METHODS FOR THE SYNTHESIS OF FUNCTIONALIZED NUCLEIC ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States provisional application serial number 61/509,526, filed July 19, 2011, the entirety of which is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0001] Oligonucleotides are useful in therapeutic, diagnostic, researchand nanomaterials applications. The use of natural sequences of DNA or RNA for therapeutics is limited because of their instability against extra and intracellular nucleases, poor cell penetration and distribution. Additionally, *in vitro* studies have shown that the properties of antisense nucleotides such as binding affinity, sequence specific binding to the complementary RNA (Cosstick and Eckstein, 1985; LaPlanche *et al.*, 1986; Latimer *et al.*, 1989; Hacia *et al.*, 1994; Mesmaeker *et al.*, 1995), stability to nucleases are affected by the configurations of the phosphorous atomsTherefore, there is a need for modified oligonucleotides to impart stability towards ubiquitous nucleases, increase binding affinity towards complementary RNA and increase cell penetration and bio-distribution for a number of *in-vitro* and *in-vivo* applications.

SUMMARY OF THE INVENTION

[0002] Described herein are methods for the synthesis of novel functionalized nucleic acids and nucleic acid prodrugs. In some embodiments, the nucleic acids comprise chiral phosphorous moieties.

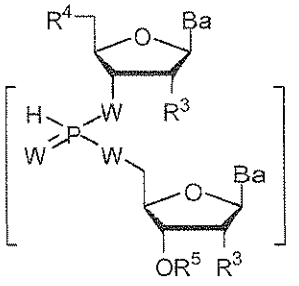
[0003] One embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa comprising the steps of:

i) reacting an H-phosphonate of structure Ia with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIa to provide a phosphorothiotriester of structure IIIa;

wherein,

the H-phosphonate of structure Ia has the following structure:



Structure Ia

wherein,

W is independently selected from O, S, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

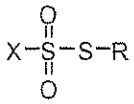
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIa has the following structure:



Structure IIa wherein,

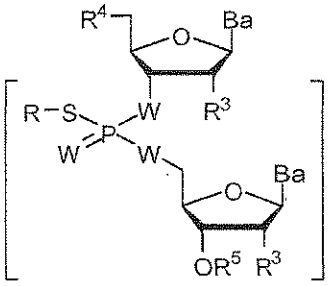
X is alkyl, cycloalkyl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R'-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the phosphorothiotriester of structure IIIa has the following structure:



Structure IIIa

wherein,

W is independently selected from O, S, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^a is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

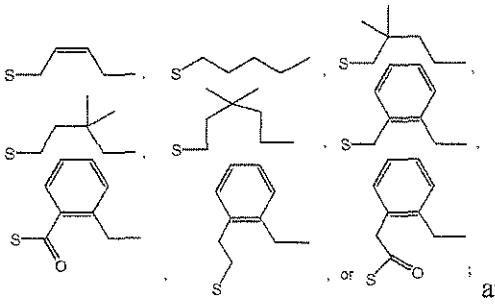
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

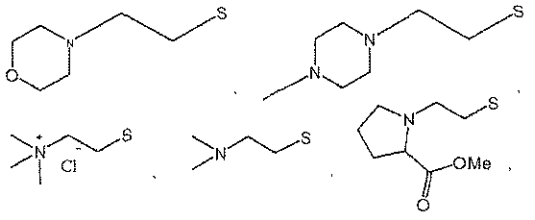
[0004] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein W is O.

[0005] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein R^1 is selected from:



and

R^2 is selected from:



[0006] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N′-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

[0007] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0008] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0009] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

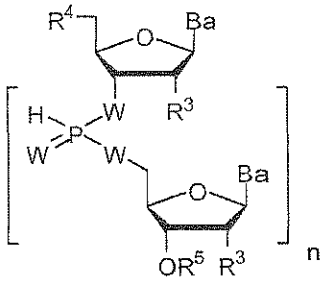
[0010] One embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb comprising the steps of:

i) reacting a H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIb to provide a phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb;

wherein,

the H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib has the following structure:



Structure Ib

wherein,

W is independently selected from O, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

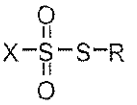
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIb has the following structure:



Structure IIb wherein,

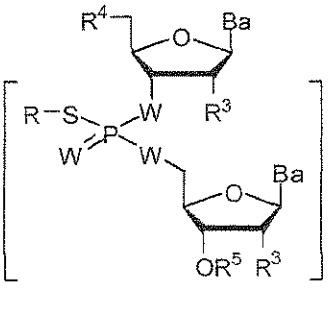
X is alkyl, cycloalkyl, aryl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the chiral phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb has the following structure:



n Structure IIIb

wherein,

W is independently selected from O, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^a is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

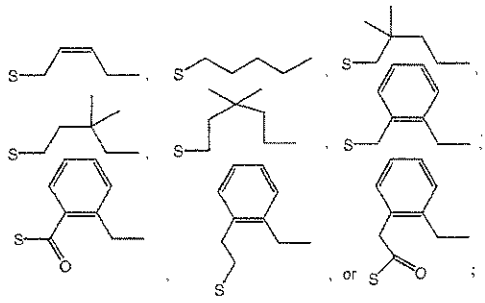
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

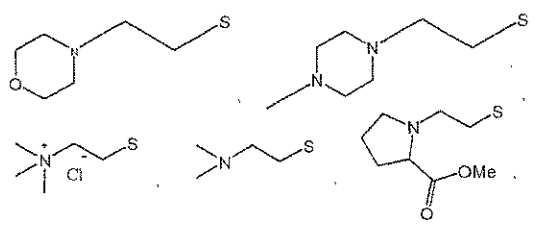
[0011] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein W is O.

[0012] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein R^1 is selected from:



and

R^2 is selected from:



[0013] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;
1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;
1-(trimethylsilyl)imidazole;
N-trimethylsilyl-N-methyl trifluoroacetamide;
bis(dimethylamino)dimethylsilane;
bromotrimethylsilane;
chlorodimethyl(pentafluorophenyl)silane;
chlorotriethylsilane;
chlorotriisopropylsilane;
chlorotrimethylsilane;
dichlorodimethylsilane;
hexamethyldisilazane;
N,N′-bis(trimethylsilyl)urea;
N,N-bis(trimethylsilyl)methylamine;
N,N-dimethyltrimethylsilylamine;
N,O-bis(trimethylsilyl)acetamide;
N,O-bis(trimethylsilyl)carbamate;
N,O-bis(trimethylsilyl)trifluoroacetamide;
N-methyl-N-(trimethylsilyl)trifluoroacetamide;
N-methyl-N-trimethylsilylacetamide;
N-methyl-N-trimethylsilylheptafluorobutyramide;
N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;
N-methyl-N-trimethylsilylheptafluorobutyramide;
trimethylsilyltriflate;
triethylsilyltriflate;
triisopropylsilyltriflate; or
tert-butyldimethylsilyltriflate.

[0014] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0015] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0016] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

[0017] One embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIc comprising the steps of:

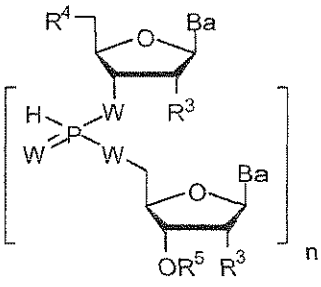
i) reacting a H-phosphonate of structure Ic with an silylating reagent to provide a silyloxyphosphonate;

ii) reacting the silyloxyphosphonate with a bis(thiosulfonate) reagent of structure IVc to provide a phosphorothiotriester comprising a thiosulfonate group of structure Vc;

iii) reacting the phosphorothiotriester comprising a thiosulfonate group of structure Vc with a nucleophile of structure VIc to provide the phosphorothiotriesters of structure IIIc;

wherein,

the H-phosphonate of structure Ic has the following structure:



Structure Ic

wherein,

W is independently selected from O, S, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^c is O, NR^u, or S;

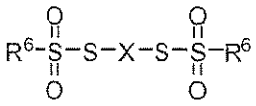
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the bis(thiosulfonate) reagent of structure IVc has the following structure:



Structure IVc wherein,

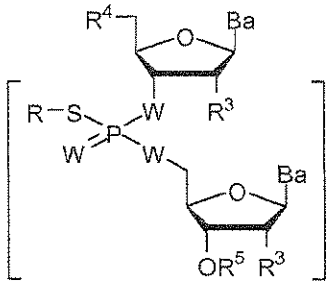
X is alkylene, alkenylene, arylene, or heteroarylene;

each R^6 is independently alkyl, cycloalkyl, aryl, or heteroaryl;

the nucleophile of structure VIc has the following structure:

R^7-SH, wherein R^7 is selected from alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

and phosphorothiotriesters of structure IIIc has the following structure:



n Structure IIIc

wherein,

W is independently selected from O, S, NH, or CH_2;

R is R^7-S-S-X-

R^7 is alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

X is alkylene, alkenylene, arylene, or heteroarylene;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^a, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid;

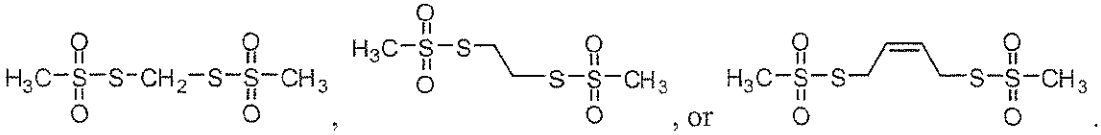
n is between 1 and about 200; and

wherein the phosphorous linkages of the H-phosphonate of structure Ic, the phosphorothiotriester comprising a thiosulfonate group of structure Vc, and the phosphorothiotriesters of structure IIIc may optionally comprise non-stereorandom phosphorous linkages.

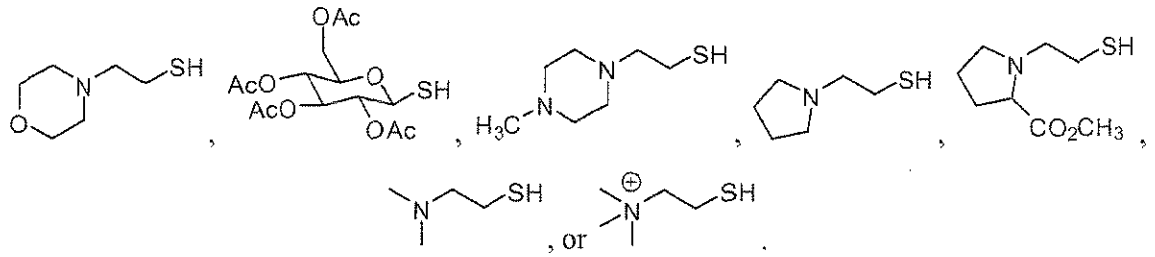
[0018] Another embodiment provides the process wherein the phosphorothiotriesters of structure IIIb comprise non-stereorandom phosphorous linkages and the H-phosphonate of structure Ic comprise non-stereorandom phosphorous linkages; and W is independently selected from O, NH, or CH_2. Another embodiment provides the process wherein W is O.

[0019] Another embodiment provides the process wherein R^6 is methyl.

[0020] Another embodiment provides the process wherein bis(thiosulfonate) reagent of structure IVc is selected from:



[0021] Another embodiment provides the process wherein the nucleophile of structure VIc has the following structure:



[0022] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N'-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

[0023] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0024] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0025] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

INCORPORATION BY REFERENCE

[0026] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0028] Figure 1 provides the ^{31}P NMR spectrum of Compound 100S in CD_3CN as described in Example 6;

[0029] Figure 2 provides the ^{31} P NMR spectrum of Compound 100S in CD_3CN after adding BSTFA as described in Example 6;

[0030] Figure 3 provides the ^{31} P NMR spectrum of Compound 100S in CD_3CN after adding BSTFA, TEA and MTS as described in Example 6;

[0031] Figure 4 provides the ^{31} P NMR spectrum of Compound 100R in CD_3CN as described in Example 6;

[0032] Figure 5 provides the ^{31} P NMR spectrum of Compound 100R in CD_3CN as described in Example 6; and

[0033] Figure 6 provides the ^{31} P NMR spectrum of Compound 100R in CD_3CN after adding BSTFA, TEA and MTS as described in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

[0034] Unless otherwise stated, the following terms used in this application, including the specification and claims, have the definitions given below. It must be noted that, as used in the specification and the appended claims, the singular forms "a" "an" and "the" include plural referents unless the context clearly dictates otherwise. Unless otherwise indicated, conventional methods of mass spectroscopy, NMR, HPLC, protein chemistry, biochemistry, recombinant DNA techniques and pharmacology are employed. In this application, the use of "or" or "and" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes" and "included" is not limiting.

Certain Chemical Terminology

[0035] Unless otherwise noted, the use of general chemical terms, such as though not limited to "alkyl," "amine," "aryl," are unsubstituted.

[0036] As used herein, C_1-C_x includes C_1-C_2, C_1-C_3 . . . C_1-C_x. By way of example only, a group designated as "C_1-C_4" indicates that there are one to four carbon atoms in the moiety, i.e. groups containing 1 carbon atom, 2 carbon atoms, 3 carbon atoms or 4 carbon atoms, as well as the ranges C_1-C_2 and C_1-C_3. Thus, by way of example only, "C_1-C_4 alkyl" indicates that there are one to four carbon atoms in the alkyl group, *i.e.*, the alkyl group is selected from among methyl, ethyl, propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl. Whenever it appears herein, a numerical range such as "1 to 10" refers to each integer in the given range; *e.g.*, "1 to 10 carbon atoms" means that the group may have 1 carbon atom, 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms, 6 carbon atoms, 7 carbon atoms, 8 carbon atoms, 9 carbon atoms, or 10 carbon atoms.

[0037] The terms "heteroatom" or "hetero" as used herein, alone or in combination, refer to an atom other than carbon or hydrogen. Heteroatoms are may be independently selected from among oxygen, nitrogen, sulfur, phosphorous, silicon, selenium and tin but are not limited to these atoms. In embodiments in which two or more heteroatoms are present, the two or more heteroatoms can be the same as each another, or some or all of the two or more heteroatoms can each be different from the others.

[0038] The term "alkyl" as used herein, alone or in combination, refers to a straight-chain or branched-chain saturated hydrocarbon monoradical having from one to about ten carbon atoms, or one to six carbon atoms. Examples include, but are not limited to methyl, ethyl, n-propyl, isopropyl, 2-methyl-1-propyl, 2-methyl-2-propyl, 2-methyl-1-butyl, 3-methyl-1-butyl, 2-methyl-3-butyl, 2,2-dimethyl-1-propyl, 2-methyl-1-pentyl, 3-methyl-1-pentyl, 4-methyl-1-

pentyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 2,2-dimethyl-1-butyl, 3,3-dimethyl-1-butyl, 2-ethyl-1-butyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, isopentyl, neopentyl, tert-amyl and hexyl, and longer alkyl groups, such as heptyl, octyl and the like. Whenever it appears herein, a numerical range such as "C_1-C_6 alkyl" or "C_{1-6} alkyl", means that the alkyl group may consist of 1 carbon atom, 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms or 6 carbon atoms. In one embodiment, the "alkyl" is substituted. Unless otherwise indicated, the "alkyl" is unsubstititued.

[0039] The term "alkenyl" as used herein, alone or in combination, refers to a straight-chain or branched-chain hydrocarbon monoradical having one or more carbon-carbon double-bonds and having from two to about ten carbon atoms, or two to about six carbon atoms. The group may be in either the *cis* or *trans* conformation about the double bond(s), and should be understood to include both isomers. Examples include, but are not limited to ethenyl ($-CH=CH_2$), 1-propenyl ($-CH_2CH=CH_2$), isopropenyl [$-C(CH_3)=CH_2$], butenyl, 1,3-butadienyl and the like. Whenever it appears herein, a numerical range such as "C_2-C_6 alkenyl" or "C_{2-6} alkenyl", means that the alkenyl group may consist of 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms or 6 carbon atoms. In one embodiment, the "alkenyl" is substituted. Unless otherwise indicated, the "alkenyl" is unsubstititued.

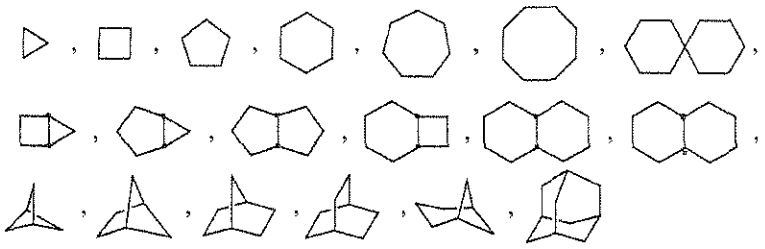
[0040] The term "alkynyl" as used herein, alone or in combination, refers to a straight-chain or branched-chain hydrocarbon monoradical having one or more carbon-carbon triple-bonds and having from two to about ten carbon atoms, or from two to about six carbon atoms. Examples include, but are not limited to ethynyl, 2-propynyl, 2-butynyl, 1,3-butadiynyl and the like. Whenever it appears herein, a numerical range such as "C_2-C_6 alkynyl" or "C_{2-6} alkynyl", means that the alkynyl group may consist of 2 carbon atoms, 3 carbon atoms, 4 carbon atoms, 5 carbon atoms or 6 carbon atoms. In one embodiment, the "alkynyl" is substituted. Unless otherwise indicated, the "alkynyl" is unsubstititued.

[0041] The terms "heteroalkyl", "heteroalkenyl" and "heteroalkynyl" as used herein, alone or in combination, refer to alkyl, alkenyl and alkynyl structures respectively, as described above, in which one or more of the skeletal chain carbon atoms (and any associated hydrogen atoms, as appropriate) are each independently replaced with a heteroatom (i.e. an atom other than carbon, such as though not limited to oxygen, nitrogen, sulfur, silicon, phosphorous, tin or combinations thereof), or heteroatomic group such as though not limited to -O-O-, -S-S-, -O-S-, -S-O-, =N-N=, -N=N-, -N=N-NH-, $-P(O)_2-$, $-O-P(O)_2-$, $-P(O)_2-O-$, $-S(O)-$, $-S(O)_2-$, $-SnH_2-$ and the like.

[0042] The terms "haloalkyl", "haloalkenyl" and "haloalkynyl" as used herein, alone or in combination, refer to alkyl, alkenyl and alkynyl groups respectively, as defined above, in which one or more hydrogen atoms is replaced by fluorine, chlorine, bromine or iodine atoms, or combinations thereof. In some embodiments two or more hydrogen atoms may be replaced with halogen atoms that are the same as each another (e.g. difluoromethyl); in other embodiments two or more hydrogen atoms may be replaced with halogen atoms that are not all the same as each other (e.g. 1-chloro-1-fluoro-1-iodoethyl). Non-limiting examples of haloalkyl groups are fluoromethyl, chloromethyl and bromoethyl. A non-limiting example of a haloalkenyl group is bromoethenyl. A non-limiting example of a haloalkynyl group is chloroethynyl.

[0043] The term "carbon chain" as used herein, alone or in combination, refers to any alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl or heteroalkynyl group, which is linear, cyclic, or any combination thereof. If the chain is part of a linker and that linker comprises one or more rings as part of the core backbone, for purposes of calculating chain length, the "chain" only includes those carbon atoms that compose the bottom or top of a given ring and not both, and where the top and bottom of the ring(s) are not equivalent in length, the shorter distance shall be used in determining the chain length. If the chain contains heteroatoms as part of the backbone, those atoms are not calculated as part of the carbon chain length.

[0044] The term "cycloalkyl" as used herein, alone or in combination, refers to a saturated, hydrocarbon monoradical ring, containing from three to about fifteen ring carbon atoms or from three to about ten ring carbon atoms, though may include additional, non-ring carbon atoms as substituents (e.g. methylcyclopropyl). Whenever it appears herein, a numerical range such as "C_3-C_6 cycloalkyl " or "C_{3-6} cycloalkyl ", means that the cycloalkyl group may consist of 3 carbon atoms, 4 carbon atoms, 5 carbon atoms or 6 carbon atoms, i.e., is cyclopropyl, cyclobutyl, cyclopentyl or cyclohepty, although the present definition also covers the occurrence of the term " cycloalkyl " where no numerical range is designated. The term includes fused, non-fused, bridged and spiro radicals. A fused cycloalkyl may contain from two to four fused rings where the ring of attachment is a cycloalkyl ring, and the other individual rings may be alicyclic, heterocyclic, aromatic, heteroaromatic or any combination thereof. Examples include, but are not limited to cyclopropyl, cyclopentyl, cyclohexyl, decalinyl, and bicyclo [2.2.1] heptyl and adamantyl ring systems. Illustrative examples include, but are not limited to the following moieties:

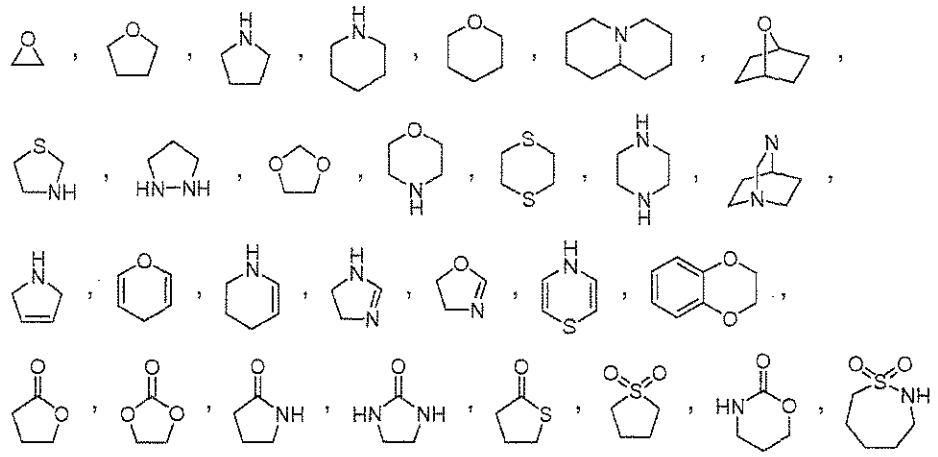


and the like.

In one embodiment, the "cycloalkyl" is substituted. Unless otherwise indicated, the "cycloalkyl" is unsubstititued.

[0045] The terms "non-aromatic heterocyclyl" and "heteroalicyclyl" as used herein, alone or in combination, refer to a saturated, partially unsaturated, or fully unsaturated nonaromatic ring monoradicals containing from three to about twenty ring atoms, where one or more of the ring atoms are an atom other than carbon, independently selected from among oxygen, nitrogen, sulfur, phosphorous, silicon, selenium and tin but are not limited to these atoms. In embodiments in which two or more heteroatoms are present in the ring, the two or more heteroatoms can be the same as each another, or some or all of the two or more heteroatoms can each be different from the others. The terms include fused, non-fused, bridged and spiro radicals. A fused non-aromatic heterocyclic radical may contain from two to four fused rings where the attaching ring is a non-aromatic heterocycle, and the other individual rings may be alicyclic, heterocyclic, aromatic, heteroaromatic or any combination thereof. Fused ring systems may be fused across a single bond or a double bond, as well as across bonds that are carbon-carbon, carbon-hetero atom or hetero atom-hetero atom. The terms also include radicals having from three to about twelve skeletal ring atoms, as well as those having from three to about ten skeletal ring atoms. Attachment of a non-aromatic heterocyclic subunit to its parent molecule can be via a heteroatom or a carbon atom. Likewise, additional substitution can be via a heteroatom or a carbon atom. As a non-limiting example, an imidazolidine non-aromatic heterocycle may be attached to a parent molecule via either of its N atoms (imidazolidin-1-yl or imidazolidin-3-yl) or any of its carbon atoms (imidazolidin-2-yl, imidazolidin-4-yl or imidazolidin-5-yl). In certain embodiments, non-aromatic heterocycles contain one or more carbonyl or thiocarbonyl groups such as, for example, oxo- and thio-containing groups. Examples include, but are not limited to pyrrolidinyl, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, dihydropyranyl, tetrahydrothiopyranyl, piperidino, morpholino, thiomorpholino, thioxanyl, piperazinyl, azetidinyl, oxetanyl, thietanyl, homopiperidinyl, oxepanyl, thiepanyl, oxazepinyl, diazepinyl, thiazepinyl, 1,2,3,6-tetrahydropyridinyl, 2-pyrrolinyl, 3-pyrrolinyl, indolinyl, 2H-pyranyl,

4H-pyranyl, dioxanyl, 1,3-dioxolanyl, pyrazolinyl, dithianyl, dithiolanyl, dihydropyranyl, dihydrothienyl, dihydrofuranyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[4.1.0]heptanyl, 3H-indolyl and quinolizinyl. Illustrative examples of heterocycloalkyl groups, also referred to as non-aromatic heterocycles, include:



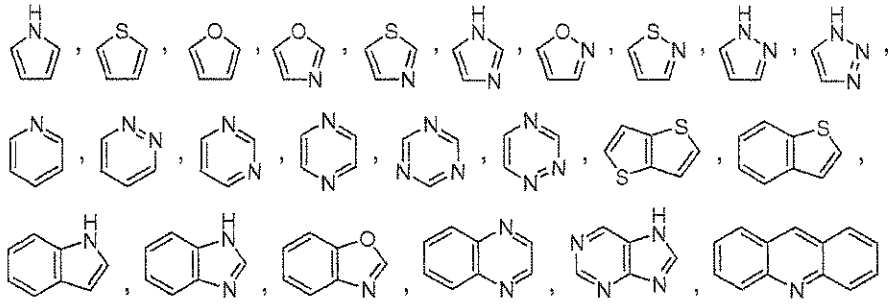
and the like.

[0046] The terms also include all ring forms of the carbohydrates, including but not limited to the monosaccharides, the disaccharides and the oligosaccharides. In one embodiment, the "non-aromatic heterocyclyl" or "heteroalicyclyl" is substituted. Unless otherwise indicated, the "non-aromatic heterocyclyl" or "heteroalicyclyl" is unsubstititued.

[0047] The term "aryl" as used herein, alone or in combination, refers to an aromatic hydrocarbon radical of six to about twenty ring carbon atoms, and includes fused and non-fused aryl rings. A fused aryl ring radical contains from two to four fused rings where the ring of attachment is an aryl ring, and the other individual rings may be alicyclic, heterocyclic, aromatic, heteroaromatic or any combination thereof. Further, the term aryl includes fused and non-fused rings containing from six to about twelve ring carbon atoms, as well as those containing from six to about ten ring carbon atoms. A non-limiting example of a single ring aryl group includes phenyl; a fused ring aryl group includes naphthyl, phenanthrenyl, anthracenyl, azulenyl; and a non-fused bi-aryl group includes biphenyl. In one embodiment, the "aryl" is substituted. Unless otherwise indicated, the "aryl" is unsubstititued.

[0048] The term "heteroaryl" as used herein, alone or in combination, refers to an aromatic monoradicals containing from about five to about twenty skeletal ring atoms, where one or more of the ring atoms is a heteroatom independently selected from among oxygen, nitrogen, sulfur, phosphorous, silicon, selenium and tin but not limited to these atoms and with the proviso that the ring of said group does not contain two adjacent O or S atoms. In

embodiments in which two or more heteroatoms are present in the ring, the two or more heteroatoms can be the same as each another, or some or all of the two or more heteroatoms can each be different from the others. The term heteroaryl includes fused and non-fused heteroaryl radicals having at least one heteroatom. The term heteroaryl also includes fused and non-fused heteroaryls having from five to about twelve skeletal ring atoms, as well as those having from five to about ten skeletal ring atoms. Bonding to a heteroaryl group can be via a carbon atom or a heteroatom. Thus, as a non-limiting example, an imidazole group may be attached to a parent molecule via any of its carbon atoms (imidazol-2-yl, imidazol-4-yl or imidazol-5-yl), or its nitrogen atoms (imidazol-1-yl or imidazol-3-yl). Likewise, a heteroaryl group may be further substituted via any or all of its carbon atoms, and/or any or all of its heteroatoms. A fused heteroaryl radical may contain from two to four fused rings where the ring of attachment is a heteroaromatic ring and the other individual rings may be alicyclic, heterocyclic, aromatic, heteroaromatic or any combination thereof. A non-limiting example of a single ring heteroaryl group includes pyridyl; fused ring heteroaryl groups include benzimidazolyl, quinolinyl, acridinyl; and a non-fused bi-heteroaryl group includes bipyridinyl. Further examples of heteroaryls include, without limitation, furanyl, thienyl, oxazolyl, acridinyl, phenazinyl, benzimidazolyl, benzofuranyl, benzoxazolyl, benzothiazolyl, benzothiadiazolyl, benzothiophenyl, benzoxadiazolyl, benzotriazolyl, imidazolyl, indolyl, isoxazolyl, isoquinolinyl, indolizinyl, isothiazolyl, isoindolyloxadiazolyl, indazolyl, pyridyl, pyridazyl, pyrimidyl, pyrazinyl, pyrrolyl, pyrazinyl, pyrazolyl, purinyl, phthalazinyl, pteridinyl, quinolinyl, quinazolinyl, quinoxalinyl, triazolyl, tetrazolyl, thiazolyl, triazinyl, thiadiazolyl and the like, and their oxides, such as for example pyridyl-N-oxide. Illustrative examples of heteroaryl groups include the following moieties:



and the like.

In one embodiment, the "heteroaryl" is substituted. Unless otherwise indicated, the "heteroaryl" is unsubstitituted.

[0049] The term "heterocyclyl" as used herein, alone or in combination, refers collectively to heteroalicyclyl and heteroaryl groups. Herein, whenever the number of carbon atoms in a

heterocycle is indicated (e.g., C_1-C_6 heterocycle), at least one non-carbon atom (the heteroatom) must be present in the ring. Designations such as "C_1-C_6 heterocycle" refer only to the number of carbon atoms in the ring and do not refer to the total number of atoms in the ring. Designations such as "4-6 membered heterocycle" refer to the total number of atoms that are contained in the ring (i.e., a four, five, or six membered ring, in which at least one atom is a carbon atom, at least one atom is a heteroatom and the remaining two to four atoms are either carbon atoms or heteroatoms). For heterocycles having two or more heteroatoms, those two or more heteroatoms can be the same or different from one another. Non-aromatic heterocyclic groups include groups having only three atoms in the ring, while aromatic heterocyclic groups must have at least five atoms in the ring. Bonding (i.e. attachment to a parent molecule or further substitution) to a heterocycle can be via a heteroatom or a carbon atom. In one embodiment, the "heterocyclyl" is substituted. Unless otherwise indicated, the "heterocycyl" is unsubstititued.

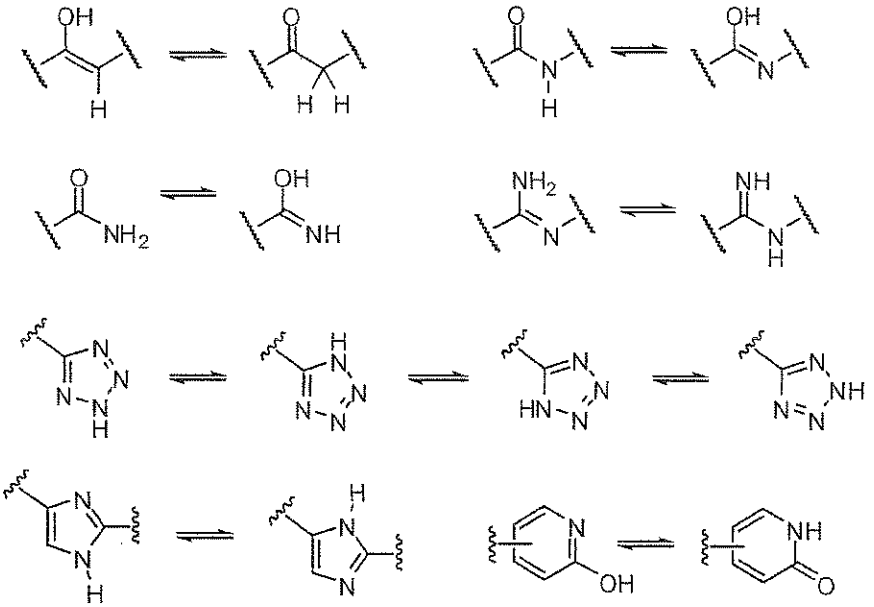
[0050] The terms "halogen", "halo" or "halide" as used herein, alone or in combination refer to fluoro, chloro, bromo and/or iodo.

[0051] The compounds, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, such as (*R*)- or (*S*)-. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *Z* and E geometric isomers (*e.g., cis* or *trans*). Likewise, all possible isomers, as well as their racemic and optically pure forms, and all tautomeric forms are also intended to be included.

[0052] A "stereoisomer" refers to the relationship between two or more compounds made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not superimposable. The term "enantiomer" refers to two stereoisomers that are nonsuperimposeable mirror images of one another. It is contemplated that the various stereoisomers of the compounds disclosed herein, and mixtures thereof, are within the scope of the present disclosure and specifically includes enantiomers.

[0053] A "tautomer" refers to a compound wherein a proton shift from one atom of a molecule to another atom of the same molecule is possible. The compounds presented herein may exist as tautomers. In solutions where tautomerization is possible, a chemical equilibrium of the tautomers will exist. The exact ratio of the tautomers depends on several

factors, including temperature, solvent, and pH. Some examples of tautomeric equilibrium are shown below.



[0054] The term "non-stereorandom phosphorous linkage(s)" as used herein refers to a chiral phosphorous atom in the phosphodiester, or other isosteric linkage type, internucleotide linkage. For embodiments comprising more than one phosphorous internucleotide linkage, the handedness of chirality at phosphorous is independently selected at each phosphorous atom. In one embodiment, the oligonucleotide described herein is a pure diastereomer. In another embodiment, the oligonucleotide is greater that 95% diastereomeric purity. In another embodiment, the oligonucleotide is greater that 90% diastereomeric purity.

[0055] "Optional" or "optionally" means that a subsequently described event or circumstance may or may not occur and that the description includes instances when the event or circumstance occurs and instances in which it does not. For example, "optionally substituted alkyl" means that the alkyl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution.

Certain Nucleic Acid Terminology

[0056] Natural nucleic acids have a phosphate backbone; artificial nucleic acids may contain other types of backbones, but contain the same bases.

[0057] The term "nucleotide" as used herein refers to a monomeric unit of a polynucleotide that consists of a heterocyclic base, a sugar, and one or more phosphate groups. 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. Nucleic acids are linked via phosphate bonds to form nucleic acids, or polynucleotides, though many other linkages are known in the art (such as, though not limited to phosphorothioates, boranophosphates and the like). Artificial nucleic acids include PNAs (peptide nucleic acids), phosphothionates, and other variants of the phosphate backbone of native nucleic acids.

[0058] The term "nucleoside" refers to a moiety wherein a nucleobase or a modified nucleobase is covalently bound to a sugar or modified sugar.

[0059] The term "sugar" refers to a monosaccharide in closed and/or open form. Sugars include, but are not limited to, ribose, deoxyribose, pentofuranose, pentopyranose, and hexopyranose moieties.

[0060] The term "modified sugar" refers to a moiety that can replace a sugar. The modified sugar mimics the spatial arrangement, electronic properties, or some other physicochemical property of a sugar.

[0061] The terms "nucleic acid" and "polynucleotide" as used herein refer to a polymeric form of nucleotides of any length, either ribonucleotides (RNA) or deoxyribonucleotides (DNA). 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 made from nucleotide analogs and modified polynucleotides such as, though not limited to, methylated and/or capped 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 phosphorous-atom bridges. The term encompasses nucleic acids containing any combinations of nucleobases, modified nucleobases, sugars, modified sugars, phosphate bridges or modified phosphorous atom bridges. Examples include, and are not limited to, nucleic acids containing ribose moieties, the nucleic acids containing deoxy-ribose moieties, nucleic acids containing both ribose and deoxyribose moieties, nucleic acids containing ribose and modified ribose moieties. The prefix poly- refers to a nucleic acid containing about 1 to about 10,000 nucleotide monomer units and wherein the prefix oligo- refers to a nucleic acid containing about 1 to about 200 nucleotide monomer units.

[0062] 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).

[0063] The term "modified nucleobase" refers to a moiety that can replace a nucleobase. The 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. 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.

[0064] The term "chiral reagent" refers to a compound that is chiral or enantiopure and can be used for asymmetric induction in nucleic acid synthesis.

[0065] The term "chiral ligand" or "chiral auxiliary" refers to a moiety that is chiral or enantiopure and controls the stereochemical outcome of a reaction.

[0066] In a condensation reaction, the term "condensing reagent" refers to a reagent that activates a less reactive site and renders it more susceptible to attack by a nucleophile.

[0067] The term "blocking group" refers to a group that transiently masks the reactivity of a functional group. The functional group can be subsequently unmasked by removal of the blocking group.

[0068] The term "moiety" refers to a specific segment or functional group of a molecule. Chemical moieties are often recognized chemical entities embedded in or appended to a molecule.

[0069] The term "solid support" refers to any support which enables synthetic mass production of nucleic acids and can be reutilized at need. As used herein, the term refers to a polymer, that is insoluble in the media employed in the reaction steps performed to synthesize nucleic acids, and is derivatized to comprise reactive groups.

[0070] The term "linking moiety" refers to any moiety optionally positioned between the terminal nucleoside and the solid support or between the terminal nucleoside and another nucleoside, nucleotide, or nucleic acid.

[0071] A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found,

inter alia, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

[0072] As used herein, an "antisense" nucleic acid molecule comprises a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule, complementary to an mRNA sequence or complementary to the coding strand of a gene. Accordingly, an antisense nucleic acid molecule can hydrogen bond to a sense nucleic acid molecule.

[0073] As used herein, a "complementary DNA" or "cDNA" includes recombinant polynucleotides synthesized by reverse transcription of mRNA and from which intervening sequences (introns) have been removed.

Synthetic methods for the preparation novel functionalized nucleic acids and nucleic acid prodrugs

[0074] Described herein are methods for the synthesis of novel functionalized nucleic acids and nucleic acid prodrugs. In some embodiments, the nucleic acids comprise chiral phosphorous moieties.

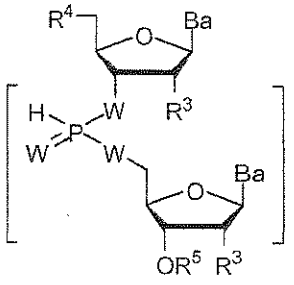
[0075] One embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa comprising the steps of:

i) reacting an H-phosphonate of structure Ia with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIa to provide a phosphorothiotriester of structure IIIa;

wherein,

the H-phosphonate of structure Ia has the following structure:



Structure Ia

wherein,

W is independently selected from O, S, NH, or CH_2;

R^5 is -OH, -SH, $-NR^aR^a$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

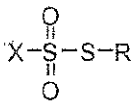
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIa has the following structure:



Structure IIa wherein,

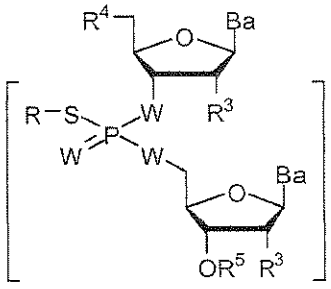
X is alkyl, cycloalkyl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the phosphorothiotriester of structure IIIa has the following structure:



Structure IIIa

wherein,

W is independently selected from O, S, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

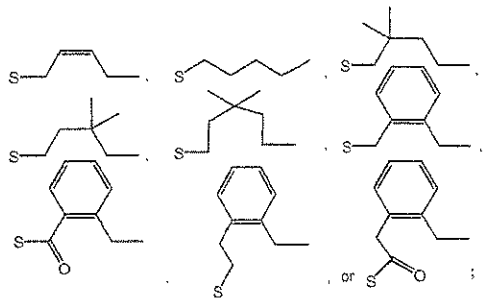
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

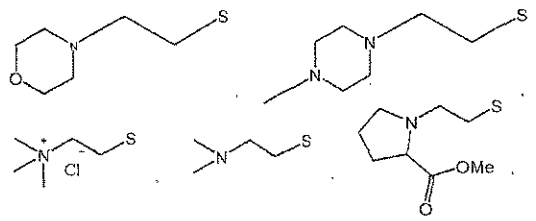
[0076] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein W is O.

[0077] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein R^1 is selected from:



and

R^2 is selected from:



[0078] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N'-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

[0079] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0080] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0081] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

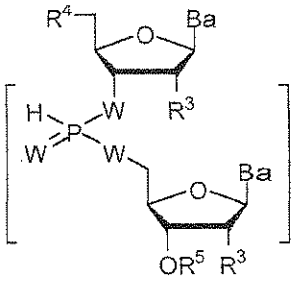
[0082] One embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb comprising the steps of:

i) reacting a H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIb to provide a phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb;

wherein,

the H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib has the following structure:



Structure Ib

wherein,

W is independently selected from O, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

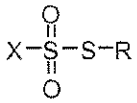
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIb has the following structure:



Structure IIb wherein,

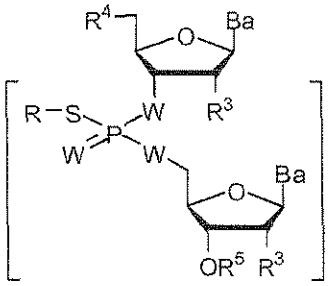
X is alkyl, cycloalkyl, aryl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the chiral phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb has the following structure:



Structure IIIb

wherein,

W is independently selected from O, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^z is O, NR^a, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

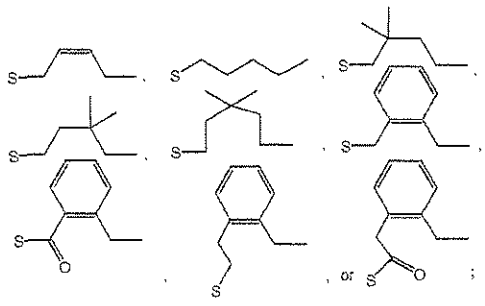
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

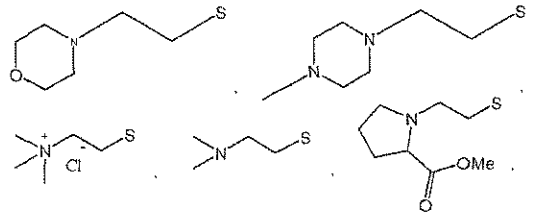
[0083] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein W is O.

[0084] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein R^1 is selected from:



and

R^2 is selected from:



[0085] Another embodiment provides a process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N'-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

[0086] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0087] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0088] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

[0089] One embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIc comprising the steps of:

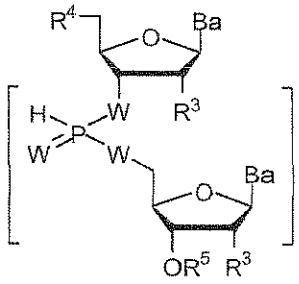
i) reacting a H-phosphonate of structure Ic with an silylating reagent to provide a silyloxyphosphonate;

ii) reacting the silyloxyphosphonate with a bis(thiosulfonate) reagent of structure IVc to provide a phosphorothiotriester comprising a thiosulfonate group of structure Vc;

iii) reacting the phosphorothiotriester comprising a thiosulfonate group of structure Vc with a nucleophile of structure VIc to provide the phosphorothiotriesters of structure IIIc;

wherein,

the H-phosphonate of structure Ic has the following structure:



Structure Ic

wherein,

W is independently selected from O, S, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

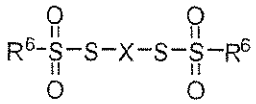
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the bis(thiosulfonate) reagent of structure IVc has the following structure:



Structure IVc wherein,

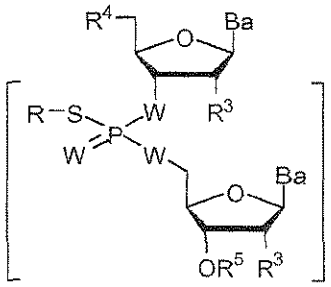
X is alkylene, alkenylene, arylene, or heteroarylene;

each R^6 is independently alkyl, cycloalkyl, aryl, or heteroaryl;

the nucleophile of structure VIc has the following structure:

R^7-SH, wherein R^7 is selected from alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

and phosphorothiotriesters of structure IIIc has the following structure:



n Structure IIIc

wherein,

W is independently selected from O, S, NH, or CH_2;

R is R^7-S-S-X-

R^7 is alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

X is alkylene, alkenylene, arylene, or heteroarylene;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid;

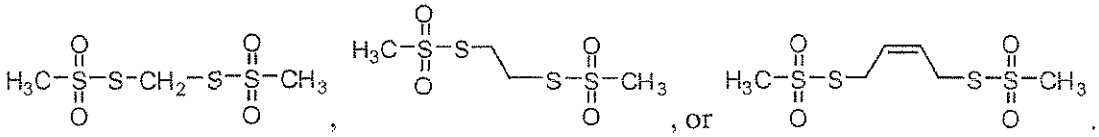
n is between 1 and about 200; and

wherein the phosphorous linkages of the H-phosphonate of structure Ic, the phosphorothiotriester comprising a thiosulfonate group of structure Vc, and the phosphorothiotriesters of structure IIIc may optionally comprise non-stereorandom phosphorous linkages.

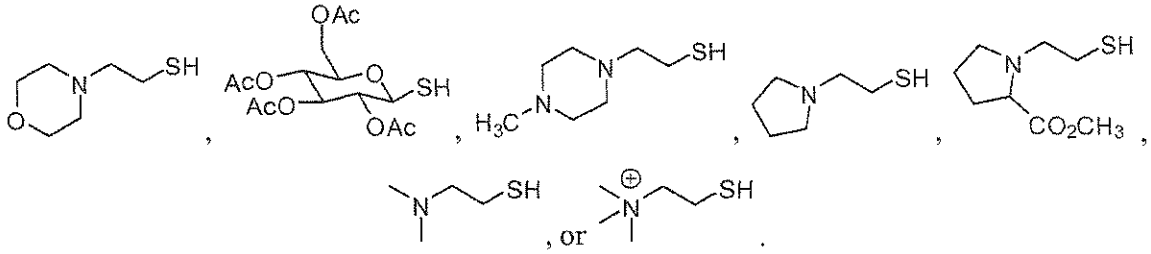
[0090] Another embodiment provides the process wherein the phosphorothiotriesters of structure IIIb comprise non-stereorandom phosphorous linkages and the H-phosphonate of structure Ic comprise non-stereorandom phosphorous linkages; and W is independently selected from O, NH, or CH_2. Another embodiment provides the process wherein W is O.

[0091] Another embodiment provides the process wherein R^6 is methyl.

[0092] Another embodiment provides the process wherein bis(thiosulfonate) reagent of structure IVc is selected from:



[0093] Another embodiment provides the process wherein the nucleophile of structure VIc has the following structure:



[0094] Another embodiment provides a process for the preparation of phosphorothiotriesters of structure IIIa, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N′-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

[0095] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

[0096] Another embodiment provides the process, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

[0097] Another embodiment provides the process, wherein the H-phosphonate is covalently linked to a solid phase.

Modified Oligonucleotides

[0098] Oligonucleotides have several pharmaceutical properties which can be improved through the application of prodrug strategies. In particular, oligonucleotides are rapidly degraded by nucleases and exhibit poor cellular uptake through the cytoplasmic cell membrane (Poijarvi-Virta et al., Curr. Med. Chem. (2006), 13(28);3441-65; Wagner et al., Med. Res. Rev. (2000), 20(6):417-51; Peyrottes et al., Mini Rev. Med. Chem. (2004), 4(4):395-408; Gosselin et al., (1996), 43(1):196-208; Bologna et al., (2002), Antisense & Nucleic Acid Drug Development 12:33-41). In one example, Vives et al., (Nucleic Acids Research (1999), 27(20):4071-76) found that tert-butyl SATE pro-oligonucleotides displayed markedly increased cellular penetration compared to the parent oligonucleotide. Described herein are methods for the synthesis of modified oligonucleotides or pronucleotides.

Reaction Conditions and Reagents used in the methods of the invention.

Conditions

[0099] The steps of reacting a molecule comprising an achiral *H*-phosphonate moiety and a nucleoside comprising a 5'-OH moiety to form a condensed intermediate can occur without isolating any intermediates. In some embodiments, the steps of reacting a molecule comprising an achiral *H*-phosphonate moiety and a nucleoside comprising a 5'-OH moiety to form a condensed intermediate occurs is a one-pot reaction. In an embodiment, a molecule comprising an achiral *H*-phosphonate moiety, condensing reagent, chiral reagent, and compound comprising a free nucleophilic moiety are added to the reaction mixture at different times. In another embodiment, a molecule comprising an achiral *H*-phosphonate moiety, condensing reagent, and chiral reagent are present in the same reaction vessel or same pot. In another embodiment, a molecule comprising an achiral *H*-phosphonate moiety, condensing reagent, chiral reagent, and compound comprising a free nucleophilic moiety are present in the same reaction or same pot. This allows the reaction to be performed without isolation of intermediates and eliminates time-consuming steps, resulting in an economical and efficient synthesis. In specific embodiments, the achiral *H*-phosphonate, condensing reagent, chiral amino alcohol, 5'-OH nucleoside are present at the same time in a reaction. In a further embodiment, the formation of the chiral intermediate for condensation is formed in situ and is not isolated prior to the condensation reaction. In another embodiment, a molecule comprising an achiral *H*-phosphonate moiety has been activated by reaction with a condensing reagent, chiral reagent in a different reaction vessel from that used when reacting the chiral intermediate with the compound comprising a free 5'- OH moiety.

Synthesis on solid support

[00100] In some embodiments, the synthesis of the nucleic acid is performed in solution. In other embodiments, the synthesis of the nucleic acid is performed on solid phase. The reactive groups of a solid support may be unprotected or protected. During oligonucleotide synthesis a solid support is treated with various reagents in several synthesis cycles to achieve the stepwise elongation of a growing oligonucleotide chain with individual nucleotide units. The nucleoside unit at the end of the chain which is directly linked to the solid support is termed "the first nucleoside" as used herein. The first nucleoside is bound to the solid support via a linker moiety, *i.e.* a diradical with covalent bonds to both the polymer of the solid support and the nucleoside. The linker stays intact during the synthesis cycles performed to assemble the oligonucleotide chain and is cleaved after the chain assembly to liberate the oligonucleotide from the support.

[00101] Solid supports for solid-phase nucleic acid synthesis include the supports described in, *e.g.*, US patents 4,659,774 , 5,141,813, 4,458,066; Caruthers U.S. Pat. Nos. 4,415,732, 4,458,066, 4,500,707, 4,668,777, 4,973,679, and 5,132,418; Andrus *et al.* U.S. Pat. Nos. 5,047,524, 5,262,530; and Koster U.S. Pat. Nos. 4,725,677 (reissued as Re34,069). In some embodiments, the solid phase is an organic polymer support. In other embodiments, the solid phase is an inorganic polymer support. In some embodiments, the organic polymer support is polystyrene, aminomethyl polystyrene, a polyethylene glycol-polystyrene graft copolymer, polyacrylamide, polymethacrylate, polyvinylalcohol, highly cross-linked polymer (HCP), or other synthetic polymers, carbohydrates such as cellulose and starch or other polymeric carbohydrates, or other organic polymers and any copolymers, composite materials or combination of the above inorganic or organic materials. In other embodiments, the inorganic polymer support is silica, alumina, controlled poreglass (CPG), which is a silica-gel support, or aminopropyl CPG. Other useful solid supports include fluorous solid supports (see *e.g.,* WO/2005/070859), long chain alkylamine (LCAA) controlled pore glass (CPG) solid supports (see *e.g.*, S. P. Adams, K. S. Kavka, E. J. Wykes, S. B. Holder and G. R. Galluppi, *J. Am. Chem. Soc.*, **1983**, *105*, 661-663; G. R. Gough, M. J. Bruden and P. T. Gilham, *Tetrahedron Lett.*, **1981**, *22*, 4177-4180). Membrane supports and polymeric membranes (see *e.g.* Innovation and Perspectives in Solid Phase Synthesis, Peptides, Proteins and Nucleic Acids, ch 21 pp 157-162, 1994, Ed. Roger Epton and U.S. Pat. No. 4,923,901) are also useful for the synthesis of nucleic acids. Once formed, a membrane can be chemically functionalized for use in nucleic acid synthesis. In addition to the attachment of a functional group to the membrane, the use of a linker or spacer group attached to the

membrane may be used to minimize steric hindrance between the membrane and the synthesized chain.

[00102] Other suitable solid supports include those generally known in the art to be suitable for use in solid phase methodologies, including, for example, glass sold as PrimerTM 200 support, controlled pore glass (CPG), oxalyl-controlled pore glass (see, *e.g.*, Alul, *et al.*, *Nucleic Acids Research*, **1991**, *19*, 1527), TentaGel Support-an aminopolyethyleneglycol derivatized support (see, *e.g.*, Wright, *et al.*, *Tetrahedron Lett.*, **1993**, *34*, 3373), and Poros-a copolymer of polystyrene/divinylbenzene.

[00103] Surface activated polymers have been demonstrated for use in synthesis of natural and modified nucleic acids and proteins on several solid supports mediums. The solid support material can be any polymer suitably uniform in porosity, has sufficient amine content, and sufficiently flexible to undergo any attendant manipulations without losing integrity. Examples of suitable selected materials include nylon, polypropylene, polyester, polytetrafluoroethylene, polystyrene, polycarbonate, and nitrocellulose. Other materials can serve as the solid support, depending on the design of the investigator. In consideration of some designs, for example, a coated metal, in particular gold or platinum can be selected (see *e.g.*, US publication No. 20010055761). In one embodiment of oligonucleotide synthesis, for example, a nucleoside is anchored to a solid support which is functionalized with hydroxyl or amino residues. Alternatively, the solid support is derivatized to provide an acid labile trialkoxytrityl group, such as a trimethoxytrityl group (TMT). Without being bound by theory, it is expected that the presence of the trialkoxytrityl protecting group will permit initial detritylation under conditions commonly used on DNA synthesizers. For a faster release of oligonucleotide material in solution with aqueous ammonia, a diglycoate linker is optionally introduced onto the support.

Linking moiety

[00104] A linking moiety or linker is optionally used to connect the solid support to the compound comprising a free nucleophilic moiety. Suitable linkers are known such as short molecules which serve to connect a solid support to functional groups (*e.g.*, hydroxyl groups) of initial nucleosides molecules in solid phase synthetic techniques. In some embodiments, the linking moiety is a succinamic acid linker, or a succinate linker (-CO-CH_2-CH_2-CO-), or an oxalyl linker (-CO-CO-). In other embodiments, the linking moiety and the nucleoside are bonded together through an ester bond. In other embodiments, the linking moiety and the nucleoside are bonded together through an amide bond. In further embodiments, the linking moiety connects the nucleoside to another nucleotide or nucleic acid. Suitable linkers are

disclosed in, for example, Oligonucleotides And Analogues A Practical Approach, Ekstein, F. Ed., IRL Press, N.Y., 1991, Chapter 1.

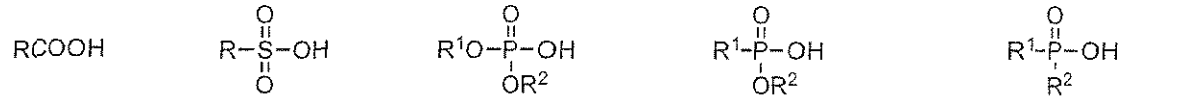
[00105] A linker moiety is used to connect the compound comprising a free nucleophilic moiety to another nucleoside, nucleotide, or nucleic acid. In some embodiments, the linking moiety is a phosphodiester linkage. In other embodiments, the linking moiety is an *H*-phosphonate moiety. In yet other embodiments, the linking moiety is an X-phosphonate moiety.

Solvents for synthesis

[00106] Synthesis of the nucleic acids is performed in an aprotic organic solvent. In some embodiments, the solvent is acetonitrile, pyridine, or NMP. In some embodiments, the solvent is acetone, acetontrile, NMP, ethyl acetate, THF, dioxane, DMF, DMSO, DCM, chloroform, pyridine, 2,6-lutidine, HMPA, HMPT, DMA, glyme, diglyme, sulfone, methyl tert-butyl ether, or combinations thereof. In some embodiments, the solvent is a polar, aprotic organic solvent. In some embodiments, the solvent is anhydrous.

Acidification conditions to remove blocking groups.

[00107] Acidification to remove blocking groups is accomplished by a Brønsted acid or Lewis acid. In some embodiments, acidification is used to remove R^1 blocking groups. Useful Brønsted acids are carboxylic acids, alkylsulfonic acids, arylsulfonic acids, phosphoric acid and its derivatives, phosphonic acid and its derivatives, alkylphosphonic acids and their derivatives, arylphosphonic acids and their derivatives, phosphinic acid, dialkylphosphinic acids, and diarylphosphinic acids which have a pKa (25 °C in water) value of -0.6 (trifluoroacetic acid) to 4.76 (acetic acid) in an organic solvent or water (in the case of 80% acetic acid). The concentration of the acid (1 to 80%) used in the acidification step depends on the acidity of the acid. Consideration to the acid strength must be taken into account as strong acid conditions will result in depurination/depyrimidination, wherein purinyl or pyrimidinyl bases are cleaved from ribose ring.



[00108] In some embodiments, acidification is accomplished by a Lewis acid in an organic solvent. Useful Lewis acids are ZnX_2 wherein X is Cl, Br, I, or CF_3SO_3.

[00109] In some embodiments, the acidifying comprises adding an amount of a Brønsted or Lewis acid effective to convert the condensed intermediate into the compound of Formula 4 without removing purine moieties from the condensed intermediate.

[00110] Acids that are useful in the acidifying step also include, but are not limited to 10% phosphoric acid in an organic solvent, 10% hydrochloric acid in an organic solvent, 1% trifluoroacetic acid in an organic solvent, 3% dichloroacetic acid in an organic solvent or 80% acetic acid in water. The concentration of any Brønsted or Lewis acid used in the process is selected such that the concentration of the acid does not exceed a concentration that causes cleavage of the nucleobase from the sugar moiety.

[00111] In some embodiments, acidification comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, acidification comprises adding about 0.1% to about 8% trifluoroacetic acid in an organic solvent. In other embodiments, acidification comprises adding 3% dichloroacetic acid in an organic solvent. In other embodiments, acidification comprises adding about 0.1% to about 10% dichloroacetic acid in an organic solvent. In yet other embodiments, acidification comprises adding 3% trichloroacetic acid in an organic solvent. In yet other embodiments, acidification comprises adding about 0.1% to about 10% trichloroacetic acid in an organic solvent. In some embodiments, acidification comprises adding 80% acetic acid in water. In some embodiments, acidification comprises adding about 50% to about 90%, or about 50% to about 80%, about 50% to about 70%, about 50% to about 60%, about 70% to about 90% acetic acid in water. In some embodiments, the acidification comprises the further addition of cation scavengers to the acidic solvent. In specific embodiments, the cation scavengers can be triethylsilane or triisopropylsilane. In some embodiments, R^1 is deblocked prior to the step of acidifying the condensed intermediate. In some embodiments, R^1 is deblocked by acidification, which comprises adding 1% trifluoroacetic acid in an organic solvent. In some embodiments, R^1 is deblocked by acidification, which comprises adding 3% dichloroacetic acid in an organic solvent. In some embodiments, R^1 is deblocked by acidification, which comprises adding 3% trichloroacetic acid in an organic solvent.

Removal of blocking moieities or groups

[00112] Functional groups such as hydroxyl or amino moieties which are located on nucleobases or sugar moieties are routinely blocked with blocking (protecting) groups (moieties) during synthesis and subsequently deblocked. In general, a blocking group renders a chemical functionality of a molecule inert to specific reaction conditions and can later be removed from such functionality in a molecule without substantially damaging the remainder

of the molecule (see *e.g.*, Green and Wuts, Protective Groups in Organic Synthesis, 2nd Ed., John Wiley & Sons, New York, 1991). For example, amino groups can be blocked with nitrogen blocking groups such as phthalimido, 9-fludrenylmethoxycarbonyl (FMOC), triphenylmethylsulfenyl, *t*-BOC, 4,4'-dimethoxytrityl (DMTr), 4-methoxytrityl (MMTr), 9-phenylxanthin-9-yl (Pixyl), trityl (Tr), or 9-(*p*-methoxyphenyl)xanthin-9-yl (MOX). Carboxyl groups can be protected as acetyl groups. Hydroxy groups can be protected such as tetrahydropyranyl (THP), *t*-butyldimethylsilyl (TBDMS), 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (Ctmp), 1-(2-fluorophenyl)-4-methoxypiperidin-4-yl (Fpmp), 1-(2-chloroethoxy)ethyl, 3-methoxy-1,5-dicarbomethoxypentan-3-yl (MDP), bis(2-acetoxyethoxy)methyl (ACE), triisopropylsilyloxymethyl (TOM), 1-(2-cyanoethoxy)ethyl (CEE), 2-cyanoethoxymethyl (CEM), [4-(*N*-dichloroacetyl-*N*-methylamino)benzyloxy]methyl, 2-cyanoethyl (CN), pivaloyloxymethyl (PivOM), levunyloxymethyl (ALE). Other representative hydroxyl blocking groups have been described (see *e.g.*, Beaucage *et al.*, *Tetrahedron*, **1992**, *46*, 2223). In some embodiments, hydroxyl blocking groups are acid-labile groups, such as the trityl, monomethoxytrityl, dimethoxytrityl, trimethoxytrityl, 9-phenylxanthin-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthin-9-yl (MOX). Chemical functional groups can also be blocked by including them in a precursor form. Thus an azido group can be considered a blocked form of an amine as the azido group is easily converted to the amine. Further representative protecting groups utilized in nucleic acid synthesis are known (see e.g. Agrawal et al., Protocols for Oligonucleotide Conjugates, Eds., Humana Press, New Jersey, 1994, Vol. 26, pp. 1-72).

[00113] Various methods are known and used for removal of blocking groups from the nucleic acids. In some embodiments, all blocking groups are removed. In other embodiments, the blocking groups are partially removed. In yet other embodiments, reaction conditions can be adjusted to remove blocking groups on certain moieties. In certain embodiments where R^2 is a blocking group, removal of the blocking group at R^2 is orthogonal to the removal of the blocking group at R^1. The blocking groups at R^1 and R^2 remain intact during the synthesis steps and are collectively removed after the chain assembly. In some embodiments, the R^2 blocking group are removed simultaneously with the cleavage of the nucleic acids from the solid support and with the removal of the nucleobase blocking groups. In specific embodiments, the blocking group at R^1 is removed while the blocking groups at R^2 and nucleobases remain intact. Blocking groups at R^1 are cleavable on solid supports with an organic base such as a primary amine, a secondary amine,

or a mixture thereof. Deblocking of the R^1 position is commonly referred to as front end deprotection.

[00114] In an embodiment, the nucleobase blocking groups, if present, are cleavable after the assembly of the respective nucleic acid with an acidic reagent. In another embodiment, one or more of the nucleobase blocking groups is cleavable under neither acidic nor basic conditions, *e.g.* cleavable with fluoride salts or hydrofluoric acid complexes. In yet another embodiment, one or more of the nucleobase blocking groups are cleavable after the assembly of the respective nucleic acid in the presence of base or a basic solvent, and wherein the nucleobase blocking group is stable to the conditions of the front end deprotection step with amines.

[00115] In some embodiments, blocking groups for nucleobases are not required. In other embodiments, blocking groups for nucleobases are required. In yet other embodiments, certain nucleobases require blocking group while other nucleobases do not require blocking groups. In embodiments where the nucleobases are blocked, the blocking groups are either completely or partially removed under conditions appropriate to remove the blocking group at the front end. For example, R^1 can denote OR^a, wherein R^a is acyl, and Ba denotes guanine blocked with an acyl group including, but not limited to isobutyryl, acetyl or 4-(*tert*-butylphenoxy)acetyl. The acyl groups at R^1 and Ba will be removed or partially removed during the same deblocking step.

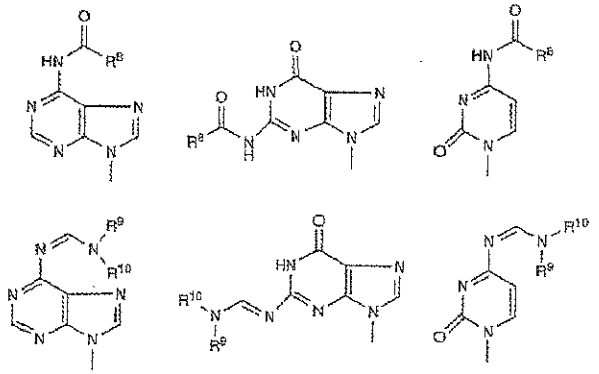
Stereochemistry of Oligonucleoside Phosphorothioate Linkages

[00116] Oligonucleoside phosphorothioates have shown therapeutic potential (Stein *et al.,* Science (1993), 261:1004-12; Agrawal *et al.,* Antisence Res. and Dev. (1992), 2:261-66; Bayever *et al.,* Antisense Res. and Dev. (1993), 3:383-390). Oligonucleoside phosphorothioates prepared without regard to the sterochemistry of the phosphorothioate exist as a mixture of 2^n diastereomers, where n is the number of internucleotide phosphorothioates linkages. The chemical and biological properties of these diastereomeric phosphorothioates can be distinct. For example, Wada *et al* (Nucleic Acids Symposium Series No. 51 p. 119-120; doi:10.1093/nass/nrm060) found that stereodefined-(Rp)-$(Ups)_9U/(Ap)_9A$ duplex showed a higher Tm value than that of natural-$(Up)_9U/(Ap)_9A$ and stereodefined-(Sp)-$(Ups)_9U$ did not form a duplex. In another example, in a study by Tang *et al.,* (Nucleosides Nucleotides (1995), 14:985-990) stereopure Rp-oligodeoxyribonucleoside phosphorothioates were found to possess lower stability to nucleases endogenous to human serum that the parent oligodeoxyribonucleoside phosphorothioates with undefined phosphorous chirality.

Nucleobases and Modified Nucleobases

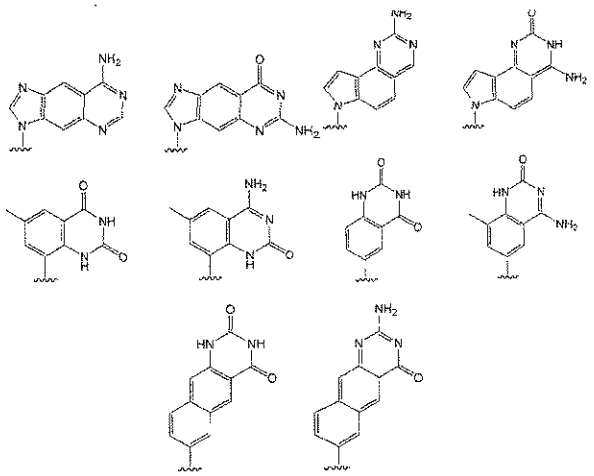
[00117] The nucleobase Ba utilized in the compounds and methods described herein is a natural nucleobase or a modified nucleobase derived from natural nucleobases. Examples include, but are not limited to, uracil, thymine, adenine, cytosine, and guanine having their respective amino groups protected 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). The modified nucleobases 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, are also contemplated as Ba moieties of the compounds and methods described herein.

[00118] Compounds represented by the following general formulae are also contemplated as modified nucleobases:

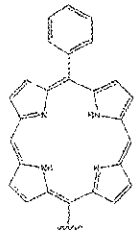


[00119] In the formulae above, R^8 is a linear or branched alkyl, aryl, aralkyl, or aryloxylalkyl group having 1 to 15 carbon atoms, including, by way of example only, a methyl, isopropyl, phenyl, benzyl, or phenoxymethyl group; and each of R^9 and R^{10} represents a linear or branched alkyl group having 1 to 4 carbon atoms.

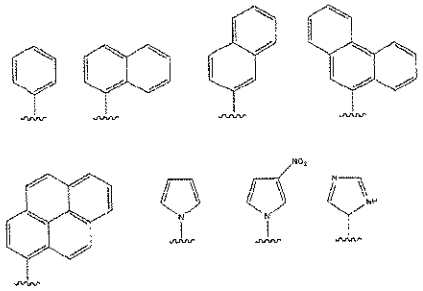
[00120] Modified nucleobases also include expanded-size nucleobases in which one or more benzene rings has been added. Nucleic base replacements described in the Glen Research catalog (www.glenresearch.com); Krueger AT *et al*, *Acc. Chem. Res.*, **2007**, *40*, 141-150; Kool, ET, *Acc. Chem. Res.*, **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; Hirao, I., *Curr. Opin. Chem. Biol.*, **2006**, *10*, 622-627, are contemplated as useful for the synthesis of the nucleic acids described herein. Some examples of these expanded-size nucleobases are shown below:



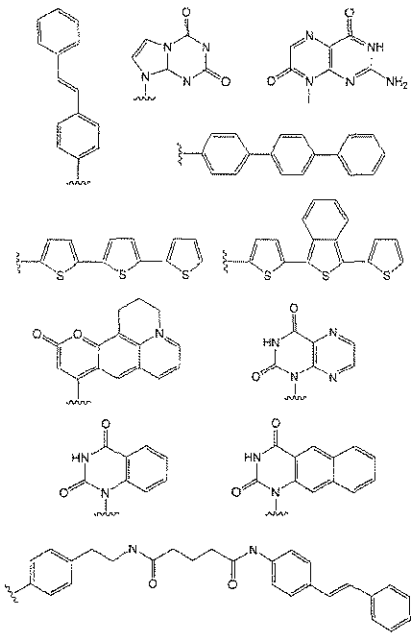
[00121] Herein, modified nucleobases also encompass structures that are not considered nucleobases but are other moieties such as, but not limited to, corrin- or porphyrin-derived rings. Porphyrin-derived base replacements have been described in Morales-Rojas, H and Kool, ET, *Org. Lett.*, **2002**, *4*, 4377-4380. Shown below is an example of a porphyrin-derived ring which can be used as a base replacement:



[00122] Other modified nucleobases also include base replacements such as those shown below:



[00123] Modified nucleobases which are fluorescent are also contemplated. Non-limiting examples of these base replacements include phenanthrene, pyrene, stillbene, isoxanthine, isozanthopterin, terphenyl, terthiophene, benzoterthiophene, coumarin, lumazine, tethered stillbene, benzo-uracil, and naphtho-uracil, as shown below:



[00124] The modified nucleobases can be unsubstituted or contain further substitutions such as heteroatoms, alkyl groups, or linking moieties connected to fluorescent moieties, biotin or avidin moieties, or other protein or peptides. Modified nucleobases also include certain `universal bases` that are not nucleobases in the most classical sense, but function similarly to nucleobases. One representative example of such a universal base is 3-nitropyrrole.

[00125] Other nucleosides can also be used in the process disclosed herein and include nucleosides that incorporate modified nucleobases, or nucleobases covalently bound to modified sugars. Some examples of nucleosides that incorporate modified nucleobases include 4-acetylcytidine; 5-(carboxyhydroxylmethyl)uridine; 2'-*O*-methylcytidine; 5-carboxymethylaminomethyl-2-thiouridine; 5-carboxymethylaminomethyluridine; dihydrouridine; 2'-*O*-methylpseudouridine; beta,D-galactosylqueosine; 2'-*O*-methylguanosine; N^6-isopentenyladenosine; 1-methyladenosine; 1-methylpseudouridine; 1-methylguanosine; l-methylinosine; 2,2-dimethylguanosine; 2-methyladenosine; 2-methylguanosine; N^7-methylguanosine; 3-methyl-cytidine; 5-methylcytidine; N^6-methyladenosine; 7-methylguanosine; 5-methylaminoethyluridine; 5-methoxyaminomethyl-2-thiouridine; beta,D-mannosylqueosine; 5-methoxycarbonylmethyluridine; 5-methoxyuridine; 2-methylthio-N^6-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.

[00126] In some embodiments, nucleosides include 6′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 6′-position and include the analogs described in US Patent No. 7,399,845. In other embodiments, nucleosides include 5′-modified bicyclic nucleoside analogs that have either (*R*) or (*S*)-chirality at the 5′-position and include the analogs described in US Patent Application Publication No. 20070287831.

[00127] In some embodiments, the nucleobases or modified nucleobases comprises biomolecule binding moieties such as antibodies, antibody fragments, biotin, avidin, streptavidin, receptor ligands, or chelating moieties. In other embodiments, Ba is 5-bromouracil, 5-iodouracil, or 2,6-diaminopurine. In yet other embodiments, Ba is modified by substitution with a fluorescent or biomolecule binding moiety. In some embodiments, the substituent on Ba is a fluorescent moiety. In other embodiments, the substituent on Ba is biotin or avidin.

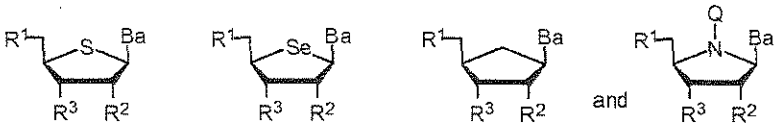
Modified sugars of the nucleotide/nucleoside.

[00128] The most common naturally occurring nucleotides are ribose sugars linked to the nucleobases adenosine (A), cytosine (C), guanine (G), and thymine (T) or uracil (U). Also contemplated are modified nucleotides wherein the phosphate group or the modified phosphorous atom moieties in the nucleotides can be linked to various positions of the sugar or modified sugar. As non-limiting examples, the phosphate group or the modified phosphorous-atom moiety can be linked to the 2′, 3′, 4′ or 5′ hydroxyl moiety of a sugar or modified sugar. Nucleotides that incorporate the modified nucleobases described above can also be used in the process disclosed herein. In some embodiments, nucleotides or modified nucleotides comprising an unprotected -OH moiety are used in the process disclosed herein.

[00129] In addition to the ribose moiety described in Schemes 1-4b, other modified sugars can also be incorporated in the nucleic acids disclosed herein. In some embodiments, the modified sugars contain one or more substituents at the 2′ position including one of the following: F; CF_3, CN, N_3, NO, NO_2, O-, S-, or N-alkyl ; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, O-alkyl-N-alkyl or N-alkyl-O-alkyl wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1-C_{10} alkyl or C_2-C_{10} alkenyl and alkynyl. Examples of substituents include, and are not limited to, $O(CH_2)_nOCH_3$, and $O(CH_2)_nNH_2$, wherein n is from 1 to about 10, MOE, DMAOE, DMAEOE. Also contemplated herein are modified sugars described in WO 2001/088198; and Martin *et al.*, *Helv. Chim. Acta*, **1995**, *78*, 486-504. In some embodiments, modified sugars comprise substituted silyl groups, an

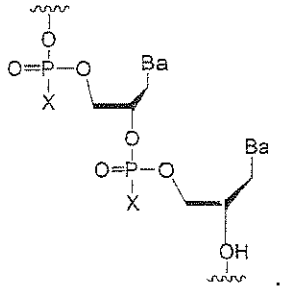
RNA cleaving group, a reporter group, a fluorescent label, an intercalator, a group for improving the pharmacokinetic properties of a nucleic acid, or a group for improving the pharmacodynamic properties of a nucleic acid, and other substituents having similar properties. The modifications may be made at the at the 2′, 3′, 4′, 5′, or 6′ positions of the sugar or modified sugar, including the 3′ position of the sugar on the 3′-terminal nucleotide or in the 5′ position of the 5′-terminal nucleotide.

[00130] Modified sugars also include sugar mimetics such as cyclobutyl or cyclopentyl moieties in place of the pentofuranosyl sugar. Representative United States patents that teach the preparation of such modified sugar structures include, but are not limited to, US Patent Nos.: 4,981,957; 5,118,800; 5,319,080 ; and 5,359,044. Some modified sugars that are contemplated include:

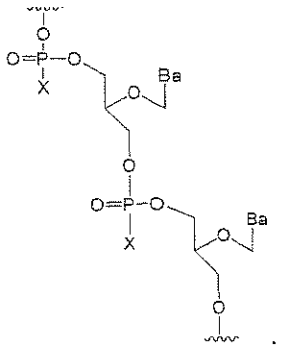


Q = Me, Et, *i*-Pr

[00131] Other non-limiting examples of modified sugars include glycerol, which form glycerol nucleic acid (GNA) analogues. One example of a GNA analogue is shown below and is 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:

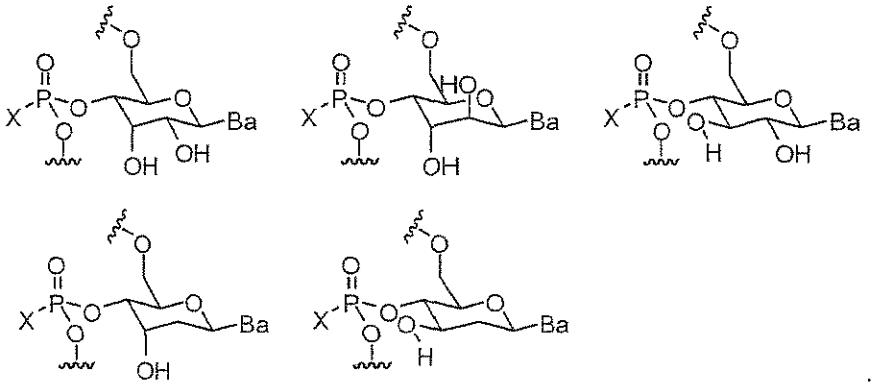


wherein X is as defined herein. Another example of a GNA derived analogue, flexible nucleic acid (FNA) based on the mixed acetal aminal of formyl glycerol, is described in Joyce GF *et al.*, *PNAS*, **1987**, *84*, 4398-4402 and Heuberger BD and Switzer C, *J. Am. Chem. Soc.*, **2008**, *130*, 412-413, and is shown below:

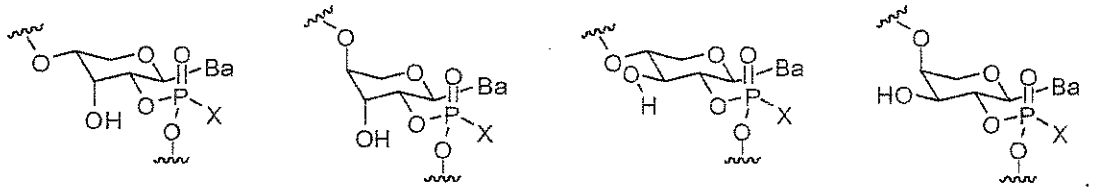


[00132] Other non-limiting examples of modified sugars include hexopyranosyl (6' to 4'), pentopyranosyl (4' to 2'), pentopyranosyl (4' to 3'), or tetrofuranosyl (3' to 2') sugars.

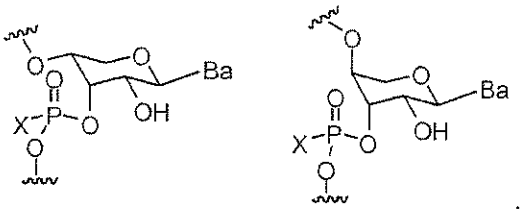
[00133] Hexopyranosyl (6' to 4') sugars contemplated include:



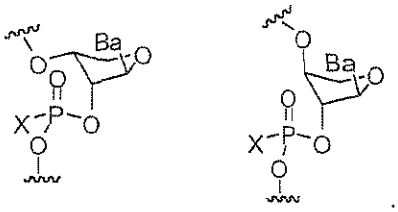
[00134] Pentopyranosyl (4' to 2') sugars contemplated include:



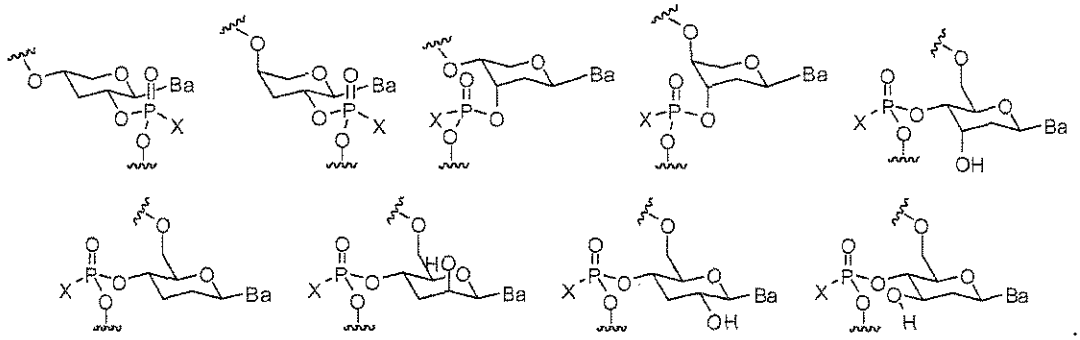
[00135] Pentopyranosyl (4' to 3') sugars contemplated include:



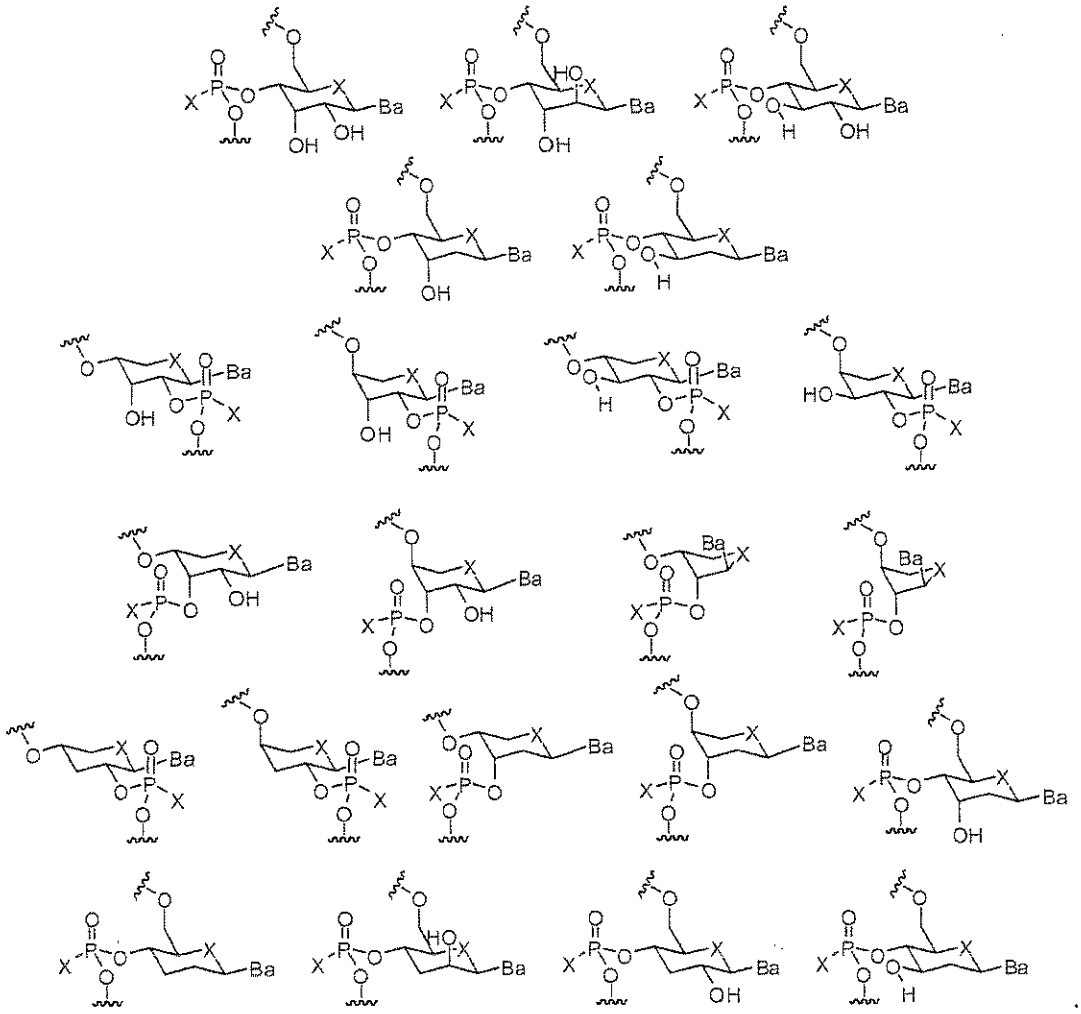
[00136] Tetrofuranosyl (3' to 2') sugars contemplated include:



[00137] Other modified sugars contemplated include:



[00138] Further contemplated are the sugar mimetics illustrated below wherein X is selected from S, Se, CH_2, N-Me, N-Et or N-iPr.



[00139] The modified sugars and sugar mimetics can be prepared by methods known in the art, including, but not limited to: A. Eschenmoser, Science (1999), 284:2118; M. Bohringer *et al,* Helv. Chim. Acta (1992), 75:1416-1477; M. Egli *et al,* J. Am. Chem. Soc. (2006), 128(33):10847-56; A. Eschenmoser in *Chemical Synthesis: Gnosis to Prognosis,* C. Chatgilialoglu and V. Sniekus, Ed., (Kluwer Academic, Netherlands, 1996), p.293; K.-U. Schoning *et al,* Science (2000), 290:1347-1351; A. Eschenmoser *et al,* Helv. Chim. Acta (1992), 75:218; J. Hunziker *et al,* Helv. Chim. Acta (1993), 76:259; G. Otting *et al,* Helv. Chim. Acta (1993), 76:2701; K. Groebke *et al,* Helv. Chim. Acta (1998), 81:375; and A. Eschenmoser, Science (1999), 284:2118.

Blocking groups

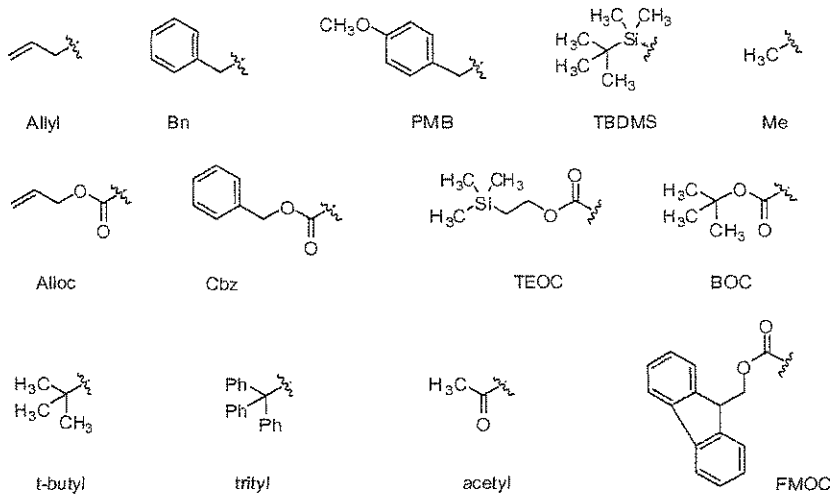
[00140] In the reactions described, it is necessary in certain embodiments to protect reactive functional groups, for example hydroxy, amino, thiol or carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions.

Protecting groups are used to block some or all reactive moieties and prevent such groups from participating in chemical reactions until the protective group is removed. In one embodiment, each protective group is removable by a different means. Protective groups that are cleaved under totally disparate reaction conditions fulfill the requirement of differential removal. In some embodiments, protective groups are removed by acid, base, and/or hydrogenolysis. Groups such as trityl, dimethoxytrityl, acetal and *t*-butyldimethylsilyl are acid labile and are used in certain embodiments to protect carboxy and hydroxy reactive moieties in the presence of amino groups protected with Cbz groups, which are removable by hydrogenolysis, and/or Fmoc groups, which are base labile. In other embodiments, carboxylic acid and hydroxy reactive moieties are blocked with base labile groups such as, but not limited to, methyl, ethyl, and acetyl in the presence of amines blocked with acid labile groups such as *t*-butylcarbamate or with carbamates that are both acid and base stable but hydrolytically removable.

[00141] In another embodiment, hydroxy reactive moieties are blocked with hydrolytically removable protective groups such as the benzyl group, while amine groups capable of hydrogen bonding with acids are blocked with base labile groups such as Fmoc. In another embodiment, carboxylic acid reactive moieties are protected by conversion to simple ester compounds, or they are, in yet another embodiment, blocked with oxidatively-removable protective groups such as 2,4-dimethoxybenzyl, while co-existing amino groups are blocked with fluoride labile silyl or carbamate blocking groups.

[00142] Allyl blocking groups are useful in the presence of acid- and base- protecting groups since the former are stable and can be subsequently removed by metal or pi-acid catalysts. For example, an allyl-blocked hydroxy groups can be deprotected with a Pd(0)-catalyzed reaction in the presence of acid labile *t*-butylcarbamate or base-labile acetate amine protecting groups. Yet another form of protecting group is a resin to which a compound or intermediate is attached. As long as the residue is attached to the resin, that functional group is blocked and cannot react. Once released from the resin, the functional group is available to react.

[00143] Typically blocking/protecting groups useful in the synthesis of the compounds described herein are, by way of example only:



[00144] Representative protecting groups useful to protect nucleotides during synthesis include base labile protecting groups and acid labile protecting groups. Base labile protecting groups are used to protect the exocyclic amino groups of the heterocyclic nucleobases. This type of protection is generally achieved by acylation. Three commonly used acylating groups for this purpose are benzoyl chloride, phenoxyacetic anhydride, and isobutyryl chloride. These protecting groups are stable to the reaction conditions used during nucleic acid synthesis and are cleaved at approximately equal rates during the base treatment at the end of synthesis.

[00145] In some embodiments, the 5'-protecting group is trityl, monomethoxy trityl, dimethoxytrityl, trimethoxytrityl, 2-chlorotrityl, DATE, TBTr, 9-phenylxanthine-9-yl (Pixyl), or 9-(*p*-methoxyphenyl)xanthine-9-yl (MOX).

[00146] In some embodiments, thiol moieties are incorporated in the compounds described herein and are protected. In some embodiments, the protecting groups include, but are not limited to, pixyl, trityl, benzyl, *p*-methoxybenzyl (PMB), or *tert*-butyl (*t*-Bu).

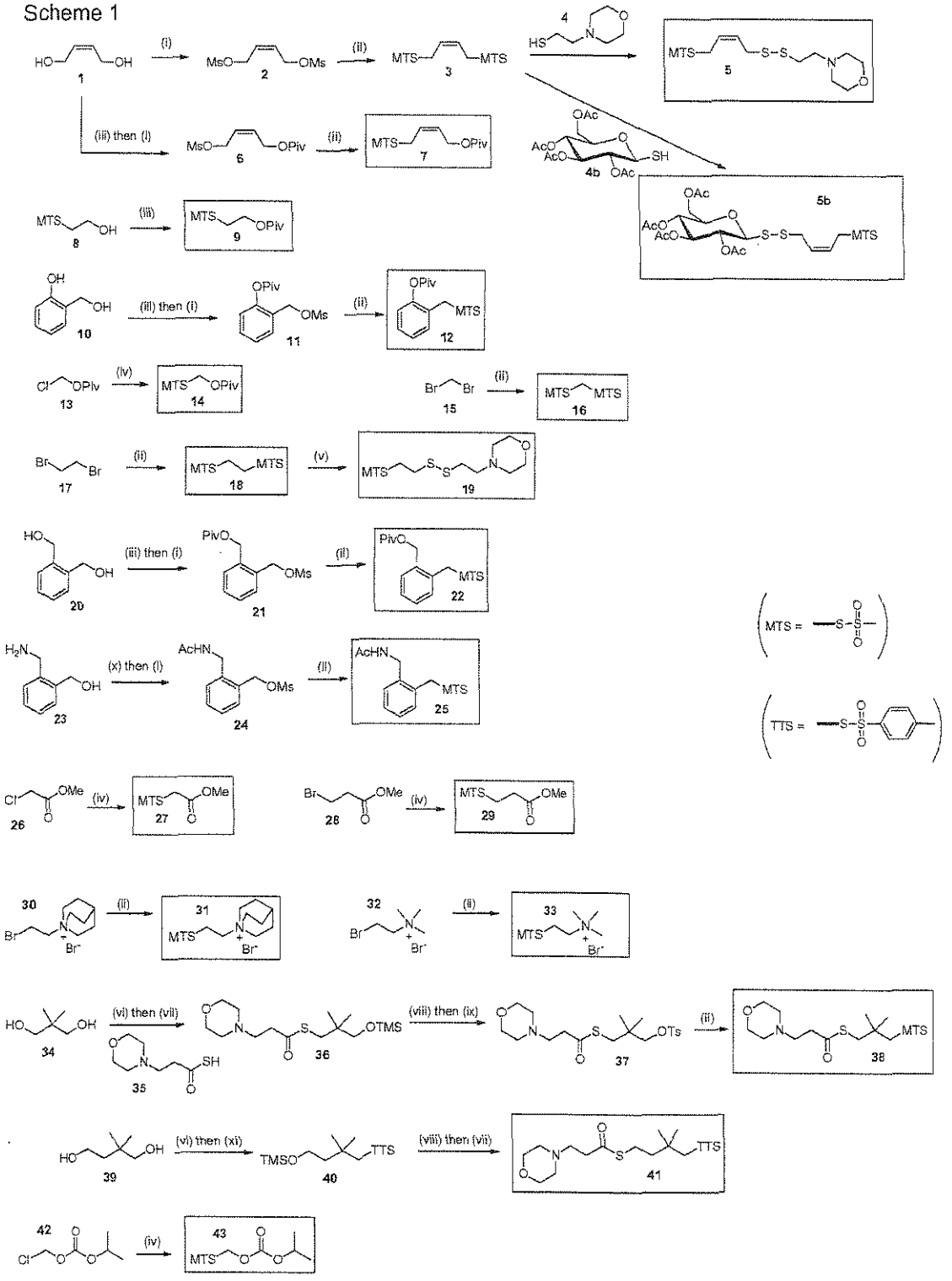
[00147] Other protecting groups, plus a detailed description of techniques applicable to the creation of protecting groups and their removal are described in Greene and Wuts, Protective Groups in Organic Synthesis, 3rd Ed., John Wiley & Sons, New York, NY, 1999, and Kocienski, Protective Groups, Thieme Verlag, New York, NY, 1994, which are incorporated herein by reference for such disclosure.

[00148] The examples provided below further illustrate and exemplify the compounds of the present invention and methods of preparing such compounds. It is to be understood that the scope of the present invention is not limited in any way by the scope of the following examples and preparations.

EXAMPLES

Example 1 – Synthesis of methanethiosulfonate reagents

Scheme 1



(i) MsCl, NEt_3, (ii) NaMTS, (iii) PivCl, NEt_3, (iv) NaMTS, NaI, (v) compound 4, (vi) TMSCl, NEt_3, (vii) compound **35**, DEAD, PPh_3, (viii) TBAF, (ix) TsCl, Pyridine, (x) Ac_2O, Pyridine (xi) NaTTS

[00149] **Compound 2:** A solution of (Z)-but-2-ene-1,4-diol (0.93 ml, 11.3 mmol) and triethylamine (3.3 ml, 24 mmol) in DCM (50 mL) was added in a dropwise fashion to a stirring ice cold solution of methanesulfonyl chloride (1.9 ml, 24 mmol) in DCM (50 mL). After stirring for 0.5h at r.t. the mixture was poured onto ice and extracted. The organic layer was collected, dried ($MgSO_4$), filtered and reduced to 2.66 g, 96% of compound **2**, which was judged by NMR to be sufficiently pure for direct use in the next step of the reaction.

[00150] ^{1}H NMR (399 MHz, $CDCl_3$) δ 5.94 (ddd, J = 5.4, 4.1, 1.3 Hz, 2H), 4.83 (dd, J = 4.1, 1.3 Hz, 4H), 3.04 (s, 6H); ^{13}C NMR 128.34, 64.38, 38.27; MS (ESI +ve): calc ($M+NH_4$): 262.04, found: 262.05. R_f = 0.3 (1:1 EtOAc/hexane).

[00151] **Compound 3:** A solution of sodium methanesulfonothioate (1.51 g, 11.3 mmol) in MeOH (20 ml) was treated with neat (Z)-but-2-ene-1,4-diyl dimethanesulfonate (1.25 g, 5.12 mmol) at r.t. After 5 min, precipitation was observed to occur. After 36 h, the mixture was partitioned between water and DCM. The organic layer was separated, dried ($MgSO_4$), filtered and reduced to afford a colorless oil. Column chromatography (ISCO) gave the pure product as a pale colorless oil. Column chromatography gave pure compound **3** (0.89 g, 63%) as a colorless oil.

[00152] ^{1}H NMR (399 MHz, $CDCl_3$) δ 5.84 (ddd, J = 6.6, 5.1, 1.5 Hz, 2H), 3.92 (dd, J = 5.1, 1.5 HZ, 4H), 3.33 (s, 6H); ^{13}C NMR 128.1, 51.47, 33.13; MS (ESI +ve): calc ($M+NH_4$): 294.00, found: 294.04. R_f = 0.4 (1:1 EtOAc/hexane).

[00153] **Compound 4:** Under argon atmosphere, morpholine (10 g, 115 mmol) was added to ethylene sulfide (15 g, 250 mmol) in a round bottom flask. The reaction was stirred for 7 hrs and was directly loaded on to a silica gel column. The column was washed with DCM first and then 2% MeOH/DCM was used to obtain compound **4** (15.3 g, 91%) as colorless oil.

[00154] ^{1}H NMR (399MHz, $CDCl_3$) δ 3.67-3.59 (m, 4H), 2.63-2.52 (m, 2H), 2.51-2.45 (m, 2H), 2.44-2.34 (m, 4H); MS (ESI +ve): calc (M+H)+= 148.07, found: 148.1.

[00155] **Compound 5:** A DCM solution (1 mL) of 2-morpholinoethanethiol (0.21 g, 1.44 mmol) was added dropwise via syringe to a stirring solution compound **3** (0.40 g, 1.44 mmol) in DCM (10 mL) at r.t. Immediately after addition, the TLC was checked, to reveal rapid formation of product and and some quantity of dimer. After 0.5 h, the mixture was partitioned by addition of water. Upon extraction, the organic layer was separated then dried

($MgSO_4$), filtered and reduced *in vacuo*. Column chromatography gave compoud **5** (0.29 g, 58%) as colorless oil.

[00156] ^{1}H NMR (399 MHz, $CDCl_3$) δ 5.78 (m, 2H), 3.92 (d, J = 7.3 Hz, 2H), 3.70 (t, J = 4.7 Hz, 4H), 3.46 (d, J = 5.5 Hz, 2H), 3.31 (s, 3H), 2.84 (dd, J = 7.8, 6.7 Hz, 2H), 2.66 (dd, J = 7.8, 6.7, 2H), 2.48 (t, J = 4.6 Hz, 4H); ^{13}C NMR 130.35, 126.27, 66.97, 58.20, 53.67, 51.52, 36.22, 35.16, 33.67; MS (ESI +ve): calc (M+H): 344.05, found: 344.06. R_f = 0.3 (EtOAc).

[00157] **Compound 5b:** A DCM solution (1mL) of compound **4b** (395 mg, 1.085 mmol) was added dropwise via syringe to a stirring DCM (15 mL) solution compound **3** (300 mg, 1.085 mmol) at r.t. After 1h, the resulting solution was partitioned by addition of water. Upon extraction, the organic layer was separated then dried ($MgSO_4$), filtered and reduced *in vacuo*.Column chromatography gave compound **5b** as a colorless oil (0.35 g, 58%). ^{1}H NMR (399 MHz, $CDCl_3$) δ 5.83 – 5.70 (m, 2H), 5.35 – 5.21 (dt, J = 26.0, 9.3 Hz, 2H), 5.16 – 5.07 (m, 1H), 4.59 – 4.54 (d, J = 9.5 Hz, 1H), 4.29 – 4.23 (m, 1H), 4.23 – 4.18 (m, 1H), 3.99 – 3.88 (dd, J = 6.7, 1.2 Hz, 2H), 3.80 – 3.72 (ddd, J = 10.1, 4.6, 2.6 Hz, 1H), 3.64 – 3.56 (m, 1H), 3.50 – 3.43 (m, 1H), 3.31 (s, 3H), 2.09 (s, 3H), 2.03 (s, 6H), 2.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.68, 170.30, 169.51, 169.30, 129.43, 127.14, 87.73, 76.49, 73.89, 69.16, 67.99, 61.99, 51.64, 35.89, 33.58, 20.95, 20.80, 20.74, 20.71; MS (ESI +ve): calc ($M+NH_4^+$): 578.07, found: 577.96. R_f = 0.5 (1:1 EtOAc/hexane).

[00158] **Compound 6:** An ice cold solution of (Z)-but-2-ene-1,4-diol (0.93 ml, 11.3 mmol) and triethylamine (1.6 mL, 11.5 mmol) in DCM (50 ml) was treated dropwise via syringe with pivaloyl chloride (1.4 ml, 11.4 mmol) over 2 min. After 1 h, TLC showed good reaction.

[00159] The resulting mixture was partitioned by addition of water. Upon extraction, the organic layer was separated then dried ($MgSO_4$), filtered and reduced *in vacuo*. This crude compound was found: by TLC (Rf = 0.6, 1:1 EtOAc/hexane) to contain no starting diol and was used crude to prepare the mesylate. The crude material was taken up in DCM (50 ml) containing triethylamine (1.7 mL, 12 mmol) and cooled on an ice bath. Methanesulfonyl chloride (0.98 ml, 12.66 mmol) was added dropwise via syringe over 2 min. TLC immediately after addition indicated complete consumption of starting material. The resulting mixture was partioned by addition of water. Upon extraction, the organic layer was separated

then dried ($MgSO_4$), filtered and reduced *in vacuo*.Column chromatography gave pure compound **6**, 1.48 g, 52%, as a colorless oil.

[00160] 1H NMR (399 MHz, CDCl3) δ 5.89 – 5.75 (m, 2H), 4.89 – 4.84 (d, J = 5.7 Hz, 2H), 4.68 – 4.63 (d, J = 5.9 Hz, 2H), 3.03 (s, 3H), 1.19 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 178.28, 130.61, 126.11, 65.08, 59.65, 38.84, 38.21, 27.25; MS (ESI +ve): calc (M+NH4): 268.12, found: 268.20; Rf = 0.3 (20% EtOAc/hexane).

[00161] **Compound 7:** A MeOH (10 ml) solution of sodium methanesulfonothioate (0.63 g, 4.70 mmol) and (Z)-4-(methylsulfonyloxy)but-2-enyl pivalate (1.00 g, 4.00 mmol) was stirred at r.t. for 18 h with formation of a white precipitate (after 10 min). The resulting mixture was partitioned by addition of water and DCM. Upon extraction into DCM, the organic layer was separated then dried ($MgSO_4$), filtered and reduced *in vacuo*. Column chromatography gave compound **7**, 0.83 g, 78% as a colorless oil.

[00162] ^{1}H NMR (399 MHz, $CDCl_3$) δ 5.82 – 5.73 (m, 2H), 4.73 – 4.66 (m, 2H), 3.95 – 3.87 (m, 2H), 3.32 (s, 3H), 1.19 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.35, 129.37, 127.32, 59.50, 51.44, 38.84, 33.61, 27.28; MS (ESI +ve): calc ($M+NH_4$): 284.10, found: 284.19; R_f = 0.4 (20% EtOAc/hexane).

[00163] **Compound 9:** Pivaloyl chloride (0.60 g, 5.0 mmol) was added in a dropwise fashion to a stirring solution of S-2-hydroxyethyl methanesulfonothioate (0.65 g, 4.16 mmol) in DCM (20 ml). After 2 h at r.t. the resulting mixture with white precipitate was partitioned with water. The organic layer was separated, dried (Ns_2SO_4), filtered and reduced to an oil. Column gave compound **9** as a colorless oil (0.45 g, 45%). ^{1}H NMR (399 MHz, $CDCl_3$) δ 4.39 – 4.34 (t, J = 6.3 Hz, 2H), 3.44 – 3.39 (t, J = 6.3 Hz, 2H), 3.36 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.10, 51.11, 38.96, 35.19, 27.24; MS (ESI +ve): calc ($M+NH_4$): 158.08, found: 158.04. R_f = 0.3 (20% EtOAc/hexane).

[00164] **Compound 11:** Pivaloyl chloride (4.96 ml, 40.3 mmol) was added dropwise via syringe to an ice cold DCM solution (50 mL) of 2-(hydroxymethyl)phenol (5 g, 40.3 mmol) and triethylamine (5.61 ml, 40.3 mmol). An ice-cold solution of the crude pivalate ester was treated with triethylamine (6.74 ml, 48.4 mmol) and 50 mL DCM. Methanesulfonyl chloride (3.43 ml, 44.3 mmol) was then added slowly (5 min) via syringe and the resulting mixture was warmed to r.t. The mixture was poured onto ice and the organic layer was

separated then washed with sat $NaHCO_3$ (aq), dried ($MgSO_4$), filtered and reduced to 10.5 g crude pale yellow oil.

[00165] Column (ISCO) gave pure **11** 5.45 g, 47%.

[00166] ^1H NMR (399 MHz, $CDCl_3$) δ 7.53 – 7.46 (dd, 7.7, 1.8 Hz, 1H), 7.46 – 7.40 (dt, 7.7, 1.8 Hz, 1H), 7.32 – 7.24 (t, 7.7 Hz, 1H), 7.13 – 7.06 (d, 7.7 Hz, 1H), 5.21 (s, 2H), 2.79 (s, 3H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.05, 150.06, 131.18, 131.07, 126.35, 125.94, 123.21, 66.88, 39.48, 38.82, 27.30, 27.26. MS (ESI +ve): calc ($M+NH_4$): 304.12, found: 303.99. R_f = 0.4 (20% EtOAc/hexane).

[00167] **Compound 12:** A MeOH (20 mL) solution of sodium methanesulfonothioate (0.825 g, 6.15 mmol) was treated with 2-((methylsulfonyloxy)methyl)phenyl pivalate (1.76 g, 6.15 mmol) at r.t. and left to stir for 18 h. The mixture was partitioned between water and DCM. The organic layer was separated, dried ($MgSO_4$), filtered and reduced to afford a colorless oil. Column chromatography gave pure compound **12** as a pale colorless oil, 0.754 g, 41%.

[00168] ^1H NMR (399 MHz, $CDCl_3$) δ 7.48 – 7.44 (dd, J = 7.7, 1.7 Hz, 1H), 7.39 – 7.34 (td, J = 7.8, 1.7 Hz, 1H), 7.25 – 7.20 (td, J = 7.6, 1.2 Hz, 1H), 7.10 – 7.06 (dd, J = 8.2, 1.2 Hz, 1H), 4.29 (s, 2H), 2.90 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.69, 149.59, 131.17, 129.85, 127.41, 126.18, 123.40, 51.43, 39.47, 36.01, 27.30; MS (ESI +ve): calc ($M+NH_4$): 320.10, found: 320.09. R_f = 0.4 (20% EtOAc/hexane).

[00169] **Compound 14:** Chloromethyl pivalate (0.478 ml, 3.32 mmol) was added to a stirring mixture of sodium iodide (0.050 g, 0.33 mmol) and sodium methanesulfonothioate (0.445 g, 3.32 mmol) in acetone (7 ml) at r.t. After 24 h, TLC showed good conversion to product. The solvent was removed, and the residue was partitioned between water and DCM. The organic layer was separated and dried ($MgSO_4$), filtered and reduced to afford a colorless oil. Column chromatography gave pure **14** as a slightly pink solid, 0.41 g, 55%.

[00170] ^1H NMR (399 MHz, $CDCl_3$) δ 5.67 (s, 2H), 3.39 (s, 3H), 1.24 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.35, 67.84, 52.20, 38.93, 27.05. R_f = 0.5 (20% EtOAc/hexane).

[00171] **Compound 16:** Prepared from **15** and NaMTS as described previously: US 3,484,473

^{1}H NMR (399 MHz, $CDCl_3$) δ 4.86 (s, 2H), 3.45 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.15, 41.50.

[00172] **Compound 18:** Prepared from **17** and NaMTS as described previously: Chem. Pharm. Bull. Vol. 12(11) p. 1271, 1964.

^{1}H NMR (399 MHz, $CDCl_3$) δ 3.55 (s, 4H), 3.40 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 50.67, 35.96.

[00173] **Compound 19:** A DCM solution (1 mL) of 2-morpholinoethanethiol (0.17 g, 1.2 mmol) was added dropwise via syringe to a stirring solution of compound **18** (300 mg, 1.2 mmol) in DCM (10 mL) at r.t. Immediately after addition, the TLC was checked, to reveal rapid formation of product and some dimer. After 0.5 h, the mixture was partitioned by addition of $NaHCO_3$. Upon extraction, the organic layer was separated then dried ($MgSO_4$), filtered and reduced *in vacuo*. Column chromatography gave pure **19** (0.20 g, 53%) as a colorless oil. ^{1}H NMR (399 MHz, $CDCl_3$) δ 3.73 – 3.67 (t, J = 4.7 Hz, 4H), 3.51 – 3.46 (m, 2H), 3.35(s, 3H), 3.07 – 3.01 (m, 2H), 2.88 – 2.83 (m, 2H), 2.69 – 2.63 (m, 2H), 2.52 – 2.43 (t, J = 4.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.96, 57.91, 53.58, 50.79, 37.66, 36.10, 35.52; MS (ESI +ve): calc (M+H): 318.03, found: 318.04. R_f = 0.3 (EtOAc).

[00174] **Compound 21:** Compound **20** is converted to compound **21** by a procedure analogous to that described for compound **11**.

[00175] **Compound 22:** Compound **21** is converted to compound **22** by a procedure analogous to that described for compound **12**.

[00176] **Compound 23:** Compound **23** is prepared according to a literature method (Journal of Medicinal Chemistry, 50(23), 5568-5570; 2007.)

[00177] **Compound 24:** An ice-cold pyridine solution (10 mL) of compound **23** (1 mmol) is treated successively, in a dropwise fashion with acetyl choride (1 mmol), then after 5 min with MsCl (1.1 mmol). The solution is warmed to room temperature then the solvent is removed. The residue is dissolved in EtOAc, washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. Purification by column chromatography affords pure compound **24**.

[00178] **Compound 25:** Compound **24** is converted to compound **25** by a procedure analogous to that described for compound **12**.

[00179] **Compound 27:** Compound **26** is converted to compound **27** by a procedure analogous to that described for compound **14**.

[00180] **Compound 29:** Compound **28** is converted to compound **29** by a procedure analogous to that described for compound **14**.

[00181] **Compound 30:** Compound **30** is prepared according to a literature method (Tetrahedron, 42(2), 601-7; 1986.)

[00182] **Compound 31:** Compound **31** is prepared from compound **30** according to a patent procedure (US 20090181444)

[00183] **Compound 33:** Compound **33** is prepared from compound **32** according to a patent procedure (US 20090181444)

[00184] **Compound 36:** An ice-cold DCM (20 mL) solution of compound **34** (1 mmol) is treated with NEt_3 (1 mmol) followed by the dropwise addition of TMS-Cl (1.1 mmol). After 1 h, the solution is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. The crude TMS protected material is redissolved in THF (10 mL), whereon PPh_3 (1.2 mmol), compound **35** (1.2 mmol), then DEAD (1.2 mmol, dropwise) are added in succession. After stirring at r.t. for 18 h, the solvent is removed under vacuum, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. Purification by column chromatography affords pure compound **36**.

[00185] **Compound 37:** A THF (10 mL) solution of compound **36** (0.5 mmol) is treated with TBAF (1 mmol of a 1M solution in THF), with monitoring by TLC. On completion of TMS cleavage, the solvent is removed under vacuum, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. The crude alcohol is redissolved in pyridine (5 mL), and TsCl (0.55 mmol) is added. After 18 h at r.t., the solvent is removed, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. Purification by column chromatography affords pure compound **37**.

[00186] **Compound 38:** Compound **37** is converted to compound **38** by a procedure analogous to that described for compound **12**.

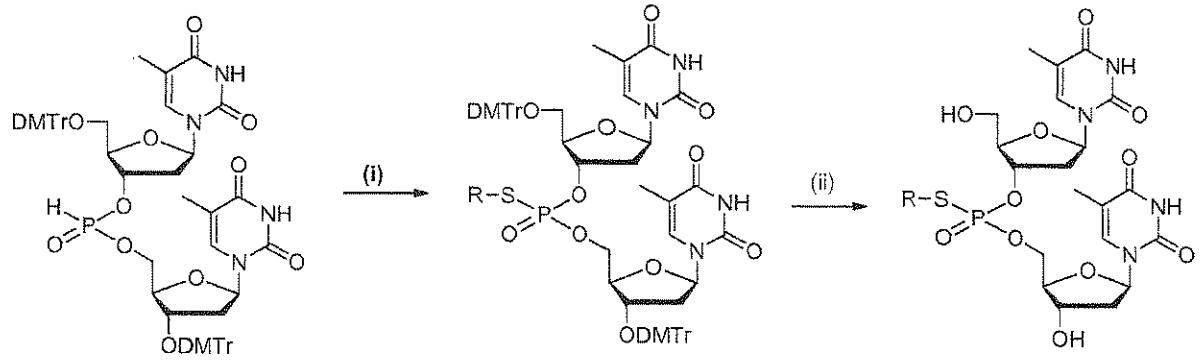
[00187] **Compound 40:** An ice-cold DCM (20 mL) solution of compound **39** (1 mmol) is treated with NEt_3 (1 mmol) followed by the dropwise addition of TMS-Cl (1.1 mmol). After 1 h, the solution is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. The crude TMS protected material is redissolved in THF (10 mL), whereon PPh_3 (1.2 mmol), potassium *p*-toluenethiosulfonate (KTTS, 1.2 mmol), anhydrous $ZnCl_2$ (1 mmol) then DEAD (1.2 mmol, dropwise) are added in succession. After stirring at r.t. for 18 h, the solvent is removed under vacuum, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. Purification by column chromatography affords pure compound **40**.

[00188] **Compound 41:** A THF (10 mL) solution of compound **40** (0.5 mmol) is treated with TBAF (1 mmol of a 1M solution in THF), with monitoring by TLC. On completion of TMS cleavage, the solvent is removed under vacuum, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. The crude alcohol is redissolved in THF (10 mL), whereon PPh_3 (1.2 mmol), compound **35** (1.2 mmol), then DEAD (1.2 mmol, dropwise) are added in succession. After stirring at r.t. for 18 h, the solvent is removed under vacuum, the residue is redissolved in DCM, the solution of which is washed with water, dried ($MgSO_4$), filtered and reduced *in vacuo*. Purification by column chromatography affords pure compound **40**.

[00189] **Compound 42:** Compound **41** is converted to compound **42** by a procedure analogous to that described for compound **14**.

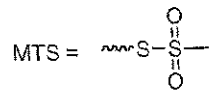
Example 2 – Thioalkylation of H-phosphonates to provide phosphorothiotriesters in solution phase

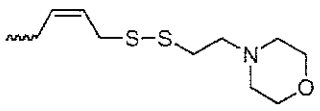
Scheme 2

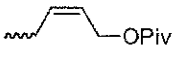


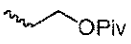
100 **101-107** **201-207**

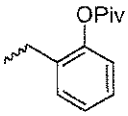
(i) BSTFA, MTS-R, (ii) 3% TCA in DCM

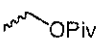
MTS = 

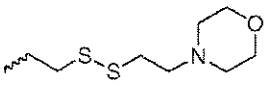
R=  Compounds **101** and **201**

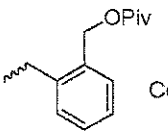
R=  Compounds **102** and **202**

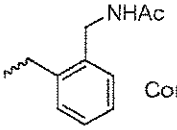
R=  Compounds **103** and **203**

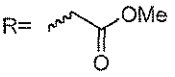
R=  Compounds **104** and **204**

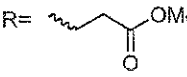
R=  Compounds **105** and **205**

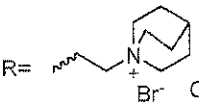
R=  Compounds **106** and **206**

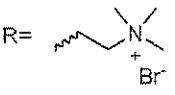
R=  Compounds **107** and **207**

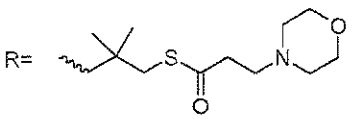
R=  Compounds **108** and **208**

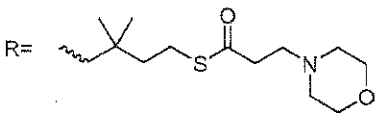
R=  Compounds **109** and **209**

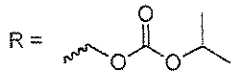
R=  Compounds **110** and **210**

R=  Compounds **111** and **211**

R=  Compounds **112** and **212**

R=  Compounds **113** and **213**

R=  Compounds **114** and **214**

R =  Compounds **115** and **215**

BSTFA = N,O-bis(trimethylsilyl)trifluoroacetamide : $CF_3C{=}NSi(CH_3)_3OSi(CH_3)_3$

[00190] **Compound 100:** The synthetic procedure for Di-DMTr H-phosphonate TT dimer (**100**) has been previously described described (Froehler, Brian C.; Ng, Peter G.; Matteucci, Mark D., Nucleic Acids Research (1986), 14(13), 5399-5407; Garegg, Per J.; Lindh, Ingvar; Regberg, Tor; Stawinski, Jacek; Stroemberg, Roger; Henrichson, Christina Tetrahedron Letters (1986), 27(34), 4051-4054).

[00191] **Compound 101:** Compound **100**, mixture of diastereomers (200 mg, 0.176 mmol) was dissolved in ACN (6 mL) then trimethylsilyl 2,2,2-trifluoro-N-(trimethylsilyl)acetimidate (227 mg, 0.882 mmol) was added. A solution of (Z)-S-4-((2-morpholinoethyl)disulfanyl)but-2-enyl methanesulfonothioate (121 mg, 0.353 mmol) in ACN (2 mL) was then added, over the course of 1 h in 3 approximately equal portions, with monitoring by TLC and HPLC/MS. After 3 h, the resulting solution was partitioned by addition of water. Upon extraction, the organic layer was separated then dried ($MgSO_4$), filtered and reduced *in vacuo*. Column chromatography gave compound **101** as a white foam, 225 mg, 91%.

[00192] ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.72 (d, br, 1H), 9.27, (d, br, 1H), 7.53 (dd, J = 25.0, 1 Hz, 1H), 7.42, (t, J = 7.0 Hz, 2H), 7.37 – 7.16 (m, 17H), 6.83 (m, 8H), 6.43 – 6.28 (m, 2H), 5.63 – 5.42 (m, 2H), 5.21 (q, J = 7.1 Hz, 1H), 4.27 (m, br, 1H), 3.94 (m, br, 2H), 3.77 (m, 12H), 3.74 – 3.60 (m, 6H), 3.51 – 3.22 (m, 5H), 2.82 – 2.76 (m, 2H), 2.68 – 2.60 (m, 2H), 2.59 – 2.46 (m, 5H), 2.44 – 2.33 (m, 2H), 2.03 – 1.88 (m, 1H), 1.84 (m, 3H), 1.75 – 1.66 (m, 1H), 1.48 – 1.32 (dd, J = 11.8, 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.10, 164.07, 164.00, 163.94, 159.14, 159.10, 150.80, 150.78, 150.75, 150.63, 145.09, 144.30, 144.27, 136.31, 136.27, 136.22, 136.18, 135.95, 135.82, 135.43, 135.35, 135.33, 135.24, 135.22, 130.52, 130.43, 130.40, 129.49, 129.30, 128.54, 128.43, 128.39, 127.64, 127.57, 113.78, 113.76, 113.73, 113.67, 112.05, 111.56, 87.77, 87.66, 87.58, 85.77, 85.59, 84.63, 84.51, 74.42, 74.33, 67.02, 66.95, 63.63, 63.49, 58.27, 58.23, 55.60, 55.58, 53.69, 53.62, 39.48, 39.26, 39.18, 35.88, 35.61, 35.43, 35.36, 28.18, 12.83, 12.79, 12.02, 11.95.; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.25, 29.12; MS (ESI +ve): calc (M+H): 1398.46, found: 1398.64. R_f = 0.4 (5% MeOH/DCM).

[00193] **Compound 201:** Compound **101** (0.150 g, 0.107 mmol) was stirred with 3% TCA/DCM (10 mL) over 10 min. TLC and HPLC/MS showed that the reaction was complete. 10 mL of MeOH was added and stirring continued for 2 min. Solvents were evaporated and the residue was purified by column chromatography to give compound **201** (85 mg, 100%) as a white solid.

[00194] ^{1}H NMR (399 MHz, CD_3OD) δ 7.78 (dd, J = 7.2, 1.3 Hz, 1H), 7.53 (d, J = 1.3 Hz, 1H), 6.33 – 6.27 (m, 2H), 5.83 – 5.70 (m, 2H), 5.25 – 5.19 (m, 1H), 4.47 – 4.30 (m, 3H), 4.27 – 4.22 (m, 1H), 4.11 – 4.05 (m, 1H), 3.89 – 3.82 (t, J = 4.8 Hz, 4H), 3.85 (m, 2H), 3.76 – 3.70 (ddd, J = 15.5, 7.2, 1.7 Hz, 2H), 3.52 (dd, J = 7.3, 3.7 Hz, 2H), 3.28 – 3.19 (br, 2H), 3.16 – 3.05 (br, 4H), 3.05 – 2.98 (ddd, J = 9.8, 5.5, 2.0 Hz, 2H), 2.62 – 2.52 (tdd, J = 11.5, 5.7, 1.9 Hz, 1H), 2.47 – 2.36 (m, 1H), 2.33 – 2.28 (m, 2H), 1.92 – 1.87 (m, 6H); ^{31}P NMR (162 MHz, CD_3OD) δ 30.22, 30.19; MS (ESI +ve): calc (M+H): 794.20, found: 794.18. R_f = 0.3 (10% MeOH/DCM).

[00195] **Compound 102:** Compound **100** (400 mg, 0.352 mmol) was converted to compound **102** by a procedure analogous to that described for compound **101** (417 mg, 90%).

[00196] ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.17 (d, J = 6.0 Hz, 1H), 9.13 – 9.00 (d, J = 25.7 Hz, 1H), 7.58 – 7.49 (dd, J = 26.3, 1.5 Hz, 1H), 7.45 – 7.40 (ddd, J = 8.0, 5.2, 1.3 Hz, 2H), 7.40 – 7.18 (m, 17H), 6.87 – 6.81 (m, 8H), 6.44 – 6.30 (m, 2H), 5.65 – 5.53 (m, 1H), 5.53 – 5.44 (m, 1H), 5.26 – 5.16 (quintet, J = 6.4 Hz, 1H), 4.61 – 4.54 (m, 2H), 4.30 – 4.24 (m, 1H), 4.19 – 4.13 (m, 1H), 3.97 – 3.88 (m, 2H), 3.80 – 3.72 (m, 12H), 3.69 – 3.57 (m, 1H), 3.54 – 3.30 (m, 5H), 2.61 – 2.49 (dt, J = 14.4, 5.4 Hz, 1H), 2.44 – 2.32 (m, 1H), 2.02 – 1.91 (dt, J = 12.5, 5.4 Hz, 1H), 1.85 – 1.80 (dd, J = 5.0, 1.3 Hz, 3H), 1.76 – 1.63 (m, 1H), 1.43 – 1.36 (dd, J = 10.2, 1.2 Hz, 3H), 1.19 – 1.14 (d, J = 2.0 Hz, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.22, 178.17, 163.82, 163.80, 163.75, 158.92, 158.88, 150.52, 150.43, 144.90, 144.88, 144.10, 144.05, 136.11, 136.08, 136.05, 136.01, 135.59, 135.28, 135.16, 135.03, 135.01, 130.30, 130.23, 130.19, 130.16, 128.69, 128.64, 128.59, 128.39, 128.34, 128.23, 128.21, 128.17, 127.42, 127.34, 113.54, 113.45, 111.85, 111.82, 111.41, 111.36, 87.59, 87.43, 87.37, 85.47, 85.33, 84.43, 84.29, 84.08, 84.00, 83.92, 74.24, 67.36, 63.38, 63.26, 59.42, 55.37, 39.22, 38.77, 27.94, 27.24, 12.57, 11.80, 11.74; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.23, 28.97; MS (ESI +ve): calc (M+H): 1338.51, found: 1338.84. R_f = 0.5 (5% MeOH/DCM).

[00197] **Compound 202:** Compound **102** (200 mg, 0.151 mmol) was converted to compound **202** by a procedure analogous to that described for compound **101** (105 mg, 97%).

[00198] ^{1}H NMR (399 MHz, CD_3OD) δ 7.81 – 7.75 (dd, J = 8.2, 1.3 Hz, 1H), 7.57 – 7.51 (dd, J = 8.2, 1.3 Hz, 1H), 6.33 – 6.23 (m, 2H), 5.85 – 5.75 (m, 1H), 5.75 – 5.66 (m, 1H), 5.26 – 5.19 (m, 1H), 4.72 – 4.66 (m, 2H), 4.47 – 4.30 (m, 3H), 4.27 – 4.20 (m, 1H), 4.11 – 4.04 (m, 1H), 3.83 – 3.76 (m, 2H), 3.74 – 3.64 (m, 2H), 2.62 – 2.51 (m, 1H), 2.45 – 2.35 (td,

J = 8.7, 6.5 Hz, 1H), 2.32 – 2.24 (m, 2H), 1.93 – 1.82 (m, 6H), 1.20 – 1.15 (d, J = 2.1 Hz, 9H); ^{13}C NMR (126 MHz, CD_3OD) δ 179.65, 166.28, 152.30, 152.28, 152.22, 137.90, 137.81, 137.79, 130.07, 130.04, 129.26, 129.24, 111.93, 111.88, 111.87, 87.26, 87.22, 86.96, 86.90, 86.76, 86.54, 86.12, 86.07, 85.98, 85.92, 85.88, 85.82, 80.54, 80.49, 80.46, 80.41, 71.84, 71.67, 68.71, 68.66, 68.45, 68.40, 62.58, 62.50, 60.72, 40.51, 40.44, 39.70, 39.52, 39.48, 28.67, 28.64, 28.61, 27.53, 12.64, 12.48; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.23, 28.97; MS (ESI +ve): calc (M+H): 717.22, found: 717.23. R_f = 0.5 (10% MeOH/DCM).

[00199] **Compound 103:** Compound **100** (400 mg, 0.352 mmol) was converted to compound **103** by a procedure analogous to that described for compound **101** (379 mg, 83%).

[00200] ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.48 (s, 1H), 9.41 – 9.29 (m, 1H), 7.60 – 7.48 (dd, J = 9.0, 1.0 Hz, 1H), 7.46 – 7.40 (dt, J = 6.9, 1.2 Hz, 2H), 7.39 – 7.17 (m, 17H), 6.89 – 6.79 (m, 8H), 6.44 – 6.31 (m, 2H), 5.27 – 5.20 (t, J = 6.5 Hz, 1H), 4.30 – 4.24 (t, J = 6.1 Hz, 1H), 4.19 – 4.15 (m, 2H), 4.13 – 4.07 (t, J = 7.1 Hz, 1H), 3.99 – 3.90 (m, 2H), 3.79 – 3.74 (m, 12H), 3.70 – 3.58 (m, 1H), 3.51 – 3.43 (td, J = 8.8, 7.2, 2.3 Hz, 1H), 3.40 – 3.32 (m, 1H), 3.02 – 2.85 (m, 2H), 2.61 – 2.49 (dt, J = 18.5, 7.0 Hz, 1H), 2.47 – 2.33 (m, 1H), 1.98 – 1.90 (dt, J = 10.2, 5.0 Hz, 1H), 1.85 – 1.81 (m, 3H), 1.74 – 1.62 (td, J = 14.2, 7.1 Hz, 1H), 1.42 – 1.36 (m, 3H), 1.19 – 1.13 (d, J = 4.9 Hz, 9H); ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.36, 29.18; ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.97, 177.89, 163.94, 163.91, 163.90, 163.86, 158.91, 158.87, 150.63, 150.54, 150.53, 150.50, 144.88, 144.85, 144.10, 144.04, 136.09, 135.99, 135.52, 135.50, 135.24, 135.16, 135.12, 135.04, 135.00, 130.31, 130.29, 130.20, 130.16, 130.13, 128.34, 128.20, 128.18, 128.14, 127.39, 127.31, 124.89, 113.55, 113.52, 113.43, 111.84, 111.38, 87.58, 87.42, 87.36, 85.30, 84.98, 84.95, 84.40, 84.33, 84.27, 83.98, 83.91, 83.84, 79.31, 79.27, 78.88, 78.84, 74.16, 74.08, 67.56, 67.50, 67.46, 67.41, 63.33, 63.24, 62.79, 62.75, 55.34, 39.21, 39.16, 39.04, 39.00, 38.85, 38.82, 29.95, 29.92, 29.66, 29.63, 27.17, 12.53, 11.80, 11.72; MS (ESI +ve): calc (M+H): 1312.69, found: 1312.49. R_f = 0.4 (5% MeOH/DCM).

[00201] **Compound 203:** Compound **103** (200 mg, 0.154 mmol) was converted to compound **203** by a procedure analogous to that described for compound **201** (103 mg, 98%).

[00202] ^{1}H NMR (399 MHz, CD_3OD) δ 7.80 – 7.76 (dd, J = 8.2, 1.2 Hz, 1H), 7.55 – 7.51 (dd, 7.1, 1.2 Hz, 1H), 6.32 – 6.24 (m, 2H), 5.26 – 5.19 (m, 1H), 4.46 – 4.20 (m, 6H), 4.10 – 4.05 (m, 1H), 3.82 – 3.78 (dd, J = 6.5, 3.2 Hz, 2H), 3.22 – 3.14 (ddd, J = 16.6, 7.0, 5.8 Hz, 2H), 2.61 – 2.51 (tdd, J = 13.0, 5.9, 2.1 Hz, 1H), 2.46 – 2.37 (ddd, J = 14.3, 8.3, 6.0 Hz,

1H), 2.31 – 2.26 (t, J = 5.8 Hz, 2H), 1.91 – 1.86 (dt, J = 11.0, 1.2 Hz, 6H), 1.21 – 1.17 (m, 9H); ^{31}P NMR (162 MHz, CD_3OD) δ 30.15; ^{13}C NMR (100 MHz, CD_3OD) δ 179.45, 179.42, 166.29, 152.31, 152.29, 152.23, 137.82, 137.80, 137.78, 111.91, 111.88, 87.21, 87.17, 86.94, 86.87, 86.63, 86.52, 86.11, 86.06, 85.92, 85.84, 85.77, 80.67, 80.60, 80.49, 80.43, 71.79, 71.64, 68.80, 68.74, 68.58, 68.52, 64.11, 64.07, 64.02, 62.54, 62.44, 40.48, 40.43, 39.81, 39.71, 39.68, 39.52, 39.47, 30.74, 30.72, 30.68, 27.52, 12.65, 12.50; MS (ESI +ve): calc (M+H): 691.21, found: 691.09. R_f = 0.5 (10% MeOH/DCM).

[00203] **Compound 104:** Compound **100** (400 mg, 0.352 mmol) was converted to compound **104** by a procedure analogous to that described for compound **101** (451 mg, 94%).

[00204] ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.17 – 9.01 (m, 2H), 7.51 – 7.46 (dd, J = 7.8, 1.5 Hz, 1H), 7.45 – 7.38 (m, 2H), 7.37 – 7.09 (m, 19H), 7.01 – 6.90 (m, 2H), 6.87 – 6.78 (m, 8H), 6.39 – 6.27 (m, 2H), 5.15 – 5.01 (m, 1H), 4.20 – 4.13 (m, 1H), 3.96 – 3.90 (m, 1H), 3.90 – 3.83 (m, 2H), 3.80 – 3.68 (m, 14H), 3.52 – 3.20 (m, 3H), 2.45 – 2.16 (m, 2H), 2.01 – 1.88 (ddd, J = 23.3, 13.6, 5.6 Hz, 1H), 1.85 – 1.79 (dd, J = 9.3, 1.2 Hz, 3H), 1.69 – 1.53 (m, 1H), 1.40 – 1.31 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.46, 176.37, 163.84, 163.78, 158.90, 158.87, 150.52, 150.50, 150.43, 149.38, 149.28, 144.95, 144.88, 144.16, 144.10, 136.13, 136.11, 136.09, 136.03, 135.57, 135.49, 135.37, 135.26, 135.21, 135.08, 135.04, 130.83, 130.74, 130.29, 130.21, 130.16, 129.51, 129.49, 129.40, 129.36, 129.35, 129.31, 128.38, 128.35, 128.27, 128.23, 128.19, 128.14, 127.39, 127.33, 126.05, 125.94, 122.94, 122.86, 113.53, 113.42, 111.77, 111.73, 111.39, 111.28, 87.55, 87.52, 87.37, 87.32, 85.33, 84.95, 84.90, 84.29, 84.20, 84.00, 83.92, 83.87, 83.79, 79.05, 79.00, 74.29, 74.24, 67.31, 67.24, 67.17, 67.11, 63.37, 55.37, 55.35, 39.37, 39.32, 39.15, 39.10, 38.64, 30.51, 30.41, 30.36, 27.28, 27.24, 12.59, 12.51, 11.75, 11.67; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.12, 28.49; MS (ESI +ve): calc ($M+NH_4$): 1374.51, found: 1374.74. R_f = 0.4 (5% MeOH/DCM).

[00205] **Compound 204:** Compound **104** (200 mg, 0.147 mmol) was converted to compound **204** by a procedure analogous to that described for compound **201** (98 mg, 88%).

[00206] ^{1}H NMR (399 MHz, CD_3OD) δ 7.77 – 7.73 (m, 1H), 7.51 – 7.43 (m, 2H), 7.38 – 7.31 (m, 1H), 7.25 – 7.19 (ddd, J = 9.2, 5.4, 1.6 Hz, 1H), 7.08 – 7.02 (ddd, J = 8.0, 3.8, 1.3 Hz, 1H), 6.28 – 6.17 (m, 2H), 5.10 – 5.01 (m, 1H), 4.30 – 4.16 (m, 3H), 4.11 – 4.03 (m, 3H), 4.03 – 3.97 (d, J = 5.3 Hz, 2H), 3.74 – 3.63 (m, 2H), 2.48 – 2.11 (m, 5H), 1.90 – 1.82 (m, 6H), 1.43 – 1.36 (d, J = 3.4 Hz, 9H); ^{13}C NMR (100 MHz, CD_3OD) δ 178.05, 166.26, 152.25, 152.19, 150.78, 137.80, 137.76, 132.13, 132.09, 130.61, 130.56, 127.24, 124.10,

111.92, 111.84, 111.79, 87.14, 87.09, 86.80, 86.71, 86.50, 85.98, 85.95, 85.92, 85.87, 85.83, 85.75, 80.55, 80.48, 80.32, 80.27, 71.97, 71.73, 68.67, 68.61, 68.35, 68.29, 62.51, 62.42, 40.41, 40.36, 40.32, 39.66, 39.64, 39.35, 39.29, 31.08, 31.04, 27.61, 12.68, 12.65, 12.49; ^{31}P NMR (162 MHz, CD_3OD) δ 29.54, 29.29; MS (ESI +ve): calc (M+H): 753.22, found: 753.12. R_f = 0.5 (10% MeOH/DCM).

[00207] **Compound 105:** Compound **100** (200 mg, 0.176 mmol) was converted to compound **105** by using compound 14 in a procedure analogous to that described for compound **101** (158 mg, 70%).

[00208] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.39 (m, 2H) 7.38 – 7.16 (m, 18H), 6.90 – 6.77 (m, 8H), 6.43 – 6.27 (m, 1H), 5.39 – 5.18 (m, 2H), 4.31 – 4.23 (dd, *J* = 12.0, 6.2 Hz, 1H), 4.20 – 4.12 (m, 1H), 3.98 – 3.86 (m, 1H), 3.82 – 3.70 (m, 12H), 3.69 – 3.52 (m, 1H), 3.50 – 3.43 (td, *J* = 9.9, 8.9, 2.7 Hz, 1H), 3.41 – 3.29 (ddd, *J* = 17.2, 10.8, 2.5 Hz, 1H), 2.59 – 2.49 (m, 1H), 2.44 – 2.30 (m, 1H), 2.03 – 1.93 (m, 1H), 1.86 – 1.79 (d, *J* = 2.9 Hz, 3H), 1.75 – 1.67 (m, 4H), 1.43 – 1.36 (d, 3H), 1.16 – 1.08 (d, *J* = 9.3 Hz, 9H); ^{31}P NMR (162 MHz, $CDCl_3$) *δ* 28.14, 27.81 (two diastereomers). MS (ESI +ve): calc (M+H): 1281.4, found: 1281.1 $(M+H)^+$ and 1298.6 $(M+NH_4)^+$

[00209] **Compound 205:** Compound **105** (137 mg, 0.107 mmol) was converted to compound **205** by a procedure analogous to that described for compound **201** (66 mg, 91%). ^{1}H NMR (399 MHz, CD_3OD) δ 7.83 – 7.76 (m, 1H), 7.56 – 7.50 (m, 1H), 6.34 – 6.22 (m, 2H), 5.51 – 5.43 (m, H), 5.28 – 5.20 (qt, *J* = 7.8, 1.8 Hz, 1H), 4.47 – 4.31 (m, 3H), 4.29 – 4.21 (m, 1H), 4.10 – 4.05 (m, 1H), 3.87 – 3.73 (dd, *J* = 7.6, 3.1 Hz, 2H), 2.62 – 2.50 (tdd, *J* = 16.9, 5.7, 1.9 Hz, 1H), 2.45 – 2.36 (m, 1H), 2.32 – 2.25 (ddd, *J* = 6.9, 5.4, 1.5 Hz, 3H), 1.92 – 1.84 (m, 6H), 1.22 – 1.18 (d, *J* = 5.3 Hz, 9H); ^{31}P NMR (162 MHz, CD_3OD) *δ* 28.71, 28.42 (two diastereomers). MS (ESI +ve): calc (M+H): 677.2, found: 677.2 $(M+H)^+$, 694.2 $(M+NH_4)^+$

[00210] **Compound 106:** Compound **100** (405 mg, 0.357 mmol) was converted to compound **106** by usi9ng compound 19 and following a procedure analogous to that described for compound **101** (0.35 g, 71%). ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.97 – 9.42 (m, 2H), 7.58 – 7.47 (m, 1H), 7.46 – 7.39 (m, 2H), 7.39 – 7.13 (m, 17H), 6.87 – 6.78 (m, 8H), 6.44 – 6.29 (dtd, *J* = 20.4, 9.2, 4.7 Hz, 2H), 5.27 – 5.16 (dt, *J* = 14.7, 7.3 Hz, 1H), 4.30 – 4.22 (m, 1H), 4.22 – 4.12 (m, 1H), 4.02 – 3.90 (q, *J* = 3.8, 3.4 Hz, 2H), 3.80 – 3.73 (m, 12H), 3.72 – 3.65 (m, 5H), 3.51 – 3.43 (m, 1H), 3.40 – 3.31 (m, 1H), 3.14 – 2.93 (m, 2H), 2.85 – 2.72

(m, 4H), 2.67 – 2.59 (m, 2H), 2.57 – 2.34 (m, 6H), 1.97 – 1.87 (td, J = 13.7, 13.1, 5.7 Hz, 1H), 1.84 (s, 3H), 1.73 – 1.61 (td, J = 14.1, 6.8 Hz, 1H), 1.42 – 1.37 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.97, 163.94, 163.91, 158.88, 158.84, 150.64, 150.60, 150.52, 144.86, 144.83, 144.09, 144.04, 136.06, 136.04, 135.95, 135.93, 135.54, 135.19, 135.09, 135.03, 134.99, 130.28, 130.17, 130.13, 128.29, 128.17, 128.14, 127.38, 127.31, 113.51, 113.42, 111.82, 111.79, 111.44, 111.38, 87.53, 87.38, 87.33, 85.29, 85.26, 84.89, 84.85, 84.41, 84.36, 84.29, 84.25, 83.88, 83.85, 83.80, 83.76, 79.28, 79.23, 78.72, 78.67, 74.04, 67.53, 67.46, 67.37, 67.29, 66.77, 63.33, 63.21, 57.84, 55.34, 53.41, 53.34, 39.23, 39.09, 39.01, 38.92, 38.55, 38.51, 38.46, 38.42, 35.64, 35.59, 30.35, 30.30, 30.26, 12.60, 11.79, 11.74; ^{31}P NMR (162 MHz, $CDCl_3$) δ 29.30, 29.14; MS (ESI +ve): calc (M+H): 1372.44, found: 1372.79. R_f = 0.4 (5% MeOH/DCM).

[00211] **Compound 206:** Compound **106** (200 mg, 0.146 mmol) was converted to compound **206** by a procedure analogous to that described for compound **201** (110 mg, 98%). ^{1}H NMR (399 MHz, CD_3OD) δ 7.83 – 7.75 (dd, J = 7.6, 1.4 Hz, 1H), 7.56 – 7.48 (d, J = 1.6 Hz, 1H), 6.35 – 6.23 (m, 2H), 5.27 – 5.20 (m, 1H), 4.48 – 4.31 (m, 3H), 4.28 – 4.21 (dd, J = 9.7, 2.1 Hz, 1H), 4.11 – 4.04 (t, J = 4.0 Hz, 1H), 3.97 – 3.84 (br, 4H), 3.83 – 3.77 (dd, J = 6.0, 3.2 Hz, 2H), 3.43 – 3.36 (m, 2H), 3.29 – 3.18 (m, 6H), 3.11 – 3.00 (m, 4H), 2.62 – 2.51 (tdd, J = 11.7, 5.7, 1.7 Hz, 1H), 2.47 – 2.38 (ddd, J = 14.3, 8.4, 6.0 Hz, 1H), 2.38 – 2.25 (q, J = 5.3, 4.8 Hz, 2H), 1.91 (s, 3H), 1.88 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD) δ 30.19, 30.12; ^{13}C NMR (100 MHz, CD_3OD) δ 166.28, 166.24, 166.23, 152.32, 152.27, 152.24, 138.05, 138.00, 137.77, 137.75, 112.08, 112.03, 111.97, 111.94, 87.28, 87.24, 87.01, 86.96, 86.62, 86.51, 86.10, 86.06, 85.76, 85.68, 71.73, 71.51, 68.91, 68.58, 68.51, 65.44, 62.60, 62.50, 57.50, 53.50, 40.25, 40.16, 39.64, 39.57, 39.20, 39.16, 39.06, 32.56, 32.55, 31.04, 31.00, 12.73, 12.69, 12.52; MS (ESI +ve): calc (M+H): 768.18, found: 768.14. R_f = 0.3 (10% MeOH/DCM).

[00212] **Compound 107:** Using compound **22** in place of compound **5**, compound **100** is converted to compound **107** by a procedure analogous to that described for compound **101**.

[00213] **Compound 207:** Compound **107** is converted to compound **207** by a procedure analogous to that described for compound **201**.

[00214] **Compound 108:** Using compound **25** in place of compound **5**, compound **100** is converted to compound **108** by a procedure analogous to that described for compound **101**.

[00215] **Compound 208:** Compound **108** is converted to compound **208** by a procedure analogous to that described for compound **201**.

[00216] **Compound 109:** Using compound **27** in place of compound **5**, compound **100** is converted to compound **109** by a procedure analogous to that described for compound **101**.

[00217] **Compound 209:** Compound **109** is converted to compound **209** by a procedure analogous to that described for compound **201**.

[00218] **Compound 110:** Using compound **29** in place of compound **5**, compound **100** is converted to compound **110** by a procedure analogous to that described for compound **101**.

[00219] **Compound 210:** Compound **110** is converted to compound **210** by a procedure analogous to that described for compound **201**.

[00220] **Compound 111:** Using compound **31** in place of compound **5**, compound **100** is converted to compound **111** by a procedure analogous to that described for compound **101**.

[00221] **Compound 211:** Compound **111** is converted to compound **211** by a procedure analogous to that described for compound **201**.

[00222] **Compound 112:** Using compound **33** in place of compound **5**, compound **100** is converted to compound **112** by a procedure analogous to that described for compound **101**.

[00223] **Compound 212:** Compound **112** is converted to compound **212** by a procedure analogous to that described for compound **201**.

[00224] **Compound 113:** Using compound **38** in place of compound **5**, compound **100** is converted to compound **113** by a procedure analogous to that described for compound **101**.

[00225] **Compound 213:** Compound **113** is converted to compound **213** by a procedure analogous to that described for compound **201**.

[00226] **Compound 114:** Using compound **41** in place of compound **5**, compound **100** is converted to compound **114** by a procedure analogous to that described for compound **101**.

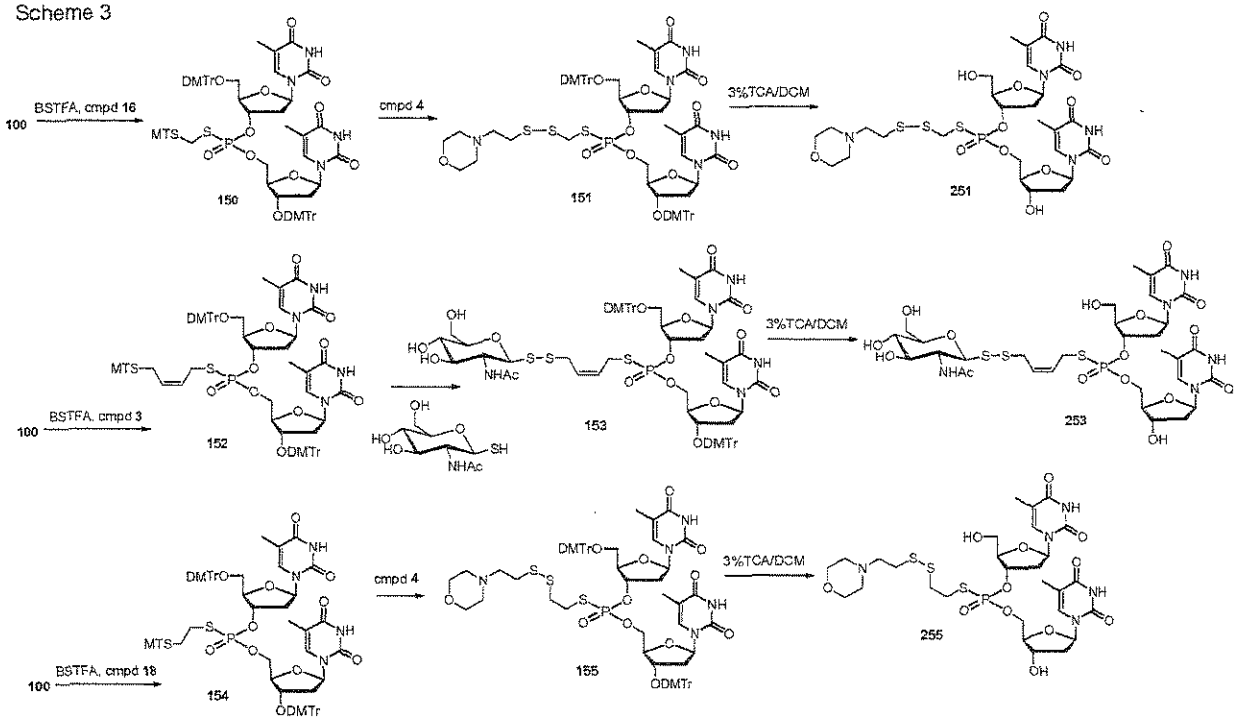
[00227] **Compound 214:** Compound **114** is converted to compound **214** by a procedure analogous to that described for compound **201**.

[00228] **Compound 115:** Using compound **43** in place of compound **5**, compound **100** is converted to compound **115** by a procedure analogous to that described for compound **101**.

[00229] **Compound 215:** Compound **115** is converted to compound **215** by a procedure analogous to that described for compound **201**.

Example 3 – Alternative synthesis of phosphorothiotriesters using bis(methanethiosulfonate) reagents

Scheme 3



[00230] **Compound 150:** Compound **100** (300 mg, 0.264 mmol) was converted to compound **150** by a procedure analogous to that described for compound **101** (170 mg, 50%).

[00231] ^{1}H NMR (399 MHz, $CDCl_3$) δ 9.34 – 9.30 (s, 1H), 9.28 – 9.17 (d, J = 30.6 Hz, 1H), 7.57 – 7.47 (m, 1H), 7.47 – 7.40 (m, 2H), 7.38 – 7.18 (m, 17H), 7.18 – 7.07 (d, J = 1.4 Hz, 1H), 6.88 – 6.77 (dd, J = 9.0, 1.5 Hz, 8H), 6.44 – 6.34 (ddd, J = 15.6, 8.9, 5.4 Hz, 1H), 6.32 – 6.21 (ddd, J = 18.9, 8.5, 5.9 Hz, 1H), 5.27 – 5.19 (q, J = 5.9 Hz, 1H), 4.46 – 4.33 (m, 2H), 4.31 – 4.16 (m, 2H), 4.03 – 3.91 (m, 2H), 3.81 – 3.67 (m, 12H), 3.54 – 3.46 (m, 1H), 3.42 – 3.34 (m, 1H), 3.34 – 3.25 (d, J = 20.2 Hz, 3H), 2.64 – 2.53 (td, J = 13.4, 5.4 Hz, 1H), 2.47 – 2.34 (dq, J = 19.9, 6.5, 5.9 Hz, 1H), 1.99 – 1.91 (m, 1H), 1.85 – 1.80 (t, J = 1.5 Hz, 3H), 1.78 – 1.65 (tt, J = 14.1, 7.5 Hz, 1H), 1.44 – 1.37 (dd, J = 7.3, 1.2 Hz, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 171.27, 163.83, 163.80, 158.95, 158.93, 158.90, 150.64, 150.53, 150.46, 150.38, 144.91, 144.88, 144.09, 144.02, 136.00, 135.98, 135.94, 135.81, 135.11, 135.04, 134.98, 134.97, 130.34, 130.27, 130.20, 128.30, 128.23, 128.20, 127.46, 127.36, 113.59, 113.56, 113.48, 111.95, 111.38, 87.60, 87.47, 87.43, 86.03, 85.83, 84.44, 84.34, 83.81, 79.82, 79.58, 73.99, 73.91, 67.85, 67.78, 63.31, 63.20, 55.39, 51.77, 51.70, 39.16, 38.99, 38.90, 37.21, 37.16, 37.12, 37.05, 12.63, 12.57, 11.85, 11.80; ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.15, 25.60; MS (ESI +ve): calc (M+H): 1308.37, found: 1308.70. R_f = 0.5 (5% MeOH/DCM).

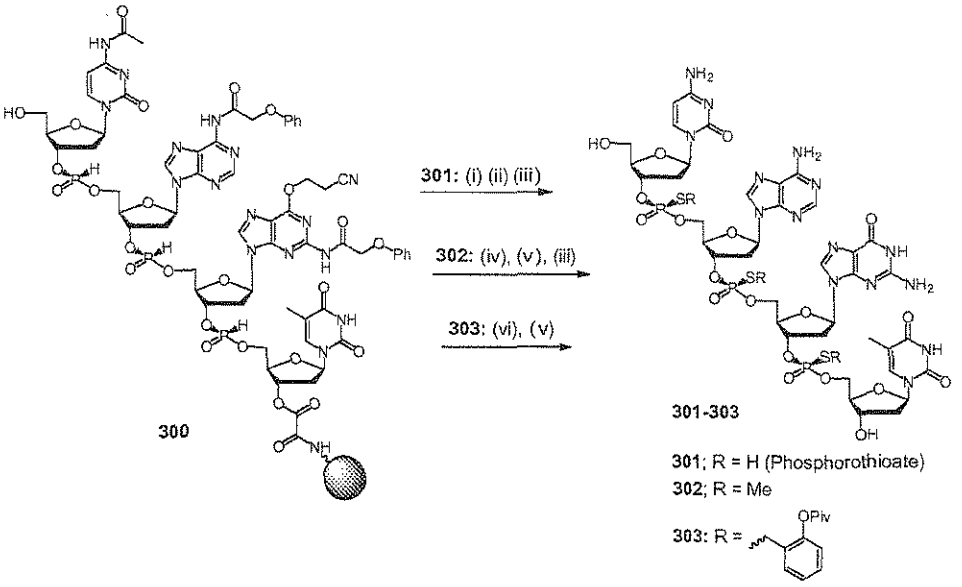
[00232] **Compound 151:** A DCM (5 mL) solution of compound **150** (150 mg, 0.116 mmol) was treated with 2-morpholinoethanethiol (17 mg, 0.116 mmol) at r.t. with monitoring by TLC. After 0.5 h, the mixture was washed with $NaHCO_3$, extracting 5x into DCM. The organic extracts were dried ($MgSO_4$), filtered and reduced. Column chromatography gave compound **151** as a colorless solid foam (81 mg, 51%).

[00233] ^1H NMR (399 MHz, $CDCl_3$) δ 9.68 – 9.54 (m, 1H), 9.44 (s, 1H), 7.59 – 7.48 (m, 1H), 7.47 – 7.40 (m, 2H), 7.40 – 7.13 (m, 17H), 6.90 – 6.76 (ddd, *J* = 9.3, 4.4, 2.7 Hz, 8H), 6.45 – 6.27 (m, 2H), 5.32 – 5.22 (dd, *J* = 8.5, 5.7 Hz, 1H), 4.34 – 4.25 (m, 1H), 4.23 – 4.14 (m, 1H), 4.07 – 3.89 (m, 2H), 3.79 – 3.74 (m, 12H), 3.74 – 3.65 (m, 6H), 3.51 – 3.33 (m, 2H), 2.90 – 2.79 (dd, *J* = 14.2, 7.6 Hz, 2H), 2.73 – 2.55 (m, 3H), 2.55 – 2.34 (m, 6H), 2.02 – 1.91 (m, 1H), 1.87 – 1.81 (dd, *J* = 4.9, 1.2 Hz, 3H), 1.77 – 1.66 (ddd, *J* = 14.2, 8.7, 6.4 Hz, 1H), 1.41 – 1.35 (dd, *J* = 6.6, 1.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.97, 163.93, 163.88, 158.90, 158.86, 158.71, 150.64, 150.59, 150.53, 150.50, 144.92, 144.88, 144.13, 144.08, 136.11, 136.07, 136.03, 136.00, 135.73, 135.60, 135.22, 135.14, 135.08, 135.04, 135.02, 130.32, 130.30, 130.23, 130.18, 128.33, 128.19, 128.17, 127.39, 127.33, 113.56, 113.52, 113.45, 111.85, 111.82, 111.38, 111.29, 87.56, 87.41, 87.38, 85.71, 85.35, 84.91, 84.38, 84.27, 84.22, 84.05, 83.97, 83.85, 83.78, 79.36, 79.11, 79.05, 74.25, 74.07, 67.39, 66.88, 66.79, 63.27, 57.80, 55.36, 53.55, 53.51, 53.40, 43.06, 40.72, 40.54, 39.25, 39.16, 39.01, 35.91, 12.64, 12.60, 11.78, 11.74; ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.76, 27.46; MS (ESI +ve): calc (M+H): 1358.43, found: 1358.74. R_f = 0.4 (5% MeOH/DCM).

[00234] **Compound 251:** Compound **151** (75 mg, 0.055 mmol) was converted to compound **251** by a procedure analogous to that described for compound **201** (10 mg, 24%). MS (ESI +ve): calc (M+H): 754.17, found: 754.19. R_f = 0.3 (10% MeOH/DCM).

[00235] **Compound 152:** Compound **100** is converted to compound **152** by a procedure analogous to that described for compound **101**.

Scheme 4. Synthesis of phosphorothioate triesters on support



(i) Beaucage Reagent, BSA, ACN, (ii) 28% NH3 aq., (iii) NH4OAc (AA) buffer, (iv) MTS-Me, BSTFA, Et3N, ACN, (v) PrNH2, ACN, (vi) Compound 12, BSTFA, ACN,

[00236] **Compound 153:** Using 1-Thio-β-D-glucose tetraacetate in place of compound **4**, compound **152** is converted to compound **153** by a procedure analogous to that described for compound **151**.

[00237] **Compound 253:** Compound **153** is converted to compound **253** by a procedure analogous to that described for compound **201**.

[00238] **Compound 154:** Compound **100** is converted to compound **154** by a procedure analogous to that described for compound **101**.

[00239] **Compound 155:** Compound **154** is converted to compound **155** by a procedure analogous to that described for compound **151**.

[00240] **Compound 255:** Compound **155** is converted to compound **255** by a procedure analogous to that described for compound **201**.

Example 4 – Thioalkylation of H-phosphonates to provide phosphorothiotriesters in solid phase

[00241] **Compound 300:** Synthesis of (*R*p)-CAGT-H-phosphonate-oxalyl linker-CPG was carried out on an Applied Biosystems 394 DNA/RNA synthesizer according to the reported methods (*Journal of American Chemical Society* **2008**, *130*, 16031-16037; *Angewandte Chemie International Edition* **2009**, *48*, 496-499).

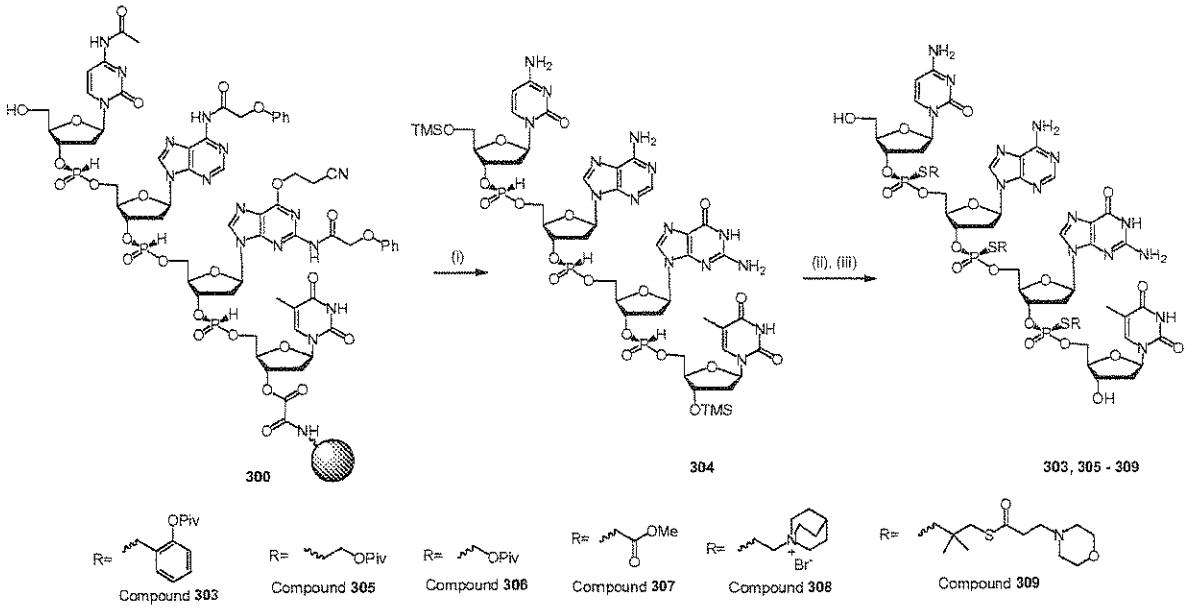
[00242] **Compound 301: (*S*p)-CAGT-phosphorothioate (R = H)**: (*R*p)-CAGT-H-phosphonate-oxalyl linker-CPG was treated by 0.2 M Beaucage Reagent/CH_3CN-BSA (9:1, v/v), stirred for 1 h at rt, then washed successively with CS_2 and acetonitrile and dried under reduced pressure. The resultant CPG was treated with 2 mL of 28% aqueous NH_3 and stirred for18 h at rt. After removal of NH_3 under reduced pressure, the resulting product was analyzed by LC/MS and HPLC.

[00243] **Compound 302: (*S*p)-CAGT-*S*-methyl phosphorothiotriester (R = Me)** : BSTFA (50 µL, 188 µmol) and acetonitrile (500 µL) were added to (*R*p)-CAGT-H-phosphonate-oxalyl linker-CPG (14.7 mg, 1 µmol) then the mixture was shaken for 20 min at rt. *S*-methyl methane sulfonothioate (20 µL, 212 µmol) and NEt_3 (50 µL) were added and shaking was continued for 1 h at rt. The CPG was washed with CH_3CN then dried *in vacuo*. 20% $PrNH_2$ in dry CH_3CN (2 mL) was added to the CPG and the mixture was stirred for 16 h at rt. Solvents were removed under reduced pressure and CH_3CN was added to the mixture. The CPG was removed by filtration and the filtrate was concentrated under reduced pressure. CH_3CN/DMSO/0.5 M AA buffer (1:1:1, v/v/v) was added, the mixture was stirred for 16 h at rt, then analyzed by LC/MS and HPLC.

[00244] **Compound 303:** Compound **303** is prepared by sulfurization of compound **300** on support followed by cleavage. ACN (450 µL), BSTFA (50 µL) and compound **12** (20 mg) are added to compound **300** (1 µmol) which is shaken for 18 h. The CPG is collected by filtration resuspended in 20% $PrNH_2$ in dry CH_3CN (2 mL) and shaken for 16 h at rt. Solvents were removed under reduced pressure and the residue is purified by RPHPLC to provide pure compound **303**.

Example 5 – Thioalkylation of H-phosphonates to provide phosphorothiotriesters in solution phase

Scheme 5. Synthesis of phosphorothioate triesters in solution.



(i) 2:1:2 v/v/v ACN:BSTFA:PrNH2, 18 h rt, (ii) Pyridine, BSTFA, R-MTS, rt, (iii) 1:1 v/v MeOH:0.5M TEAA, 18 h rt.

[00245] **Compound 305:** Compound **300** (0.5 μmol) was taken up in ACN (125 μL) then BSTFA (62 μL) was added and the mixture was shaken for 20 min. $PrNH_2$ (125 μL) was added and the vial was rotated for 18 h. After filtration and washing with 1 mL ACN, the solvent was removed *in vacuo* and the residue was co-evaporated 3x with toluene to provide crude compound **304**. The residue was redissolved in pyridine (375 μL) and treated with BSTFA for (16 μl, 60.0 μmol) followed by compound **9** (7.2 mg, 30.0 μmol) with stirring under Ar. After 2 h at r.t. the solvent was removed and the residue was treated with MeOH (0.125 mL) for 1 h, then AA (0.5 M, 0.125 mL) was added and the mixture was stirred at r.t. for 2 h. The product was purified by RPHPLC to provide compound **305**.

[00246] **Compound 303:** Substituting compound **12** for compound **9**, compound **303** was prepared by a procedure analogous to that described for compound **305**.

[00247] **Compound 306:** Substituting compound **12** for compound **14**, compound **306** was prepared by a procedure analogous to that described for compound **305**.

[00248] **Compound 307:** Substituting compound **12** for compound **29**, compound **307** is prepared by a procedure analogous to that described for compound **305**.

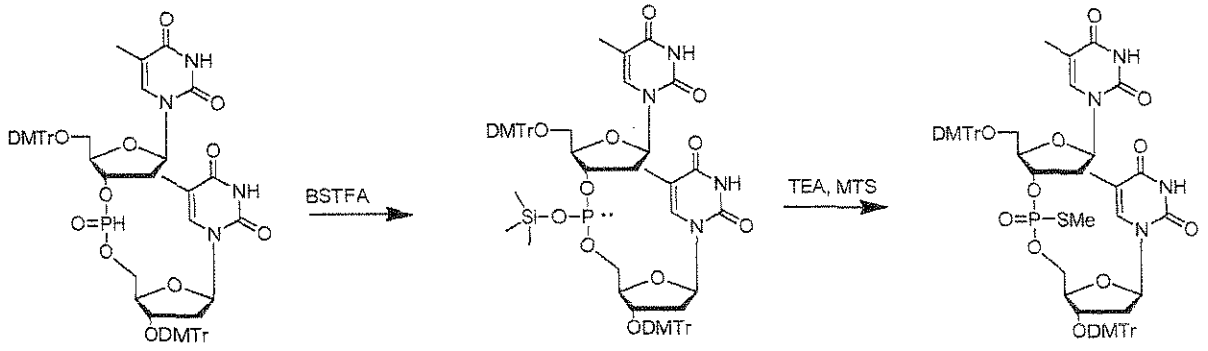
[00249] **Compound 308:** *Substituting compound 12 for compound 31, compound 308* is prepared by a procedure analogous to that described for compound **305**.

[00250] **Compound 309:** Substituting compound **12** for compound **38**, compound **309** is prepared by a procedure analogous to that described for compound **305**.

Example 6 – Stereoselective thioalkylation of H-phosphanates

[00251] **Objective**: To demonstrate that the reaction of MTS reagents to H-phosphonate to generate phosphorothio triester is stereospecific. ^{31}P NMR was used to trace the changes during the course of the reaction.

Scheme 5



100S Pure Stereoisomer Sp

100R Pure Stereoisomer Rp

Intermediate

Pure Stereoisomer Sp

Pure Stereoisomer Rp

Pure Stereoisomer R

Pure Stereoisomer Sp

[00252] Experimental procedure: In an NMR tube was added compound 100S 5′-O-(4,4'-dimethoxytrityl)thymidin-3′-yl 3′-O-(4,4'-dimethoxytrityl)thymidin-5′-yl H-phosphonate (20 mg, 18 μmol) in 0.8 mL CD_3CN and the ^{31}P NMR spectrum was recorded. BSTFA (17 μL, 176 μmol) was added to same NMR tube and after 5 min ^{31}P NMR spectrum was recorded again. Triethylamine (49 μL, 352 μmol) and S-methyl methanethiosulfonate (22 μL, 88 μmol) were added to same NMR tube and ^{31}P NMR spectrum was recorded immediately.

[00253] The same procedure was repeated for Rp isomer (compound 100R). The ^{31}P NMR spectrum recorded for the starting material, intermediate and the product show that the stereochemistry at phosphorus atom is retained during the reaction.

[00254] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

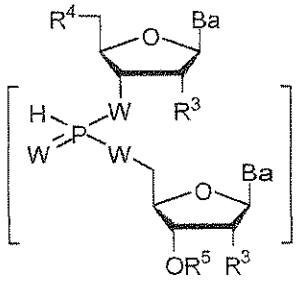
1. A process for the preparation of phosphorothiotriesters of structure IIIa comprising the steps of:

i) reacting an H-phosphonate of structure Ia with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIa to provide a phosphorothiotriester of structure IIIa;

wherein,

the H-phosphonate of structure Ia has the following structure:



Structure Ia

wherein,

W is independently selected from O, S, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

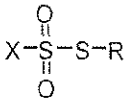
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIa has the following structure:



Structure IIa wherein,

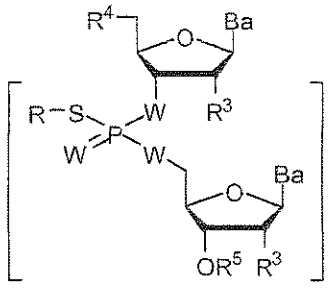
X is alkyl, cycloalkyl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the phosphorothiotriester of structure IIIa has the following structure:



n Structure IIIa

wherein,

W is independently selected from O, S, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

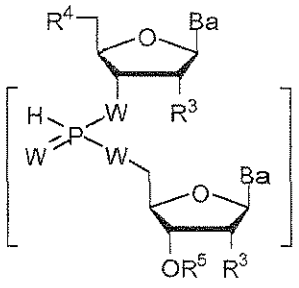
2. A process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb comprising the steps of:

i) reacting a H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib with an silylating reagent to provide a silyloxyphosphonate; and

ii) reacting the silyloxyphosphonate with a thiosulfonate reagent of structure IIb to provide a phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb;

wherein,

the H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib has the following structure:



Structure Ib

wherein,

W is independently selected from O, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

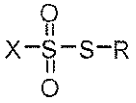
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the thiosulfonate reagent of structure IIb has the following structure:



Structure IIb wherein,

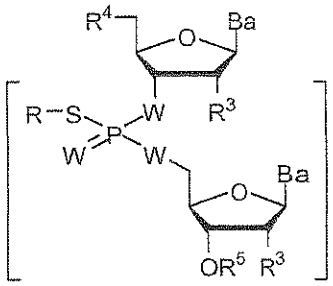
X is alkyl, cycloalkyl, aryl, or heteroaryl;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

and the chiral phosphorothiotriester comprising non-stereorandom phosphorous linkages of structure IIIb has the following structure:



Structure IIIb

wherein,

W is independently selected from O, NH, or CH_2;

R is alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or R^1-R^2;

R^1 is selected from -S-alkenylene-, -S-alkylene-, -S-alkylene-aryl-alkylene-, -S-CO-aryl-alkylene-, or -S-CO-alkylene-aryl-alkylene-;

R^2 is selected from heterocyclo-alkylene-S-, heterocyclo-alkenylene-S-,aminoalkyl-S-,or $(alkyl)_4N$-alkylene-S-;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

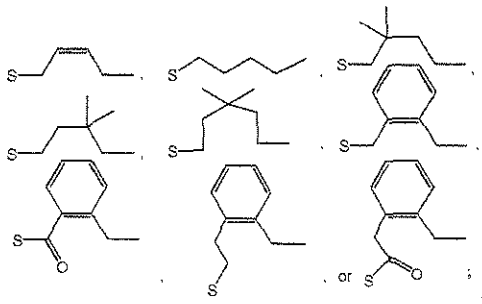
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200.

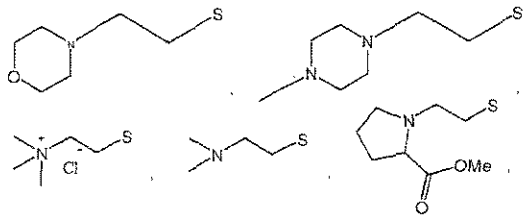
3. The process of claim 1 or 2, wherein W is O.

4. The process of claim 1 or 2, wherein R^1 is selected from:



and

R^2 is selected from:



5. The process of claim 1 or 2, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N′-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

6. The process of claim 5, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

7. The process of claim 6, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

8. The process of claim 1 or 2, wherein the H-phosphonate is covalently linked to a solid phase.

9. A process for the preparation of phosphorothiotriesters of structure IIIc comprising the steps of:

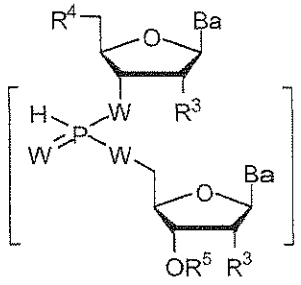
i) reacting a H-phosphonate of structure Ic with an silylating reagent to provide a silyloxyphosphonate;

ii) reacting the silyloxyphosphonate with a bis(thiosulfonate) reagent of structure IVc to provide a phosphorothiotriester comprising a thiosulfonate group of structure Vc;

iii) reacting the phosphorothiotriester comprising a thiosulfonate group of structure Vc with a nucleophile of structure VIc to provide the phosphorothiotriesters of structure IIIc;

wherein,

the H-phosphonate of structure Ic has the following structure:



Structure Ic

wherein,

W is independently selected from O, S, NH, or CH_2;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

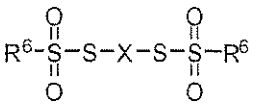
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid; and

n is between 1 and about 200; and

the bis(thiosulfonate) reagent of structure IVc has the following structure:



Structure IVc wherein,

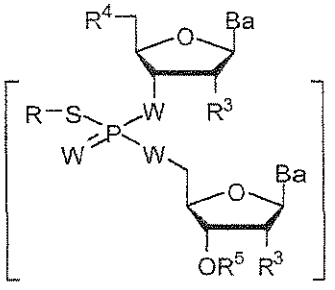
X is alkylene, alkenylene, arylene, or heteroarylene;

each R^6 is independently alkyl, cycloalkyl, aryl, or heteroaryl;

the nucleophile of structure VIc has the following structure:

R^7-SH, wherein R^7 is selected from alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

and phosphorothiotriesters of structure IIIc has the following structure:



n Structure IIIc

wherein,

W is independently selected from O, S, NH, or CH_2;

R is R^7-S-S-X-

R^7 is alkyl, alkenyl, aryl, heterocyclo, aminoalkyl, or (heterocyclo)alkyl;

X is alkylene, alkenylene, arylene, or heteroarylene;

R^3 is -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-P(O)(R^e)_2$, $-HP(O)(R^e)$, $-OR^a$ or $-SR^c$;

Y^1 is O, NR^d, S, or Se;

R^a is a blocking group;

R^c is a blocking group;

each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, carbamate, $-P(O)(R^e)_2$, or $-HP(O)(R^e)$;

each instance of R^e is independently hydrogen, alkyl, aryl, alkenyl, alkynyl, alkyl-Y^2-, alkenyl-Y^2-, alkynyl-Y^2-, aryl-Y^2-, or heteroaryl-Y^2-, or a cation which is Na^{+1}, Li^{+1}, or K^{+1};

Y^2 is O, NR^d, or S;

each instance of R^a is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

R^5 is hydrogen, a blocking group, a linking moiety connected to a solid support or a linking moiety connected to a nucleic acid;

n is between 1 and about 200; and

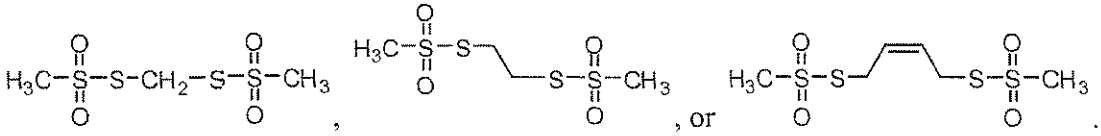
wherein the phosphorous linkages of the H-phosphonate of structure Ic, the phosphorothiotriester comprising a thiosulfonate group of structure Vc, and the phosphorothiotriesters of structure IIIc may optionally comprise non-stereorandom phosphorous linkages.

10. The process of claim 8, wherein the phosphorothiotriesters of structure IIIb comprise non-stereorandom phosphorous linkages and the H-phosphonate of structure Ic comprise non-stereorandom phosphorous linkages; and W is independently selected from O, NH, or CH_2.

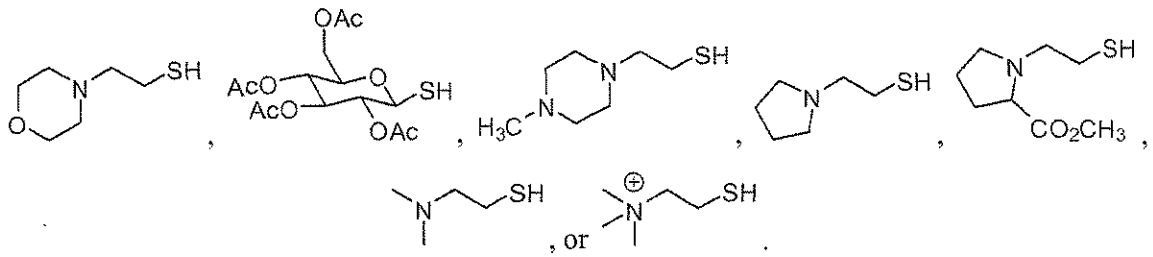
11. The process of claim 9 or 10, wherein W is O.

12. The process of claim 9, wherein R^6 is methyl.

13. The process of claim 9, wherein bis(thiosulfonate) reagent of structure IVc is selected from:



14. The process of claim 9, wherein the nucleophile of structure VIc has the following structure:



15. The process of claim 9, wherein the silylating reagent is selected from

1,1,3,3-tetramethyl-1,3-diphenyldisilazane;

1,3-dimethyl-1,1,3,3-tetraphenyldisilazane;

1-(trimethylsilyl)imidazole;

N-trimethylsilyl-N-methyl trifluoroacetamide;

bis(dimethylamino)dimethylsilane;

bromotrimethylsilane;

chlorodimethyl(pentafluorophenyl)silane;

chlorotriethylsilane;

chlorotriisopropylsilane;

chlorotrimethylsilane;

dichlorodimethylsilane;

hexamethyldisilazane;

N,N′-bis(trimethylsilyl)urea;

N,N-bis(trimethylsilyl)methylamine;

N,N-dimethyltrimethylsilylamine;

N,O-bis(trimethylsilyl)acetamide;

N,O-bis(trimethylsilyl)carbamate;

N,O-bis(trimethylsilyl)trifluoroacetamide;

N-methyl-N-(trimethylsilyl)trifluoroacetamide;

N-methyl-N-trimethylsilylacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide;

N-methyl-N-trimethylsilylheptafluorobutyramide;

trimethylsilyltriflate;

triethylsilyltriflate;

triisopropylsilyltriflate; or

tert-butyldimethylsilyltriflate.

16. The process of claim 15, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, or 1-(trimethylsilyl)imidazole.

17. The process of claim 16, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide.

18. The process of claim 17, wherein the H-phosphonate is covalently linked to a solid phase.