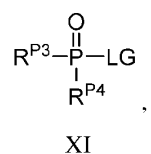
LG is a leaving group;

each of R^{P3} and R^{P4} is independently $-O-R^{SP}$, wherein R^{SP} is an optionally substituted optionally substituted C_{1-6} aliphatic.

108. The method of claim 107, wherein each of R^{P3} and R^{P4} is $-OMe$.
109. The method of any one of claims 107-108, wherein LG is $-I$.
110. The method of any one of claims 75-95, comprising a method of any one of claims 107-109.
111. A method for preparing a compound of formula IX or a salt thereof:



comprising reacting $\equiv\text{-MgBr}$ with a compound of formula XI or a salt thereof:

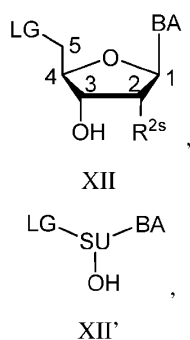


wherein:

LG is a leaving group

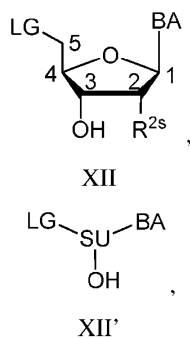
each of R^{P3} and R^{P4} is independently $-O-R^{SP}$, wherein each R^{SP} is independently optionally substituted C_{1-6} aliphatic.

112. The method of claim 111, wherein each of R^{P3} and R^{P4} is $-\text{OMe}$.
113. The method of any one of claims 111-112, wherein LG is $-\text{Cl}$.
114. The method of any one of claims 75-95 and 107-110, comprising a method of any one of claims 111-113.
115. A method for preparing a compound of any one of claims 48-69, wherein the compound is a compound of formula III or formula III', or a salt thereof, comprising reacting a compound of formula XII or formula XII' or a salt thereof:

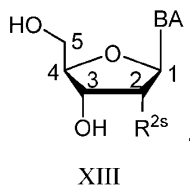


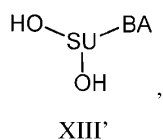
wherein LG is a leaving group, with an azide.

116. The method of claim 115, wherein LG is $-\text{I}$.
117. The method of any one of claims 115-116, wherein the azide is sodium azide.
118. The method of any one of claims 75-114, comprising a method of any one of claims 115-117.
119. A method for preparing a compound of formula XII or XII' or a salt thereof:



wherein LG is $-\text{I}$, comprising reacting a compound of formula XIII or formula XIII' or a salt thereof:





with I₂.

120. The method of any one of claims 115-119, wherein the reaction is performed in the presence of a phosphine.
121. The method of any one of claims 115-119, wherein the reaction is performed in the presence of a PPh₃.
122. The method of any one of claims 119-121, wherein the reaction is performed in the presence of a base.
123. The method of any one of claims 119-121, wherein the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises –N= or –N(R)–.
124. The method of any one of claims 119-121, wherein the reaction is performed in the presence of a heteroaryl base, wherein the heteroaryl ring comprises –N= or –NH–.
125. The method of any one of claims 119-121, wherein the reaction is performed in the presence of imidazole.
126. The method of any one of claims 75-118, comprising a method of any one of claims 119-125.
127. A compound or method of any one of Embodiments 1-205.