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.

[0002a] In a first aspect of the present disclosure, there is provided a process for the preparation of phosphorothiotriesters of structure IIIc comprising the steps of:

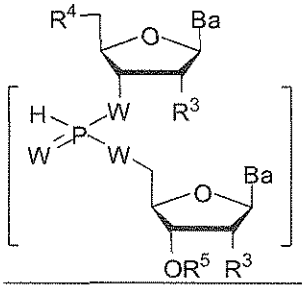
i) reacting an H-phosphonate of structure Ic with a silylating reagent to provide a silyloxyphosphonate;

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-, $P(O)(R^c)_2$, $-HP(O)(R^c)$, $-OR^a$ 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, $-P(O)(R^c)_2$, or $-HP(O)(R^c)$;

each instance of R^c 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};

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;

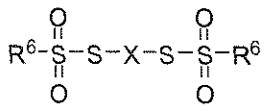
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

Y^2 is O, S or NR^d wherein R^d is hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, or carbamate;

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,

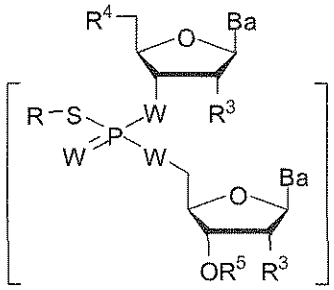
X is alkylene, alkenylene, arylene, or heteroarylene;

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};

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;

Y^2 is O, S or NR^d wherein R^d is hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, or carbamate;

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.

[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:

CLAIMS

WHAT IS CLAIMED IS:

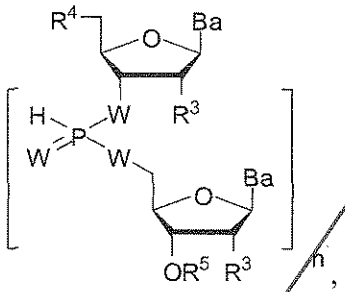
1. A process for the preparation of phosphorothiotriesters of structure IIIa comprising the steps of:

i) reacting an H-phosphonate of structure Ia with a 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 O;

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;

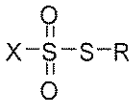
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

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:

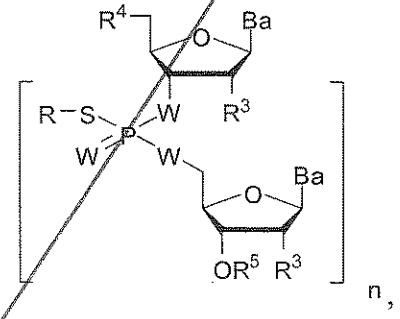
X is alkyl, cycloalkyl, or heteroaryl;

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:



Structure IIIa

wherein:

W is O;

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.

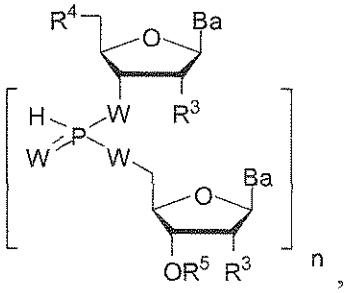
2. A process for the preparation of phosphorothiotriesters comprising non-stereorandom phosphorous linkages of structure IIIb comprising the steps of:

i) reacting an H-phosphonate comprising non-stereorandom phosphorous linkages of structure Ib with a 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 O;

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;

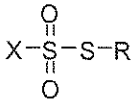
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

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:

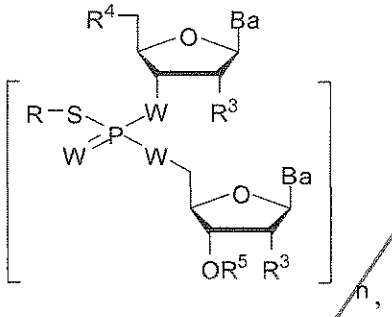
X is alkyl, cycloalkyl, aryl, or heteroaryl;

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 O;

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;

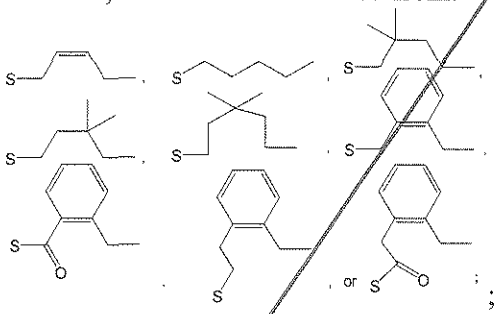
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

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

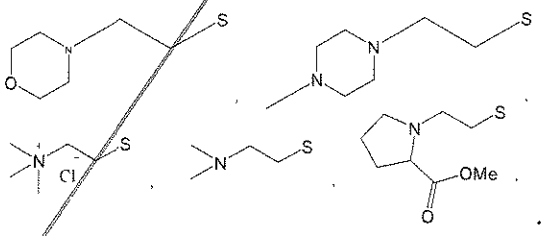
n is between 1 and about 200.

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



; and

R^2 is selected from:



4. 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; and
tert-butyldimethylsilyltriflate.

5. The process of claim 4, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, and 1-(trimethylsilyl)imidazole.

6. The process of claim 5, wherein the silylating reagent is N,O-bis(trimethylsilyl)trifluoroacetamide.

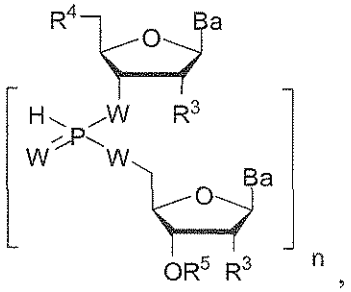
7. The process of claim 1 or 2, wherein the H-phosphonate is covalently linked to a solid phase.

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

i) reacting an H-phosphonate of structure Ic with a 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;
iii) reacting the phosphorothiotriester comprising a thiosulfonate group 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 O;

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;

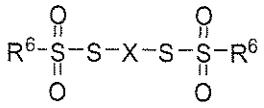
each instance of R^4 is independently hydrogen, -OH, -SH, $-NR^dR^d$, $-N_3$, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1-, alkenyl-Y^1-, alkynyl-Y^1-, aryl-Y^1-, heteroaryl-Y^1-, $-OR^b$,or $-SR^c$, and R^b is a blocking group;

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:

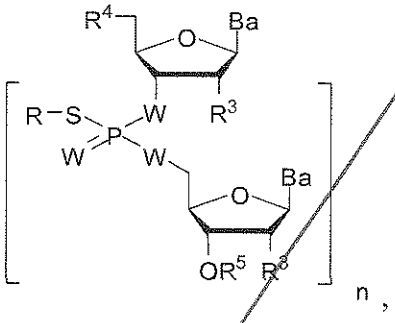
X is alkylene, alkenylene, arylene, or heteroarylene;

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:



Structure IIIc ,

wherein:

W is O;

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;

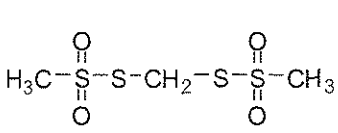
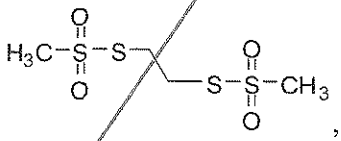
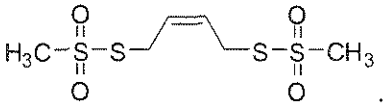
n is between 1 and about 200; and

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

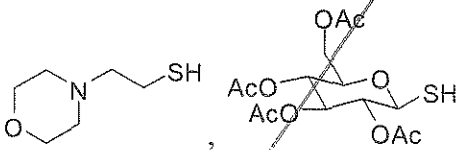
9. The process of claim 7, wherein the phosphorothiotriesters of structure IIIb comprise non-stereorandom phosphorous linkages and the H-phosphonate of structure Ia or Ib comprise non-stereorandom phosphorous linkages.

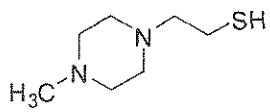
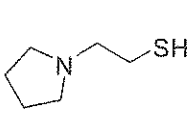
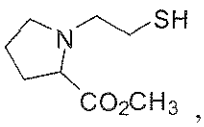
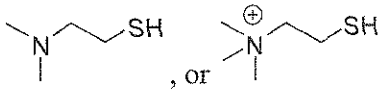
10. The process of claim 8, wherein R^6 is methyl.

11. The process of claim 8, wherein bis(thiosulfonate) reagent of structure IVc is selected from:


,

, and

.

12. The process of claim 8, wherein the nucleophile of structure VIc has the following structure:


,

,

,

,

,

, or

.

13. The process of claim 8, 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; and
tert-butyldimethylsilyltriflate.

14. The process of claim 13, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, trimethylsilyltriflate, chlorotrimethylsilane, and 1-(trimethylsilyl)imidazole.

15. The process of claim 14, wherein the silylating reagent is N,O-bis(trimethylsilyl)trifluoroacetamide.

16. The process of claim 15, wherein the H-phosphonate is covalently linked to a solid phase.

17. The process of any one of the preceding claims, wherein Y^1 is O.

18. The process of any one of the preceding claims, wherein:

each instance of R^3 is independently hydrogen, −OH, halogen, alkyl, alkenyl, alkynyl, alkyl-Y^1−, alkenyl-Y^1−, alkynyl-Y^1−, aryl-Y^1−, heteroaryl-Y^1−, or $-OR^a$;

each instance of R^4 is independently −OH, hydrogen, alkyl, alkenyl, alkynyl, alkyl-Y^1−, alkenyl-Y^1−, alkynyl-Y^1−, aryl-Y^1−, heteroaryl-Y^1−, or $-OR^b$; and

R^5 is hydrogen or a blocking group.

19. The process of any one of the preceding claims, wherein each instance of R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, or acyl.

20. The process of any one of the preceding claims, wherein Y^2 is O or S.

CLAIMS

WHAT IS CLAIMED IS:

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

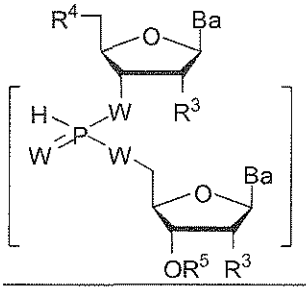
i) reacting an H-phosphonate of structure Ic with a silylating reagent to provide a silyloxyphosphonate;

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^c 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};

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;

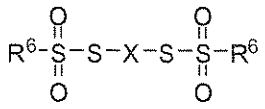
each instance of Ba is independently a blocked or unblocked adenine, cytosine, guanine, thymine, uracil or modified nucleobase;

Y^2 is O, S or NR^d wherein R^d is hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, or carbamate;

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,

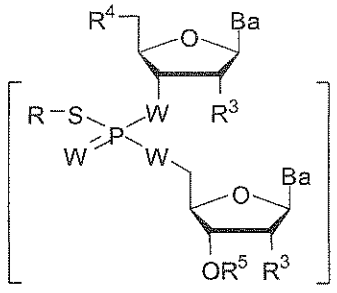
X is alkylene, alkenylene, arylene, or heteroarylene;

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};

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;

Y^2 is O, S or NR^d wherein R^d is hydrogen, alkyl, alkenyl, alkynyl, aryl, acyl, substituted silyl, or carbamate;

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

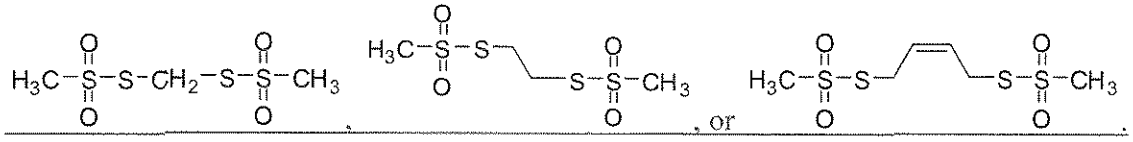
wherein the phosphorous linkages of the H-phosphonate of structure Ic, the phosphorothiotriester comprising a thiosulfonate group of structure Vc, and the phosphorothiotriesters of structure IIIc may optionally comprise non-stereorandom phosphorous linkages.

2. The process of claim 1, wherein the phosphorothiotriesters of structure IIIc 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.

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

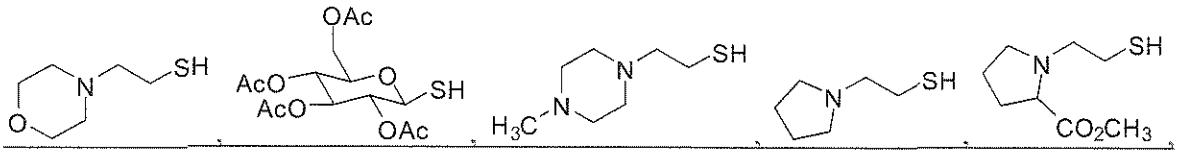
4. The process of claim 1, wherein R^6 is methyl.

5. The process of claim 1, wherein the bis(thiosulfonate) reagent of structure IVc is selected from:



, or

6. The process of claim 1, wherein the nucleophile of structure VIc has the following structure:



, or

7. The process of claim 1, 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.

8. The process of claim 7, wherein the silylating reagent is selected from N,O-bis(trimethylsilyl)trifluoroacetamide, wherein the H-phosphonate is preferably covalently linked to a solid phase.