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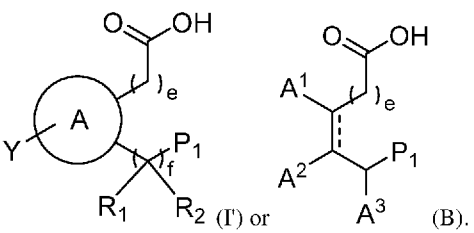
(54) Title: OLIGONUCLEOTIDES, REAGENTS AND PREPARATION THEREOF

(57) Abstract: The present disclosure describes novel reagents and processes for preparing oligonucleotides, which have two or more nucleotides. In one embodiment, the reagent is represented by Formula I' or B.



(I') or

(B).

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OLIGONUCLEOTIDES, REAGENTS, AND PREPARATION THEREOF

RELATED APPLICATION

[01] This application claims the benefit of the filing date, under 35 U.S.C. § 119(e), of U.S. Provisional Application No. 63/112,281, filed on November 11, 2020, the entire contents of which is incorporated herein by reference.

FIELD OF THE INVENTION

[02] The present invention relates to oligonucleotides, reagents, and methods for preparing oligonucleotides.

BACKGROUND

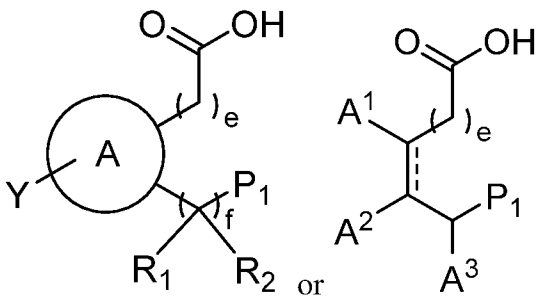
[03] Oligonucleotides are short DNA or RNA oligomers that can be chemically synthesized for a wide range of applications. Recent developments in utilizing synthetic oligonucleotides as therapeutic agents have increased demand for synthetic methods that can produce oligonucleotides in large quantities with high efficiency and purity.

[04] Traditionally, oligonucleotides are synthesized by a solid phase automated synthesizer utilizing phosphoramidite chemistry, limited to a scale of less than 2 moles. Thus, the solid phase synthesis is insufficient for the production of materials needed for clinical development and commercialization of oligonucleotide drugs in large indications. In addition, the solid phase synthesis often requires the use of excess reagents and consequently increases the cost associated with the production of the target oligonucleotides.

[05] Hence, there is a need for novel reagents and robust methods for synthesizing oligonucleotides that are suitable for large scale manufacturing process with high efficiency and purity.

SUMMARY OF THE INVENTION

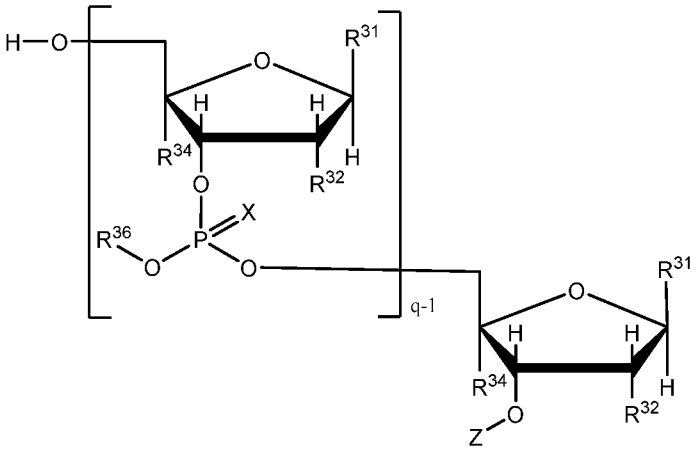
[06] One aspect of the present disclosure is directed to a compound of Formula I' or B:



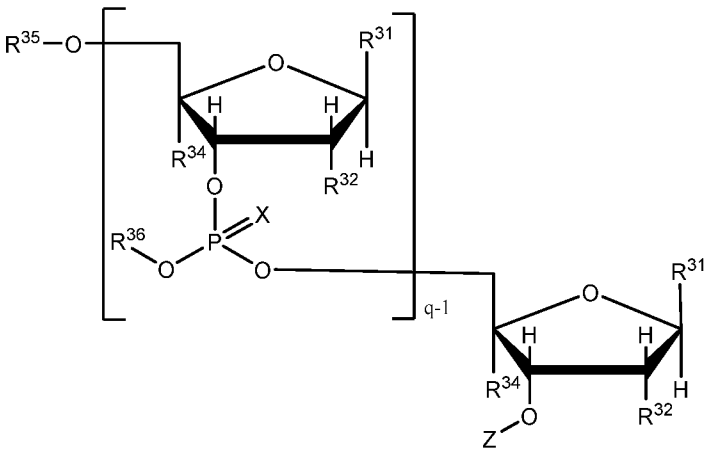
I' B

or a salt thereof; wherein ring A, A^1, A^2, A^3, R_1, R_2, P_1, Y, e, and f are defined below.

[07] One aspect of the present disclosure is directed to a nucleotide or an oligonucleotide represented by Formula III or IIIP,



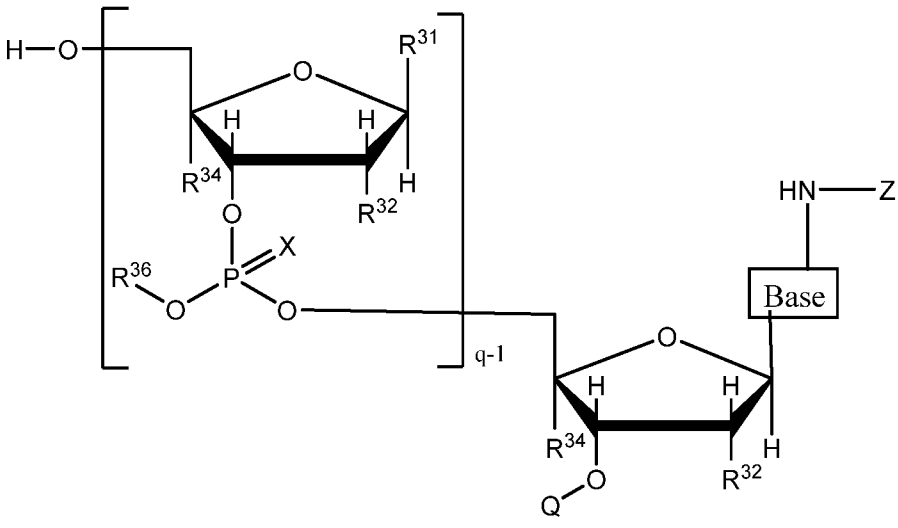
(III), or



(IIIP);

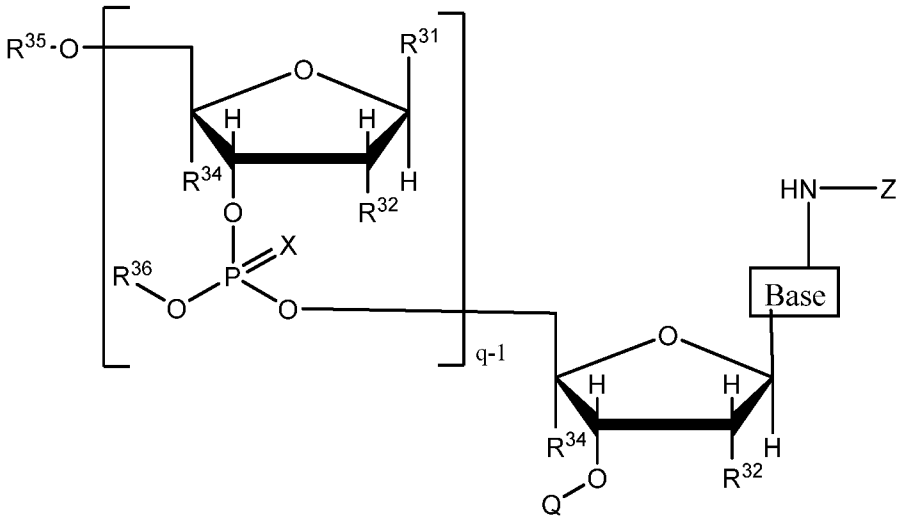
or a salt thereof, wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X, and Z are defined below.

[08] One aspect of the present disclosure is directed to a nucleotide or an oligonucleotide represented by Formula III' or IIIP',



(III'),

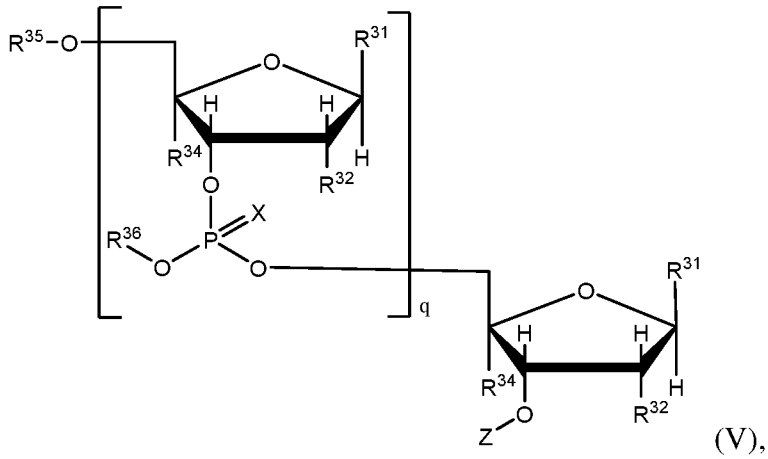
or



(IIIP');

or a salt thereof, wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, Q, X, Base and Z are defined below.

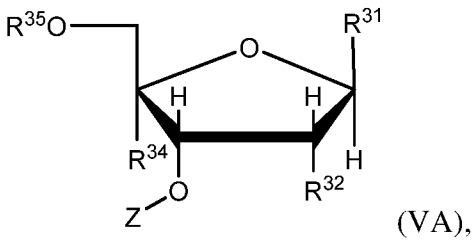
[09] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (V),



(V),

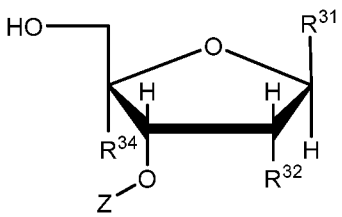
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



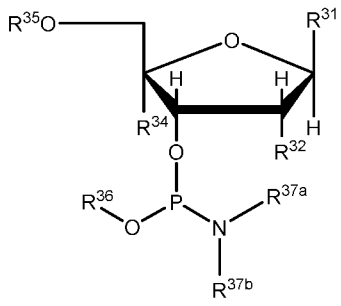
(VA),

or a salt thereof, to form a compound of formula (VB):



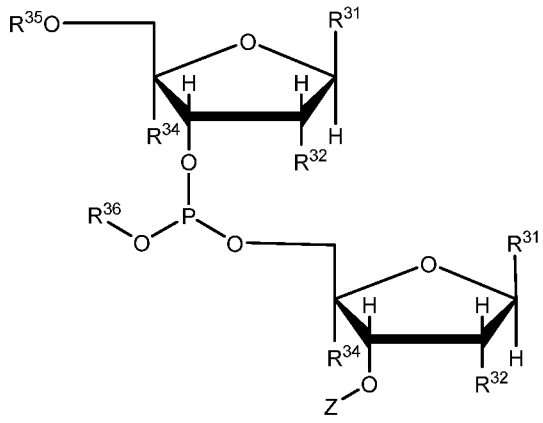
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC):



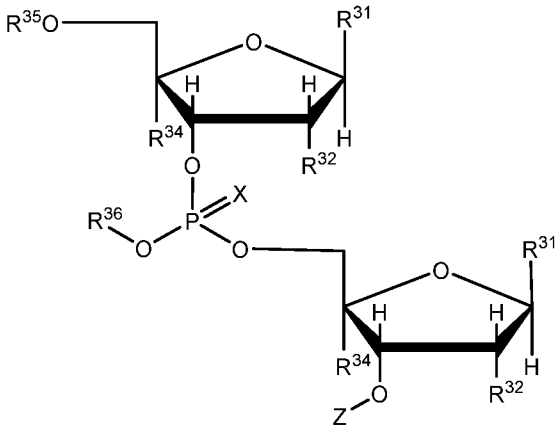
(VC),

or a salt thereof, to form a compound of formula (VD),



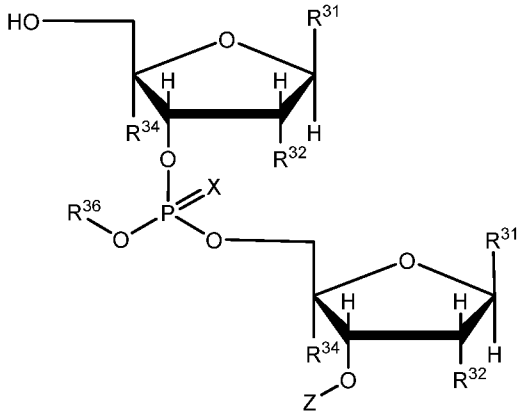
(VD), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE):



(VE), or a salt thereof;

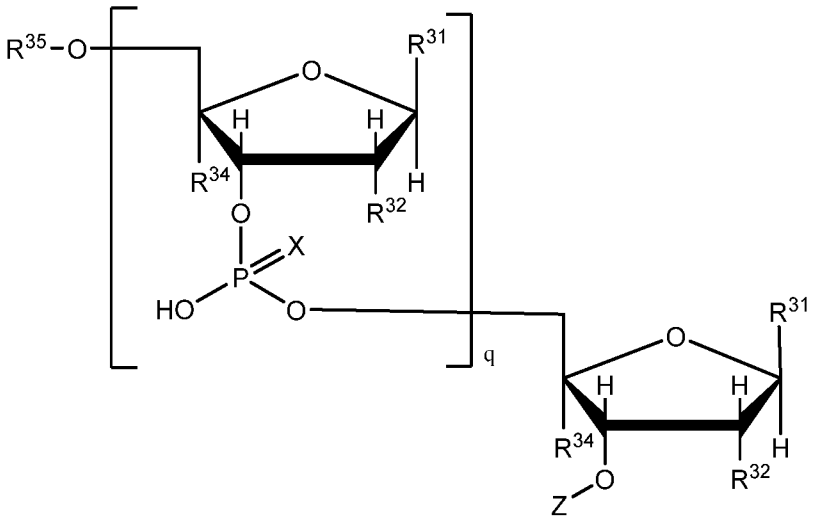
4) deprotecting the compound of formula (VE), or a salt thereof to form a compound of formula (VF):



(VF), or a salt thereof;

5) when q is equal or greater than 2, starting with the compound of formula (VF), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V), or a salt thereof; wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X, and Z are defined below.

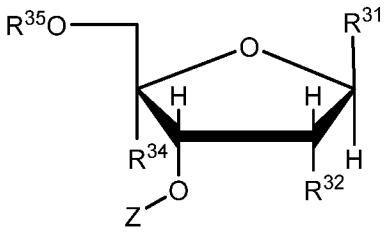
[010] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula(V'),



(V'),

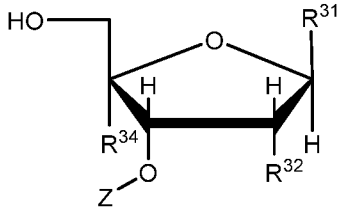
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



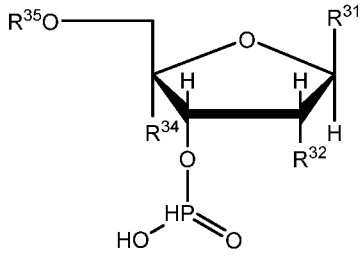
(VA),

or a salt thereof, to form a compound of formula (VB):



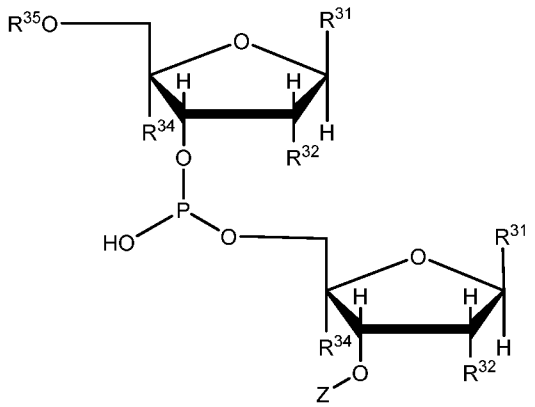
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC'):



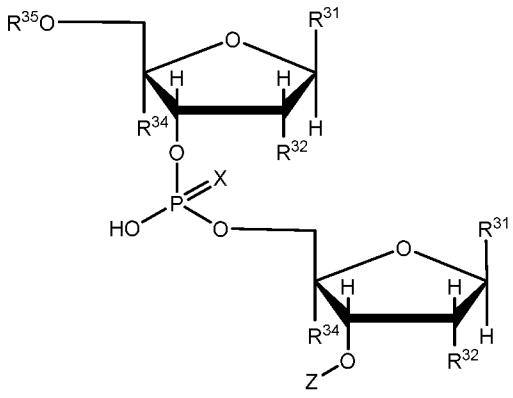
(VC'),

or a salt thereof, to form a compound of formula (VD'),



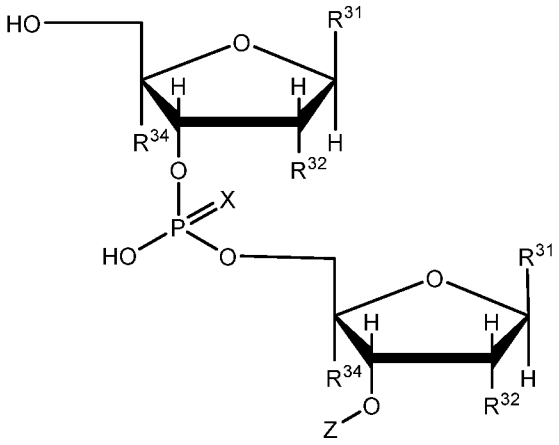
(VD'), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD'), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE'):



(VE'), or a salt thereof;

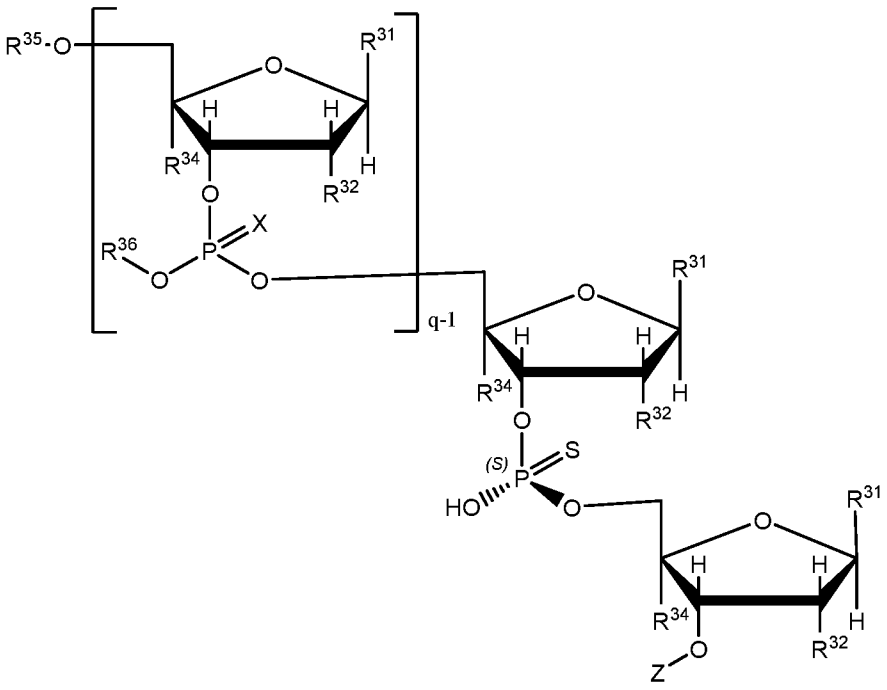
4) deprotecting the compound of formula (VE'), or a salt thereof to form a compound of formula (VF'):



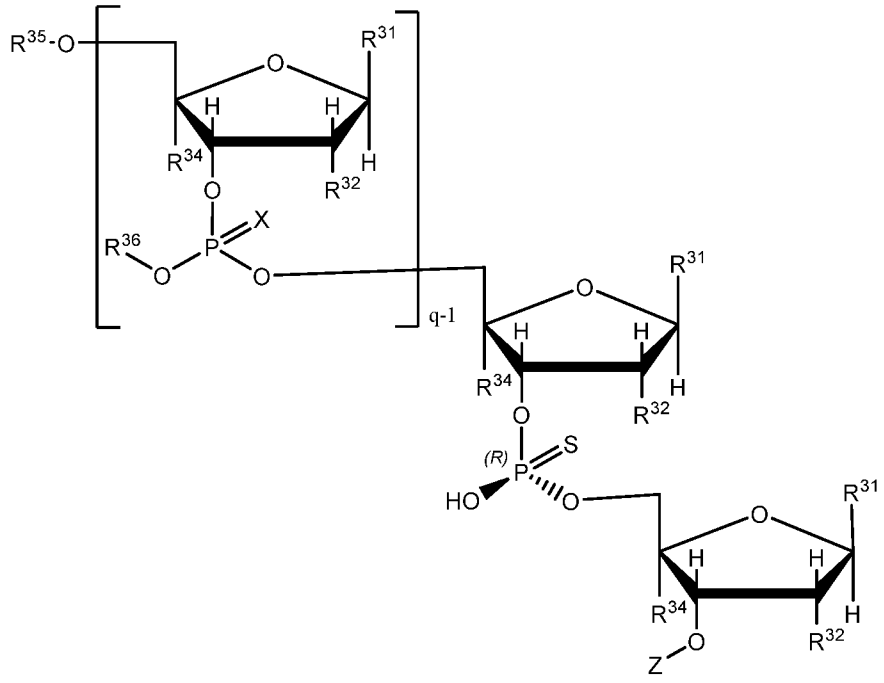
(VF'), or a salt thereof;

5) when q is equal or greater than 2, starting with the compound of formula (VF'), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V'), or a salt thereof, wherein R^{31}, R^{32}, R^{34}, R^{35}, q, X, and Z are defined below.

[011] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



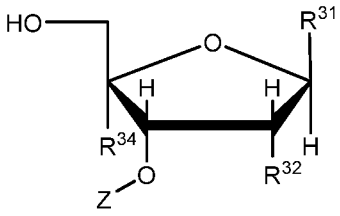
(V-C1), or



(V-C2)

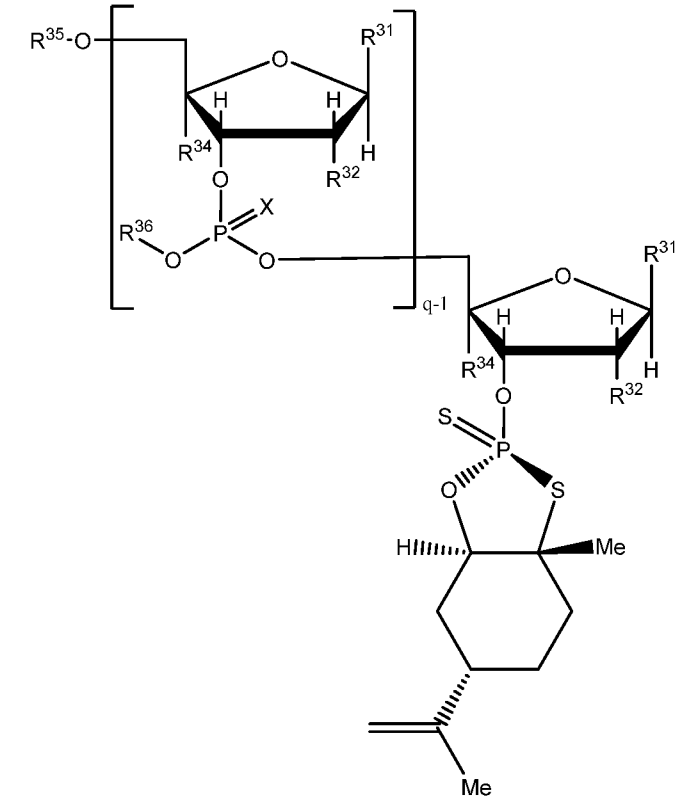
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

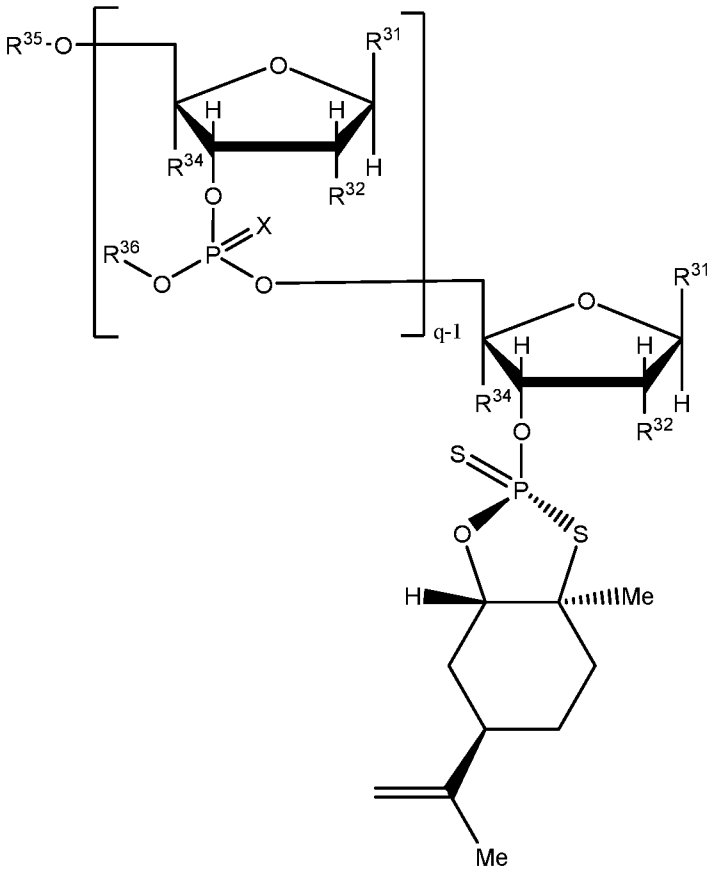


(VB),

or a salt thereof, with a compound of formula (V-CR1) or (V-CR2),



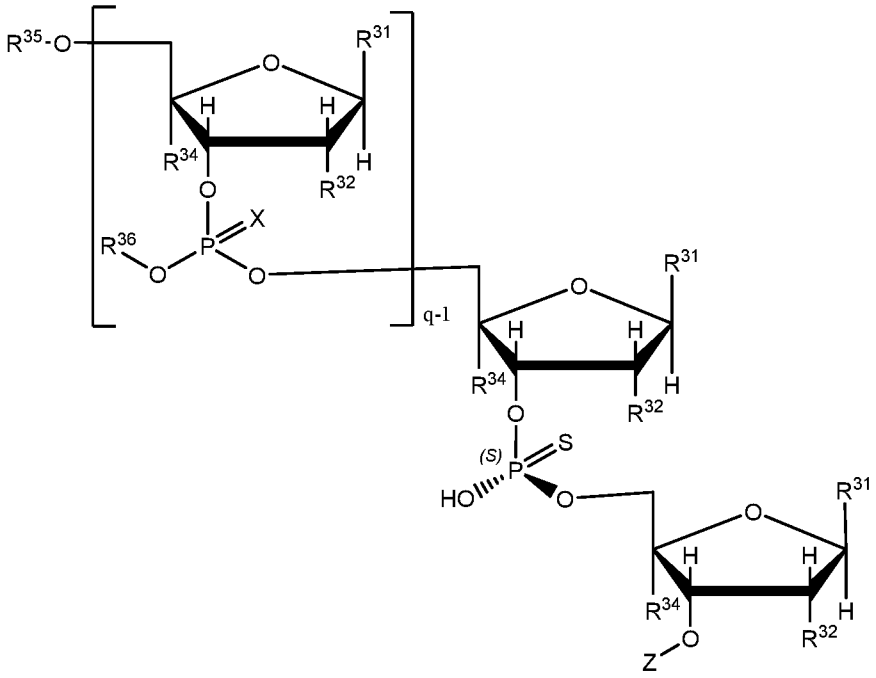
(V-CR1),



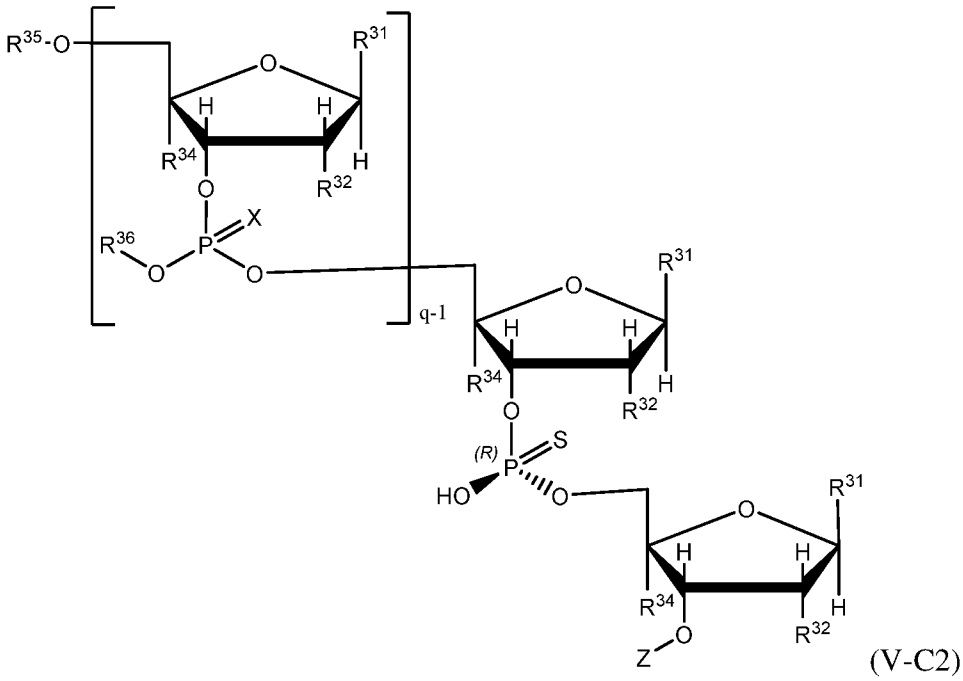
(V-CR2)

or a salt thereof, and a base, to form a compound of formula (V-C1) or (V-C2), wherein R^{31}, R^{32}, R^{34}, R^{35}, q, X, and Z are defined below.

[012] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



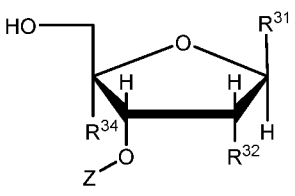
(V-C1), or



(V-C2)

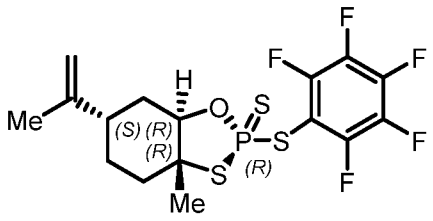
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

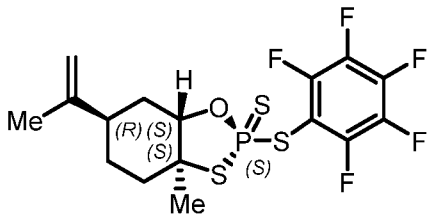


(VB),

or a salt thereof, with a reagent of formula (VR1) or (VR2),

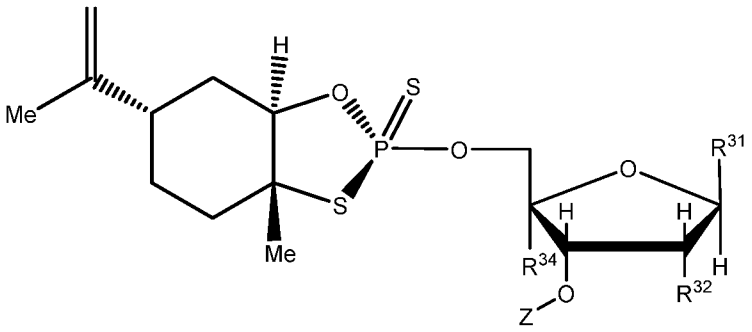


(VR1), or

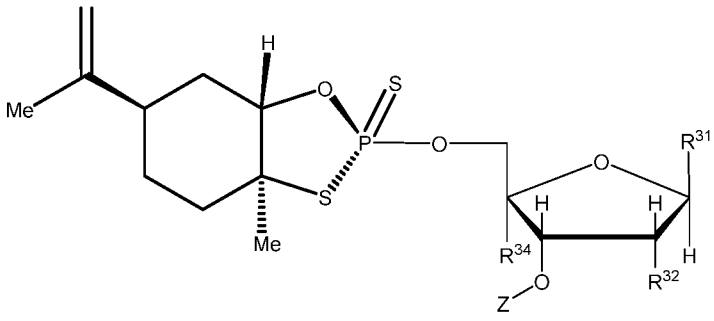


(VR2)

to form a compound of formula (V-CR3) or (V-CR4),



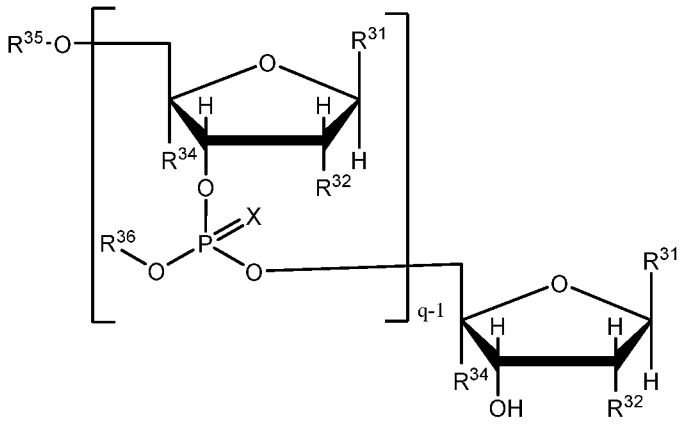
(V-CR3), or



(V-CR4)

or a salt thereof;

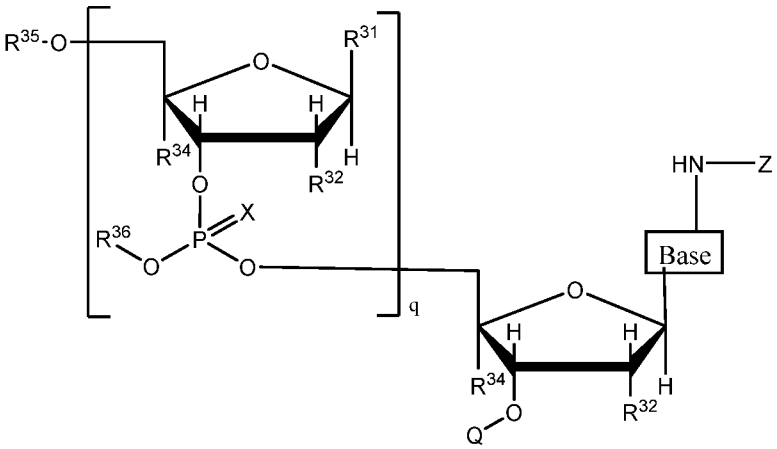
2) reacting the compound of formula (V-CR3) or (V-CR4), or a salt thereof, with a compound of formula (VG):



(VG),

or a salt thereof, and a base, to form the compound of formula (V-C1) or (V-C2), wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X, and Z are defined below.

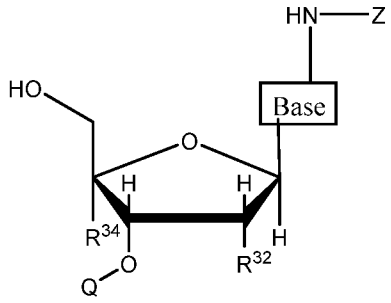
[013] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (VBZ),



(VBZ),

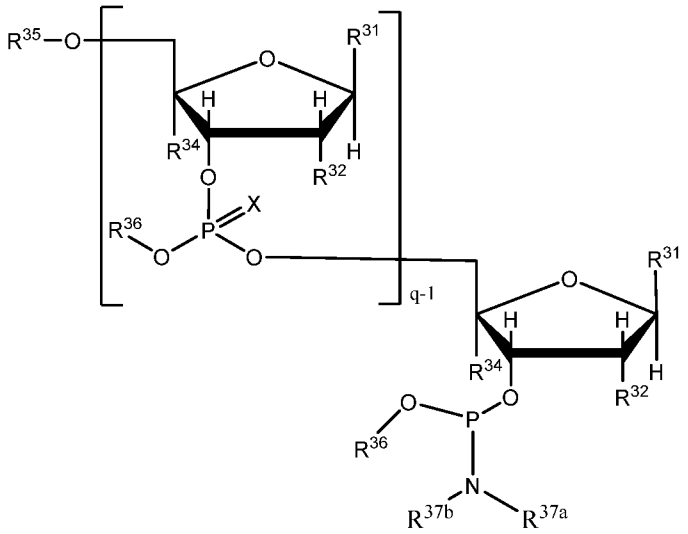
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VBZ-1),



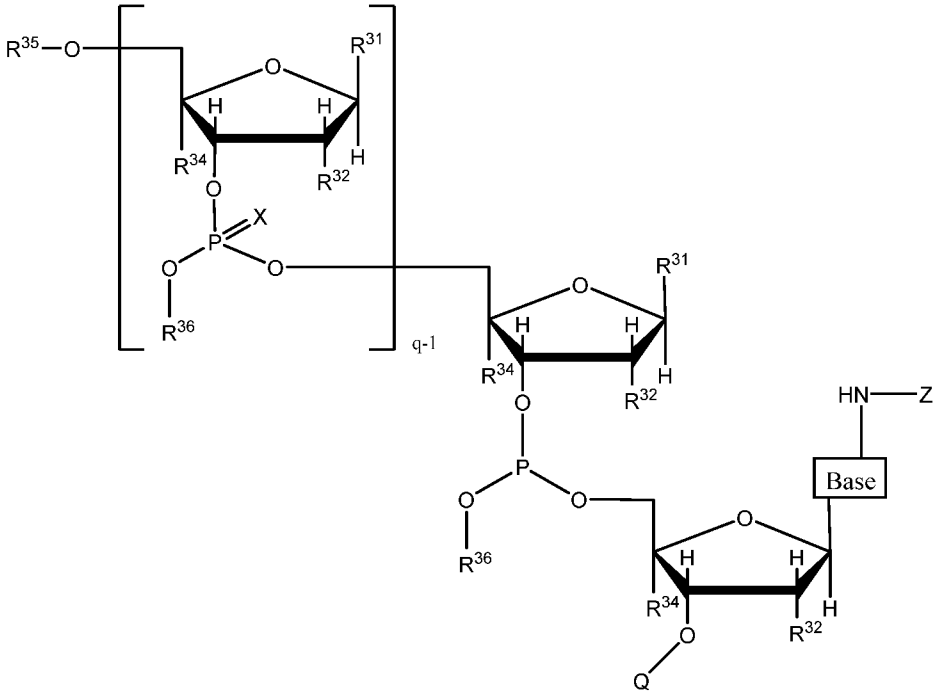
(VBZ-1),

or a salt thereof, with a compound of formula (VBZ-2):



(VBZ-2),

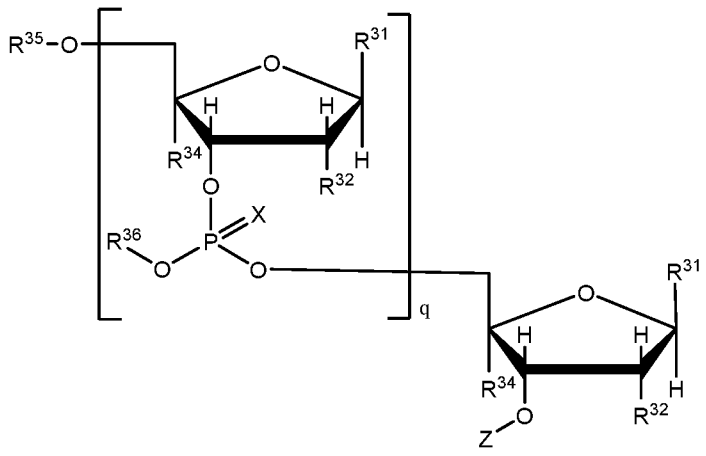
or a salt thereof, to form a compound of formula (VBZ-3),



(VBZ-3), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VBZ-3), or a salt thereof, with a sulfurization or oxidation agent to form the compound of formula (VBZ), or a salt thereof; wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X, and Z are defined below.

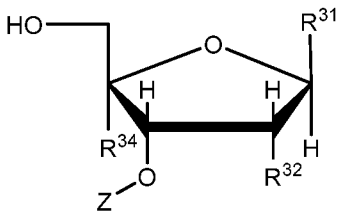
[014] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (V),



(V),

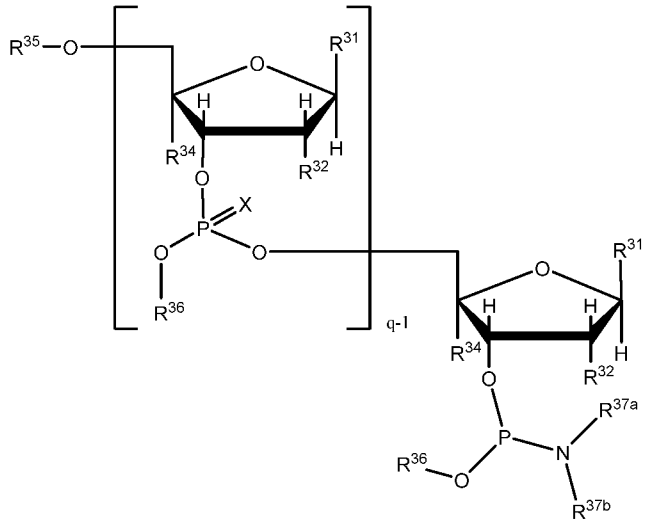
or a salt thereof, comprising the steps of:

a) coupling a nucleotide of formula (V-1):



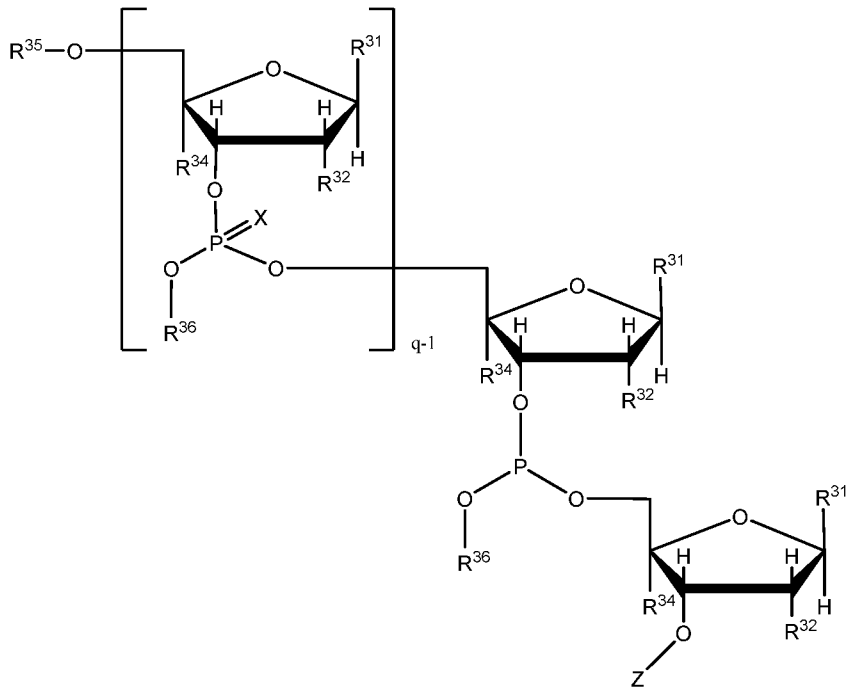
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2):



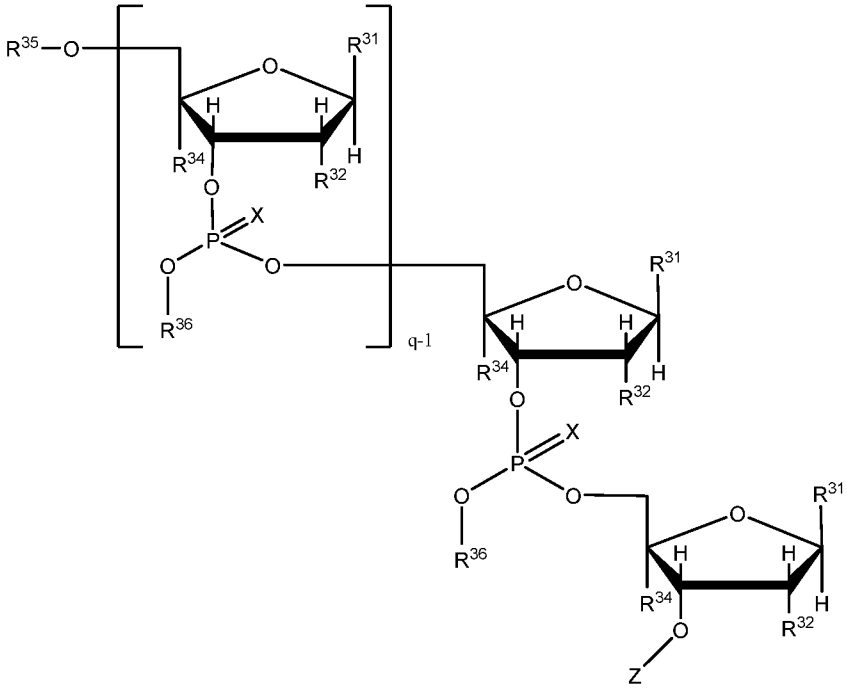
(V-2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3),



(V-3) or a salt thereof; and

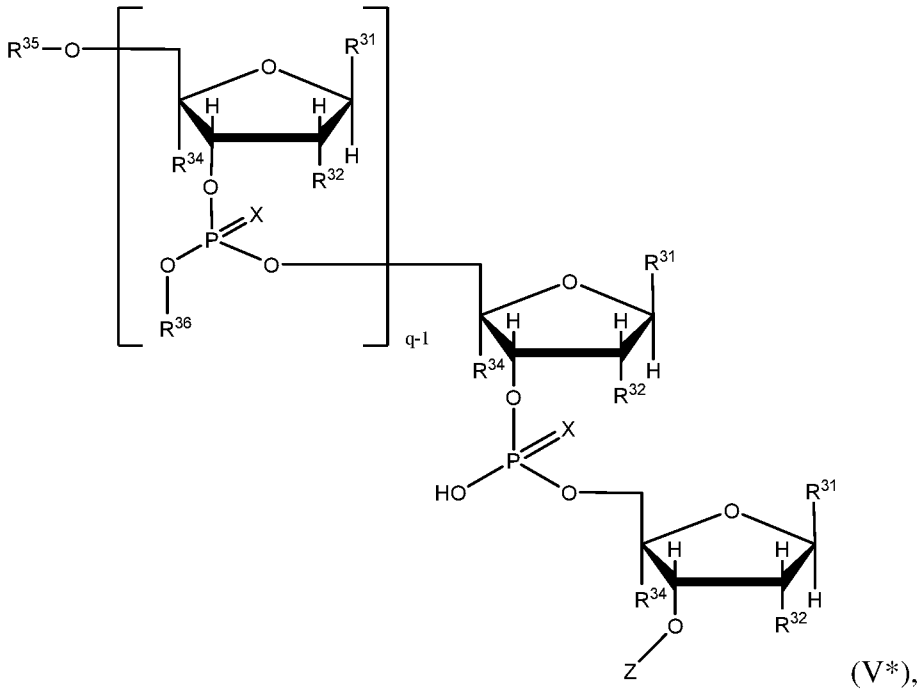
b) sulfurizing or oxidizing the oligonucleotide of formula (V-3), or a salt thereof, to form an oligonucleotide of formula (V):



(V) or a salt thereof;

wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a}, R^{37b}, q, X, and Z are defined below.

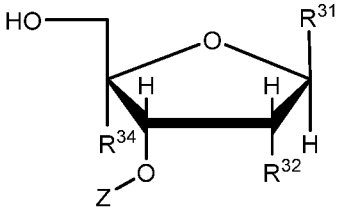
[015] One aspect of the present disclosure is directed to a process for preparing an oligonucleotide fragment of formula (V*),



(V*),

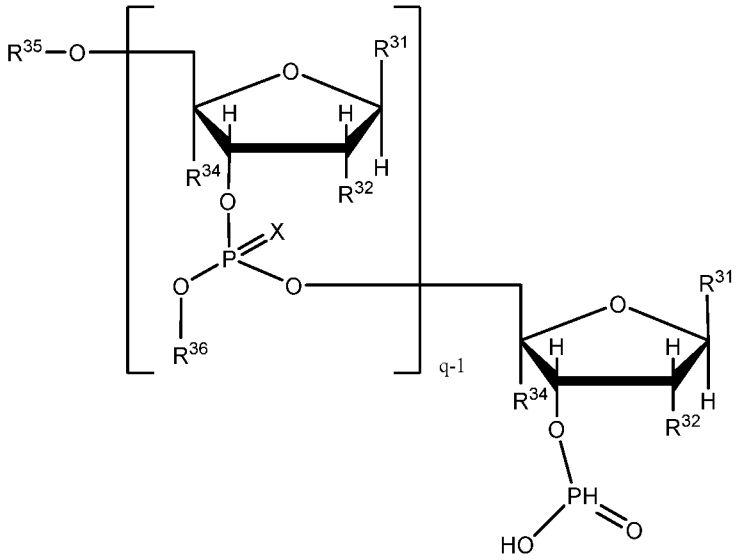
or a salt thereof, comprising the steps of:

a) coupling a nucleotide of formula (V-1):



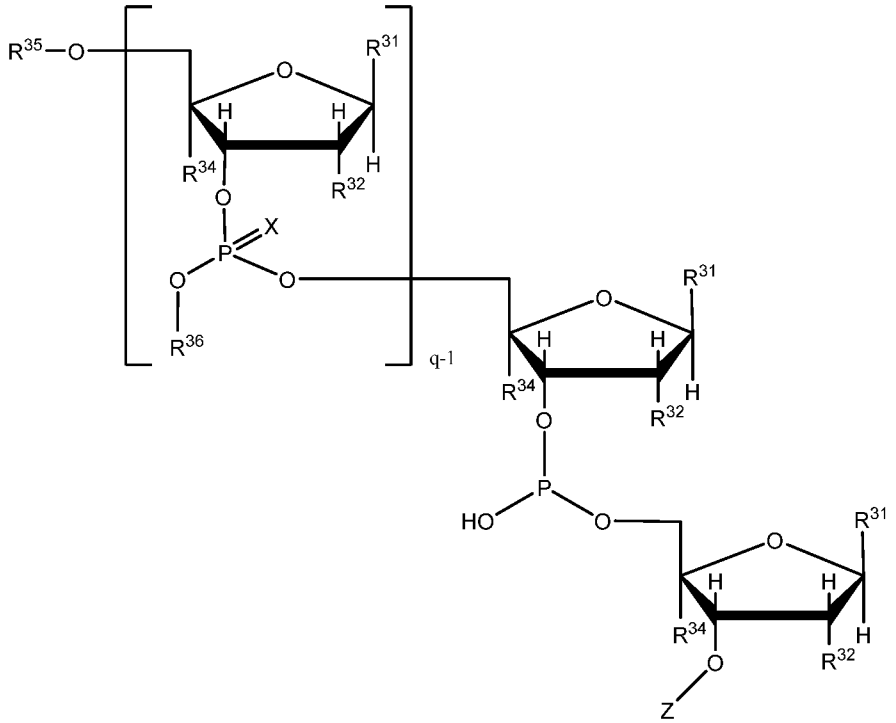
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2'):



(V-2'), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3'),



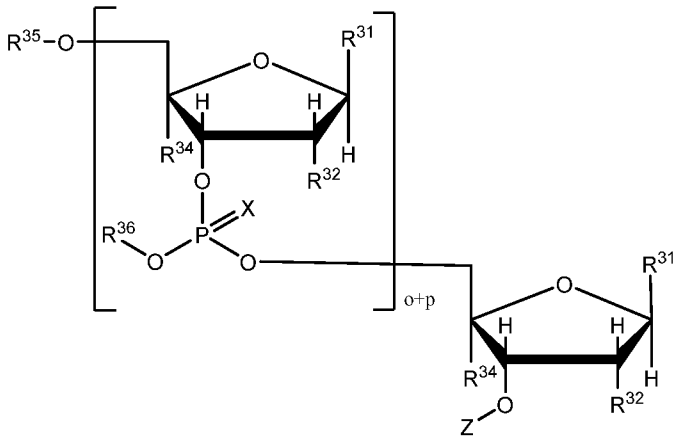
(V-3') or a salt thereof;

and

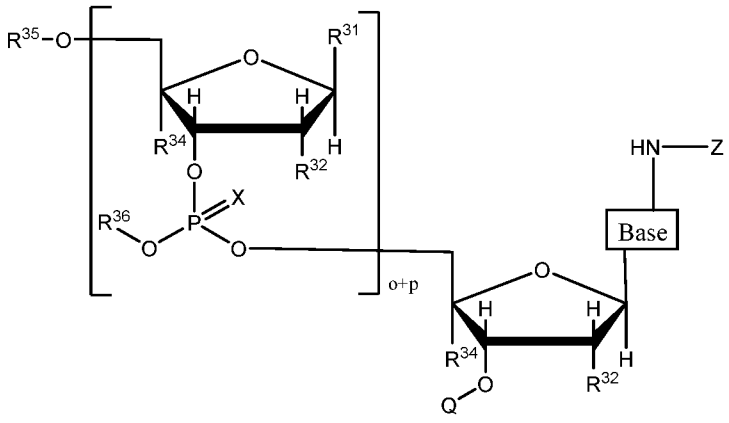
b) sulfurizing or oxidizing the oligonucleotide of formula (V-3'), or a salt thereof, to form the oligonucleotide of formula (V*) or a salt thereof;

wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X, and Z are defined below.

[016] One aspect of the present disclosure is directed to a process for preparing a target oligonucleotide of formula (VI) or (VI-1),



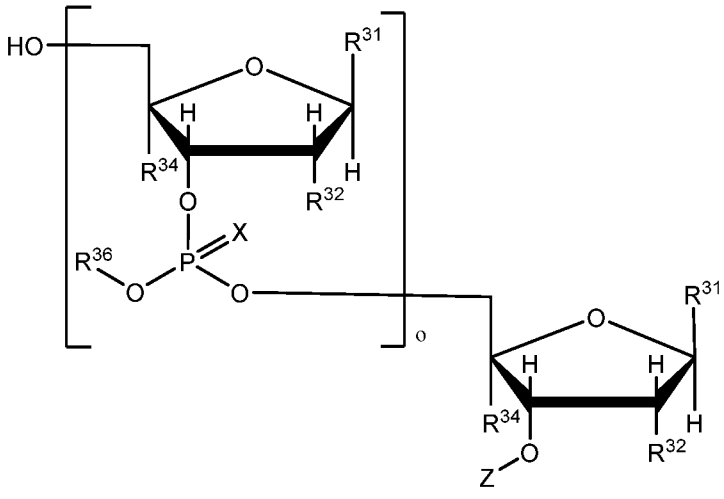
(VI),



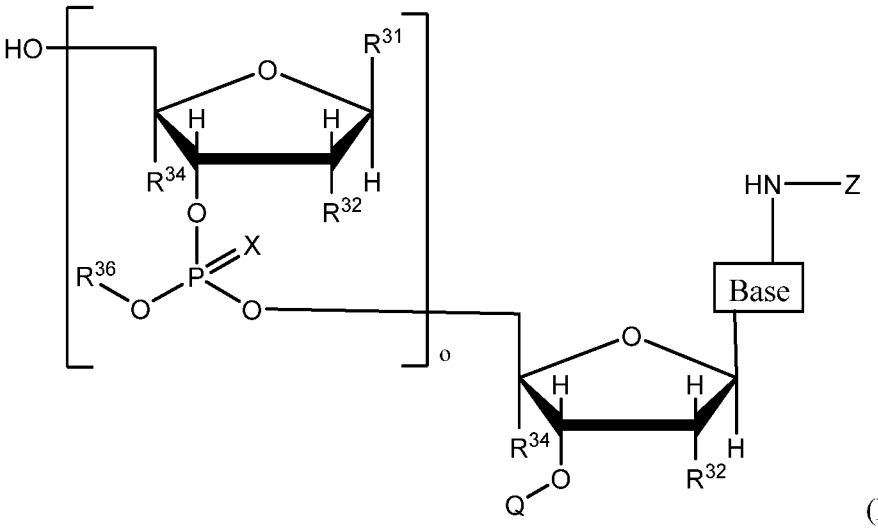
(VI-1),

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):



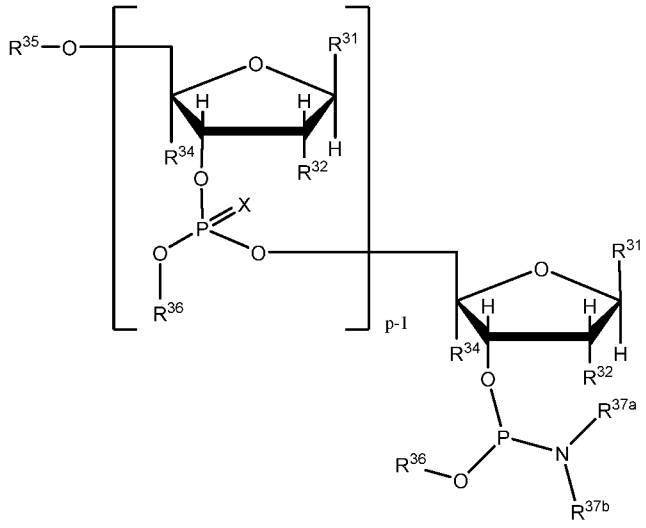
(F1),



(F1-1),

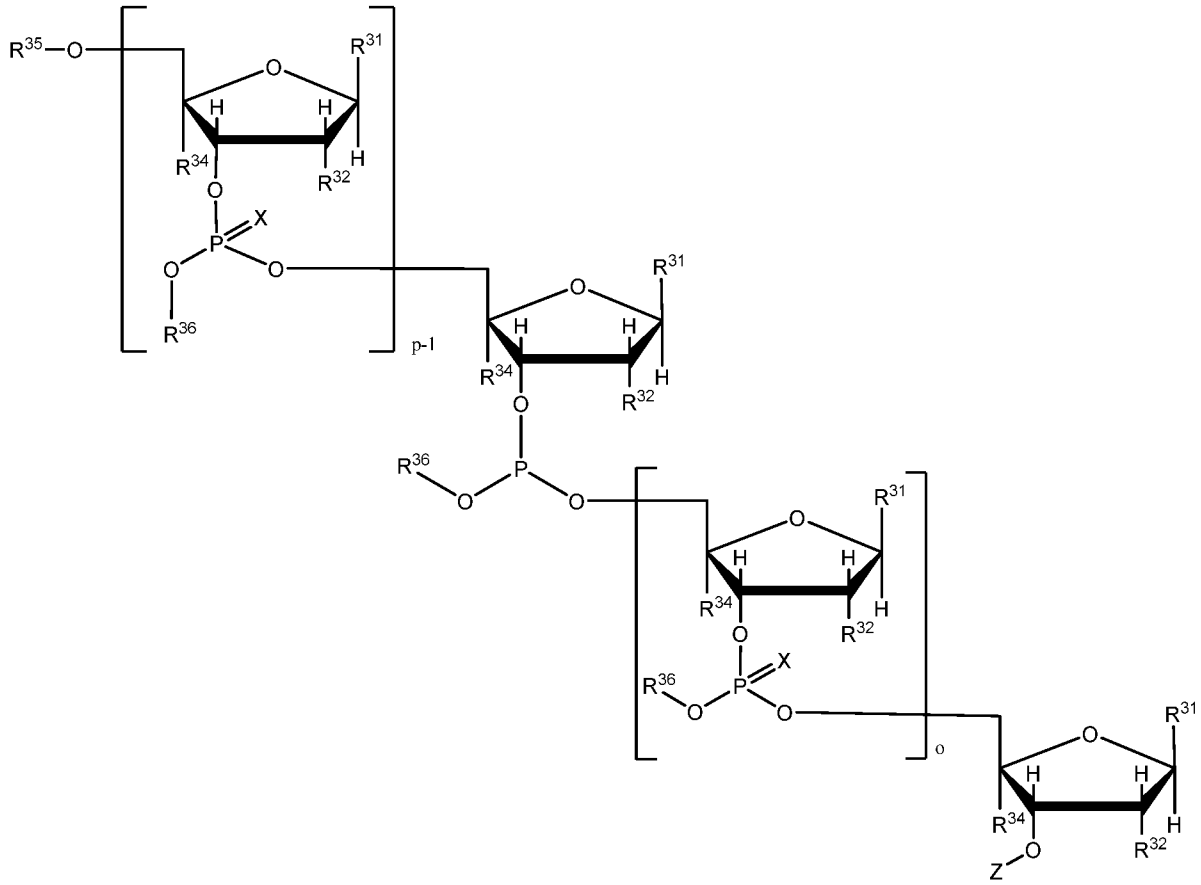
or a salt thereof,

with an oligonucleotide fragment of formula (F2):

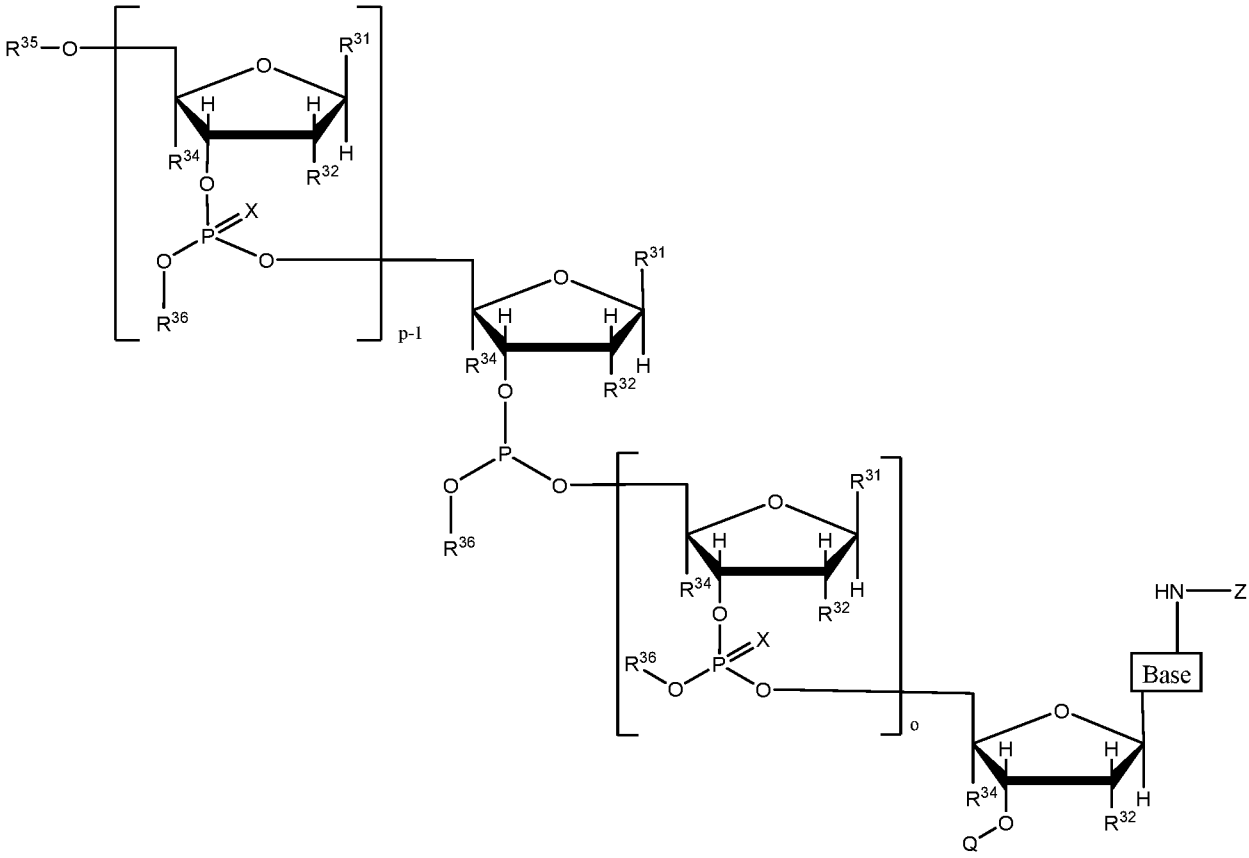


(F2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (F3) or (F3-1),



(F3),



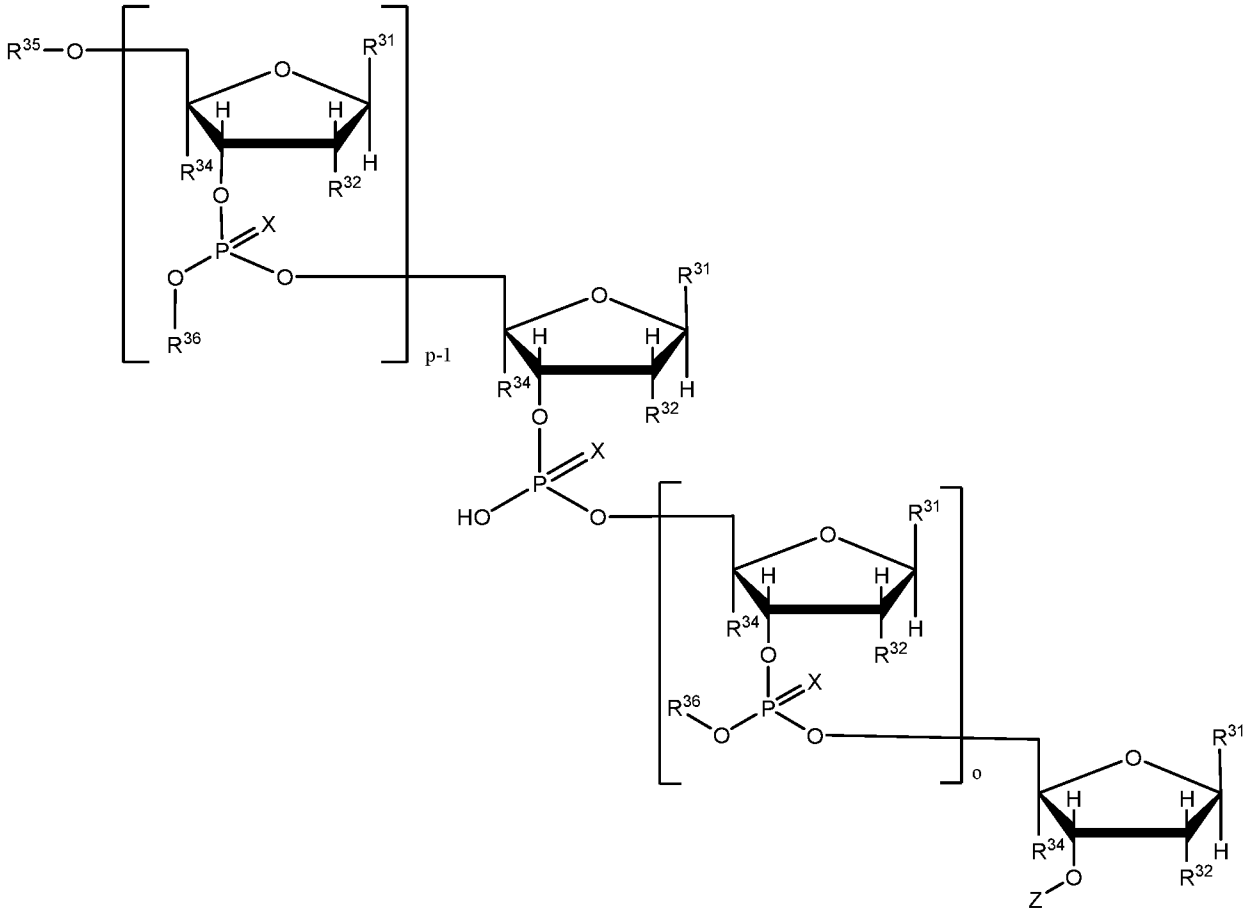
(F3-1),

or a salt thereof; and

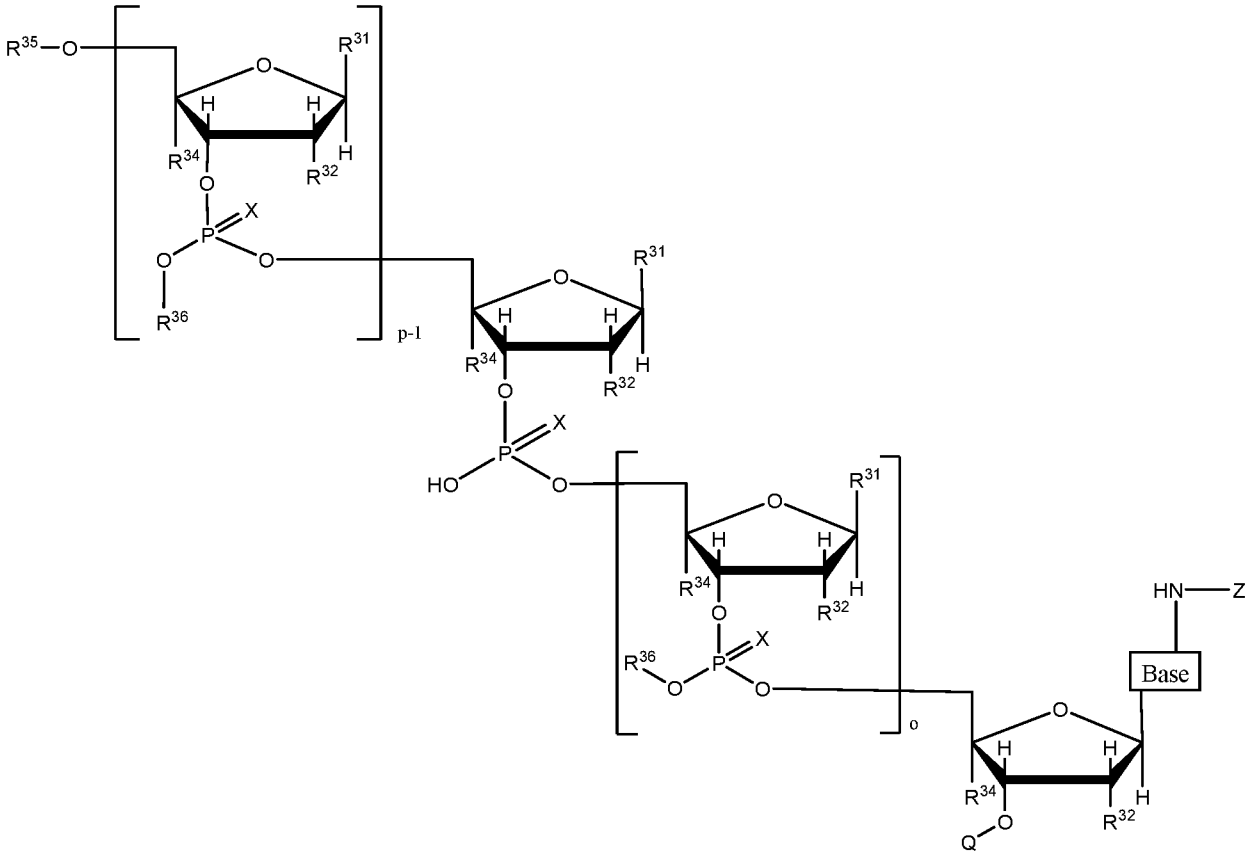
b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3) or (F3-1), or a salt thereof, to form the oligonucleotide of formula (VI) or (VI-1) or a salt thereof;

wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a}, R^{37b}, o, p, Q, X, Base and Z are defined below.

[017] One aspect of the present disclosure is directed to a process for preparing a target oligonucleotide of formula (VI') or (VI'-1),



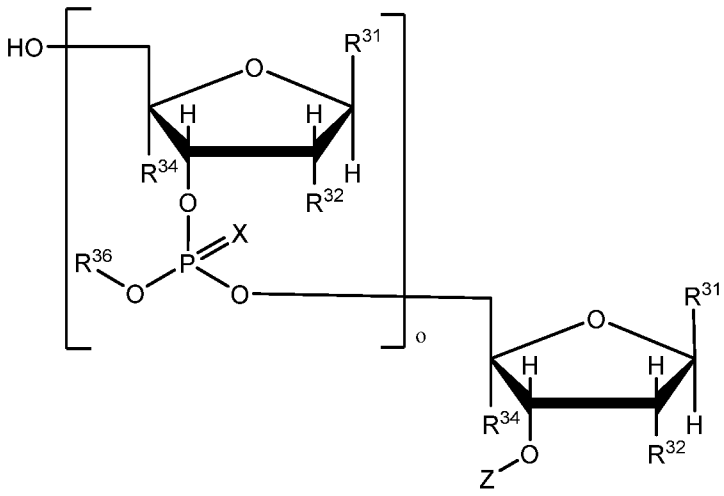
(VI'),



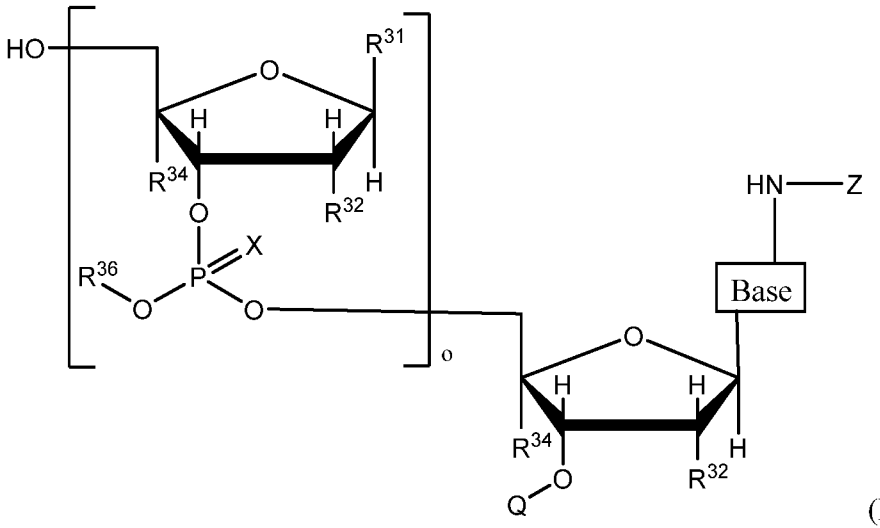
(VI'-1)

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):

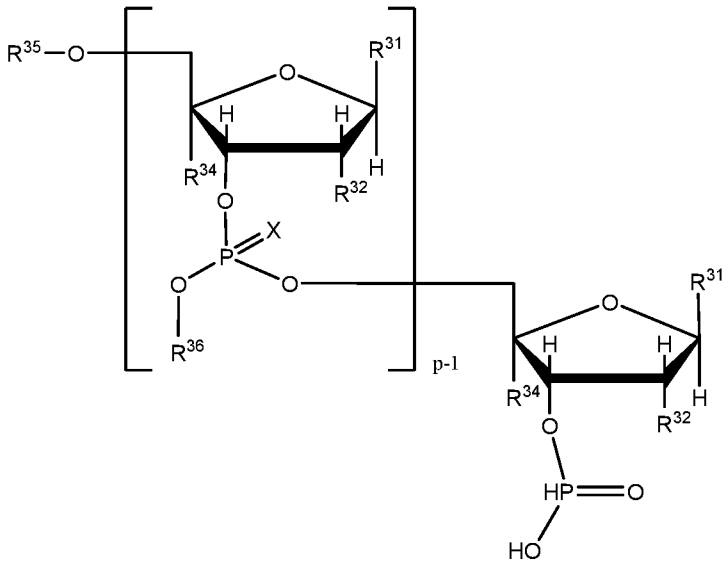


(F1),



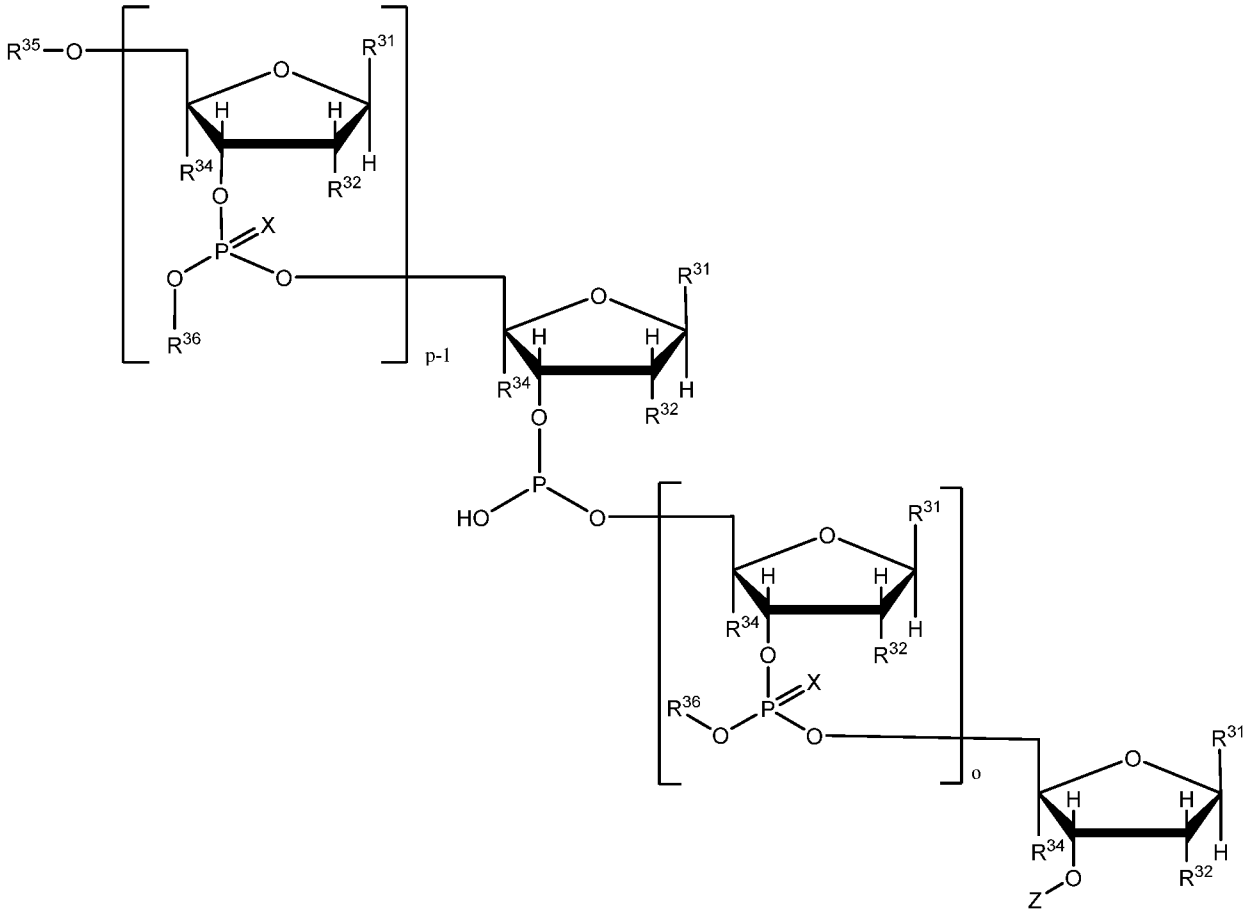
(F1-1),

or a salt thereof, with an oligonucleotide fragment of formula (F2'):

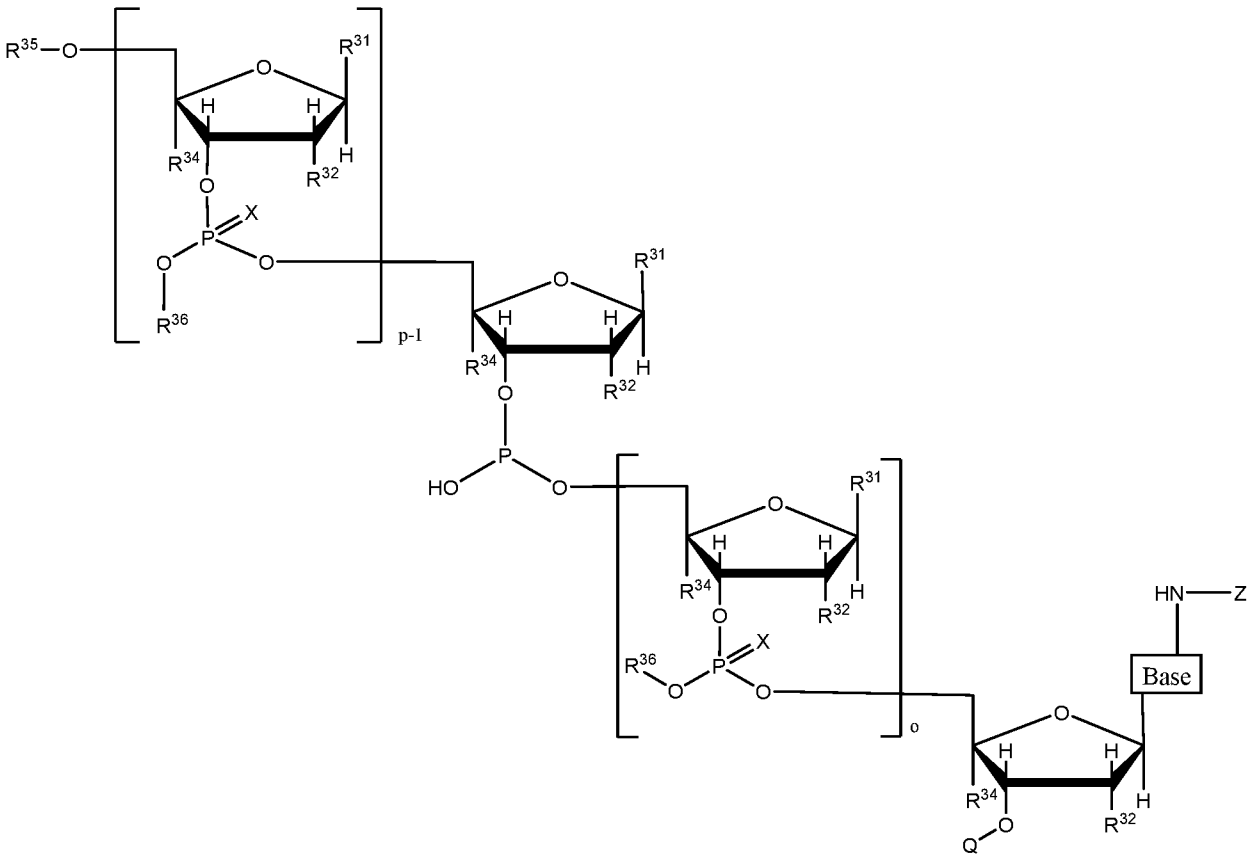


(F2'),

or a salt thereof, in a solution to form an oligonucleotide fragment of formula (F3') or (F3'-1),



(F3')



(F3'-1),

or a salt thereof; and

b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3') or (F3'-1), or a salt thereof, to form the oligonucleotide of formula (VI') or (VI'-1) or a salt thereof,

wherein: R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a}, R^{37b}, o, p, Q, X, Base and Z are defined below.

BRIEF DESCRIPTION OF THE FIGURES

[018] FIG. 1 shows a retro-synthesis scheme for preparing oligonucleotide I.

[019] FIG. 2 shows a synthetic scheme for preparing oligonucleotide fragment A.

[020] FIG. 3 shows a synthetic scheme for preparing oligonucleotide fragment B from reagent M19.

[021] FIG. 4 shows a synthetic scheme for preparing oligonucleotide fragment C.

[022] FIG. 5 shows a synthetic scheme for preparing oligonucleotide fragment D.

[023] FIG. 6 shows a synthetic scheme for preparing oligonucleotide fragment E.

[024] FIG. 7 shows a synthetic scheme for preparing oligonucleotide fragment F.

[025] FIG. 8 shows a synthetic scheme for preparing oligonucleotide fragment J.

[026] FIG. 9 shows a synthetic scheme for preparing oligonucleotide fragment K.

[027] FIG. 10 shows a synthetic scheme for preparing oligonucleotide fragment O.

[028] FIG. 11 shows synthetic scheme for preparing oligonucleotide fragment B from reagent M40.

[029] FIG. 12 shows the reaction product and by-products of the one-pot procedure for the preparation of P=O linkage.

[030] FIG. 13 shows a synthetic scheme for preparing oligonucleotide I on a large scale.

DETAILED DESCRIPTION

[031] Reagents for facilitating the preparation of oligonucleotides, especially in large scale are described. The synthetic processes based on reagents of the present disclosure produce protected target oligonucleotides on a large-scale with high purity without the need for chromatographic purification from the assembly of oligonucleotide fragments. Further, the protected target oligonucleotides can be easily deprotected selectively based on the conditions of the present disclosure. After deprotection and standard downstream purification, high purity ASO oligonucleotides suitable for therapeutic uses are obtained.

Accordingly, the novel reagents and synthetic processes of the present disclosure provide great advantages over traditional preparation of oligonucleotides.

Definitions

[032] The term "nucleobase" means the heterocyclic base portion of a nucleoside. Nucleobases may be naturally occurring or may be modified. In certain embodiments, a nucleobase may comprise any atom or group of atoms capable of hydrogen bonding to a nucleobase of another nucleic acid. In particular, the nucleobase is a heterocyclic base, typically purines and pyrimidines. In addition to "unmodified" or "natural" nucleobases such as the purine nucleobases adenine (A) and guanine (G), and the pyrimidine nucleobases thymine (T), cytosine (C) and uracil (U), many modified nucleobases or nucleobase mimetics known to those skilled in the art are amenable to incorporation into the compounds synthesized by the method described herein. In certain embodiments, a modified nucleobase is a nucleobase that is fairly similar in structure to the parent nucleobase, such as for example a 7-deaza purine, a 5-methyl cytosine, or a G-clamp. In certain embodiments, nucleobase mimetic include more complicated structures, such as for example a tricyclic phenoxazine nucleobase mimetic. Methods for preparation of the above noted modified nucleobases are well known to those skilled in the art.

[033] The term "nucleoside" means a compound comprising a heterocyclic base moiety and a sugar moiety, which can be modified at the 2'-end.

[034] The term "nucleotide" means a nucleoside comprising a phosphate or thiophosphate or dithiophosphate linking group.

[035] The term "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

[036] As used herein, "target oligonucleotide" refers to the oligonucleotide product that can be prepared based on the reagents and the processes of the present disclosure. In certain embodiments, the target oligonucleotide comprises at least 10 or at least 15 nucleotides. In certain embodiments, the target oligonucleotide has 10 to 500, 15 to 500, 15 to 200, 15 to 100, 15 to 50, 15 to 40, 15 to 30 or 16 to 30 nucleotides.

[037] As used herein, "oligonucleotide fragments" refers to short oligonucleotides that are assembled to make the target oligonucleotide. In certain embodiments, the oligonucleotide

fragment has 3 to 10, 3 to 8, 3 to 6 or 4 to 6 nucleotides. In certain embodiments, the oligonucleotide fragment has 4 or 5 nucleotides.

[038] As used herein, the term "alkyl" refers to a fully saturated branched or unbranched hydrocarbon moiety. In some embodiments, the alkyl comprises 1 to 30 carbon atoms, 1 to 20 carbon atoms, 1 to 16 carbon atoms, 1 to 10 carbon atoms, 1 to 6 carbon atoms, or 1 to 4 carbon atoms. In some embodiments, an alkyl comprises from 6 to 20 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, or n-decyl.

[039] As used herein, "carbocyclyl" refers to a saturated or unsaturated monocyclic, bicyclic or tricyclic (e.g., fused, bridged or spiro ring systems) ring system which has from 4- to 12-ring members, all of which are carbon. The term “carbocyclyl” encompasses cycloalkyl groups, cycloalkenyl group and aromatic groups (i.e., aryl). “Cycloalkyl” refers to completely saturated monocyclic hydrocarbon groups of 3-7 carbon atoms, including cyclopropyl, cyclobutyl, cyclpentyl, cyclohexyl and cyclopentyl; and “cycloalkyenyl” refers to unsaturated non-aromatic monocyclic hydrocarbon groups of 3-7 carbon atoms, including cyclpenteneyl, cyclohexenyl and cyclopentenyl.

[040] The term "aryl" refers to monocyclic, bicyclic or tricyclic aromatic hydrocarbon groups having from 6 to 14 carbon atoms in the ring portion. In one embodiment, the term aryl refers to monocyclic and bicyclic aromatic hydrocarbon groups having from 6 to 10 carbon atoms. Representative examples of aryl groups include phenyl, naphthyl, fluorenyl, and anthracenyl.

[041] The term "aryl" also refers to a bicyclic or tricyclic group in which at least one ring is aromatic and is fused to one or two non-aromatic hydrocarbon ring(s). Nonlimiting examples include tetrahydronaphthalene, dihydronaphthalenyl and indanyl.

[042] The term “bridged ring system,” as used herein, is a ring system that has a carbocyclyl or heterocyclyl ring wherein two non-adjacent atoms of the ring are connected (bridged) by one or more (preferably from one to three) atoms selected from C, N, O, or S. A bridged ring system may have from 6-7 ring members.

[043] The term “spiro ring system,” as used herein, is a ring system that has two rings each of which are independently selected from a carbocyclyl or a heterocyclyl, wherein the two ring structures having one ring atom in common. Spiro ring systems have from 5 to 7 ring members.

[044] As used herein, the term "heterocyclyl" refers to a saturated or unsaturated, monocyclic or bicyclic (e.g., bridged or spiro ring systems) ring system which has from 3- to 7-ring members, or 3- to 6- ring members or 5- to 7- ring members, at least one of which is a heteroatom, and up to 4 (*e.g.*, 1, 2, 3, or 4) of which may be heteroatoms, wherein the heteroatoms are independently selected from O, S and N, and wherein C can be oxidized (e.g., C(O)), N can be oxidized (e.g., N(O)) or quaternized, and S can be optionally oxidized to sulfoxide and sulfone. Unsaturated heterocyclic rings include heteroaryl rings. As used herein, the term "heteroaryl" refers to an aromatic 5 or 6 membered monocyclic ring system, having 1 to 4 heteroatoms independently selected from O, S and N, and wherein N can be oxidized (e.g., N(O)) or quaternized, and S can be optionally oxidized to sulfoxide and sulfone. In one embodiment, a heterocyclyl is a 3-to 7-membered saturated monocyclic or a 3-to 6-membered saturated monocyclic or a 5-to 7-membered saturated monocyclic ring. In one embodiment, a heterocyclyl is a 3-to 7-membered monocyclic or a 3-to 6-membered monocyclic or a 5-to 7-membered monocyclic ring. In another embodiment, a heterocyclyl is a 6 or-7-membered bicyclic ring. The heterocyclyl group can be attached at a heteroatom or a carbon atom. Examples of heterocyclyls include aziridinyl, oxiranyl, thiiranyl, oxaziridinyl, dioxiranyl, azetidinyl, oxetanyl, thietanyl, pyrrolidinyl, tetrahydrofuranyl, thiolanyl, imidazolidinyl, pyrazolidinyl, oxazolidinyl, isoxazolidinyl, thiazolidinyl, isothiazolidinyl, dioxolanyl, dithiolanyl, oxathiolanyl, piperidinyl, tetrahydropyranyl, thianyl, piperazinyl, morpholinyl, thiomorpholinyl, dioxanyl, dithianyl, trioxanyl, trithianyl, azepanyl, oxepanyl, thiepanyl, dihydrofuranyl, imidazolinyl, dihydropyranyl, and heteroaryl rings including azirinyl, oxirenyl, thiirenyl, diazirinyl, azetyl, oxetyl, thietyl, pyrrolyl, furanyl, thiophenyl (or thienyl), imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, furazanyl, oxadiazolyl, thiadiazolyl, dithiazolyl, triazolyl, tetrazolyl, pyridinyl, pyranyl, thiopyranyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazinyl, thiazinyl, dioxinyl, dithiinyl, oxathianyl, triazinyl, tetrazinyl, azepinyl, oxepinyl, thiepinyl, diazepinyl, and thiazepinyl and the like. Examples of bicyclic heterocyclic ring systems include 3-azabicyclo[3.1.0]hexanyl, 3-azabicyclo[3.1.1]heptanyl, 2-azaspiro[3.3]heptanyl, 2-oxa-6-azaspiro[3.3]heptanyl, and 5-azaspiro[2.3]hexanyl.

[045] "Halogen" or "halo" may be fluoro, chloro, bromo or iodo.

[046] As used herein, a “hydroxyl protecting group” refers to a group that is suitable for protecting a hydroxyl group, -OH, from reacting with other reagents. Examples of hydroxyl protecting groups can be found in Greene, TW et al., *Protective Groups in Organic Synthesis*, 4th Ed., John Wiley and Sons (2007).

[047] In certain embodiments, the hydroxyl protecting groups can be selected from, for example, acetyl (Ac); benzoyl (Bz); benzyl (Bn); β-methoxyethoxymethyl ether (MEM); methoxymethyl ether (MOM); methoxytrityl [(4-methoxyphenyl)diphenylmethyl, MMT); 4,4′-dimethoxytrityl (DMT); methoxyethyl (MOE); p-methoxybenzyl ether (PMB); methylthiomethyl ether; pivaloyl (Piv); tetrahydropyranyl (THP); tetrahydrofuran (THF); silyl ether (including, but not limited to, trimethylsilyl (TMS), tert-butyldiphenylsilyl (TBDPS), tert-butoxydiphenylsilyl (TBoDPS), triphenylsilyl (TPS), tert-butyldimethylsilyl (TBDMS), tri-iso-propylsilyloxymethyl (TOM), and triisopropylsilyl (TIPS) ethers); methyl ethers, and ethoxyethyl ethers (EE).

[048] In certain embodiments, the hydroxyl protecting group protects the 3' –hydroxyl of a nucleoside (referred to as 3'-hydroxyl protecting group). In certain embodiments, the 3'-hydroxyl protecting groups include a silyl hydroxyl protecting group, such as trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylthexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-t-butylmethylsilyl tri(trimethylsilyl)silyl, t-butylmethoxyphenylsilyl, and t-butoxydiphenylsilyl. In certain embodiments, the 3'-hydroxyl protecting group is TBDPS. In certain embodiments, the 3'-hydroxyl protecting group is a large hydrophobic protecting group (LHPG), such as those described herein.

[049] The suffic "yl" added to the end of a chemical name indicates that the named moiety is bonded to the molecule at point. The suffix "ene" added to the end of a chemical name indicates that the named moiety is bonded to the molecule at two points.

[050] In certain embodiments, the hydroxyl protecting group protects the 5' –hydroxyl of a nucleoside (referred to as 5'-hydroxyl protecting group). Exemplary 5'-hydoxyl groups include, but are not limited to those as described herein (*e.g.*, R^{35} in any of the aspects or embodiments). In a specific embodiment, 5'-hydoxyl protecting group is an acid-labile 4,4'-dimethoxytrityl (or bis-(4-methoxyphenyl)phenylmethyl) (DMT or DMTr) protecting group. In certain embodiments, the 5'-hydroxyl protecting group is a large hydrophobic protecting group (LHPG), such as those described herein.

[051] As used herein, "selective precipitation" refers to a purification method that separates the desired product from one or more impurities in a solution by adding the solution to a solvent that precipitates out the product; while leaving the one or more impurities in the solution. Alternatively, the solvent can be added to the solution comprising the crude product and the one or more impurities to precipitate out the product. In certain embodiments, the desired compound or oligonucleotide of the present disclosure comprises a hydrophobic

group (*e.g.*, hydrophobic 3'-hydroxyl protecting group or hydrophobic 5'-hydroxyl protecting group (*e.g.*, LHPG group described herein)) and the addition of a polar solvent (*e.g.* CH_3CN) into the solution containing the compound or oligonucleotide and one or more impurities to precipitate out the desired oligonucleotide. In certain embodiments, the desired compound or oligonucleotide of the present disclosure can be purified by adding a co-solvent or solvent mixture (*e.g.*, heptane, tert-butylmethylether (TBME or MBTE), heptane/MBTE mixture (*e.g.* a heptane/MBTE mixture with volume ratio of heptane to MBTE in the range of 20:1 to 1:20, 9:1 to 1:9, or 4:1 to 1:4, or a heptane/MBTE mixture with heptane to MBTE volume ratio of 9:1, 4:1, 2:1, 1:1, 2:5, 1:2, 1:4 or 1:9) to a solution comprising the crude product and the one or more impurities in an organic solvent (*e.g.*, dichloromethane (DCM) or ethylacetate (EtOAc)) to precipitate out the product. Alternatively, the solution comprising the crude product and the one or more impurities can be added to the non-polar or less polar solvent or solvent mixture to precipitate out the product. Suitable co-solvent can be determined based on the hydrophobicity of the product. In certain embodiments, the co-solvent is less polar than the organic solvent the product is dissolved in.

[052] As used herein, "extraction" refers to a purification method that separates the desired product from one or more impurities in a solution by contacting the solution with a solvent that the product is soluble in; while the one or more impurities are insoluble. Alternatively, the solution containing the product and one or more impurities can be contacted with a solvent that the one or more impurities are soluble in; while the product is insoluble. In certain embodiments, the solution (*e.g.*, a reaction mixture or a solution of crude product) containing the product and one or more impurities in an organic solvent (*e.g.*, DCM, EtOAc or THF) or an organic solvent mixture can be contacted (extracted or washed) with water or an aqueous solution (*e.g.*, $NaHCO_3/H_2O$ solution or $NaCl/H_2O$ solution) to remove hydrophilic impurities.

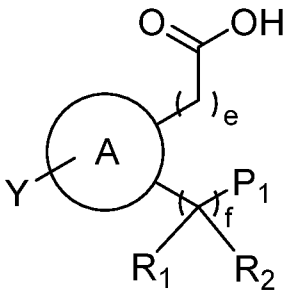
[053] As used herein the term "base" refers to a substance that can produce hydroxide ion (OH^-) in water solutions or a substance that can donate a pair of nonbonding electrons. Exemplary bases include, but are not limited to, alkaline hydroxide, alkaline earth hydroxide, alkylamines (*e.g.*, tert-butylamine, sec-butylamine, trimethylamine, triethylamine, diisopropylethylamine, 2-methylpropan-2-amine), 8-diazabicyclo[5.4. 0]undec-7-ene (DBU), imidazole, N-methylimidazole, pyridine and 3-picoline.As used herein, the term "salt" refers to an organic or inorganic salt of a compound, nucleotide or oligonucleotide described herein. In certain embodiments, the salt is a pharmaceutically acceptable salt thereof. The phrase "pharmaceutically acceptable" indicates that the substance or composition must be

compatible chemically and/or toxicologically, with the other ingredients comprising a formulation, and/or the mammal being treated therewith. In certain embodiments, the salt of the compound, nucleotide or oligonucleotide described herein is a sodium salt, a potassium salt or an ammonium salt. In certain embodiments, the salt is a sodium salt or ammonium salt.

1. Reagent

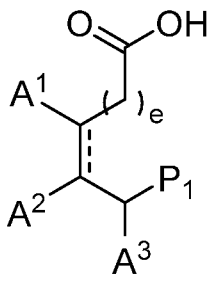
[054] In a first aspect, the present disclosure provides Reagents for facilitating the synthesis of oligonucleotides. In one embodiment, the Reagents of the disclosure serve as a protecting group protecting a 3'-hydroxyl group of a nucleotide/oligonucleotide fragment. In another embodiment, the nucleotide, oligonucleotide fragment, or target oligonucleotide which is protected by the Reagents of the disclosure can be selectively precipitated from the reaction mixture. As such, the nucleotide, oligonucleotide fragment, or target oligonucleotide is easily collected by filtration without chromatograph.

[055] In a first embodiment of the first aspect, the present disclosure provides a compound of Formula I' or B:



I'

or



B

or a salt thereof, wherein:

one of A^1, A^2 and A^3 is Y^A and the others are H;

$\equiv$ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms;

wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, $-OC(O)R^{8A}$, $-C(O)OR^{8A}$, $NR^{8A}R^{9A}$, $-NR^{8A}COR^{9A}$, $-CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

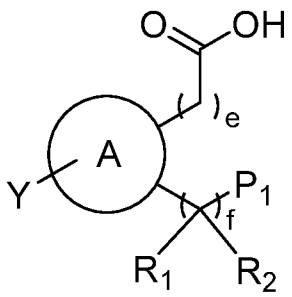
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

[056] In a second embodiment of the first aspect, the present disclosure provides a compound of formula I'



I'

or a salt thereof, wherein:

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} is independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, $-OC(O)R^{8A}$, $-C(O)OR^{8A}$, $NR^{8A}R^{9A}$, $-NR^{8A}COR^{9A}$, $-CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

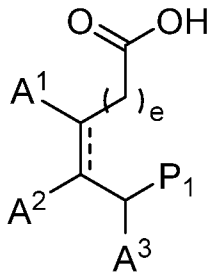
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

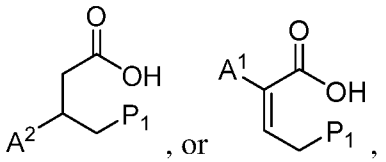
[057] In a third embodiment, the present disclosure provides a compound of formula B:



B

or a salt thereof. The remainder of the variables in formula B are described in the first embodiment.

[058] In a fourth embodiment, the present disclosure provides a compound of formula B-1 or B-2:



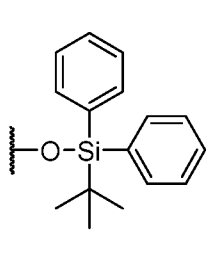
B-1 B-2

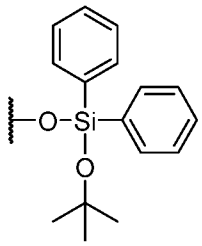
or a salt thereof. The remainder of the variables in formula B are described in the third embodiment.

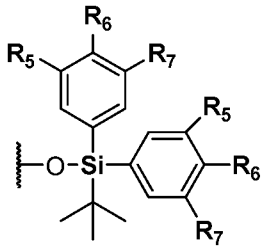
[059] In a fifth embodiment, the present disclosure provides a compound of formula I' or B or a salt thereof, Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms. The remainder of the variables in formula I' or B are described in the first, second, third or fourth embodiment.

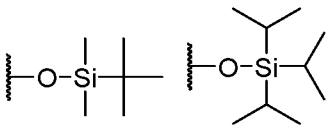
[060] In a sixth embodiment, the present disclosure provides a compound of formula I' or a salt thereof, wherein ring A is phenyl or naphthalenyl. The remainder of the variables in Formula I' are described in the second and/or fifth embodiments.

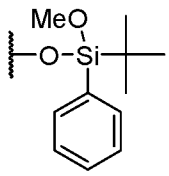
[061] In a seventh embodiment, the present disclosure provides a compound of formula I' or B or a salt thereof, wherein P_1 is a silyl hydroxyl protecting group selected from the following:

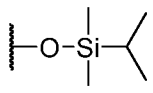

-O-TBDPS ,

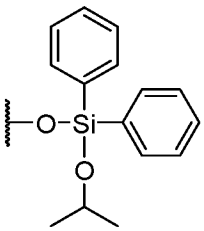

-O-TBoDPS ,

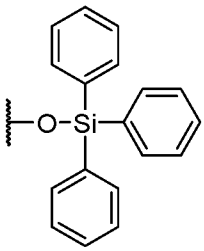

-O-TBDAS ,

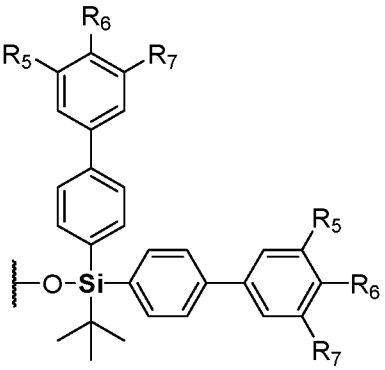

-O-TBDMS , -O-TIPS ,

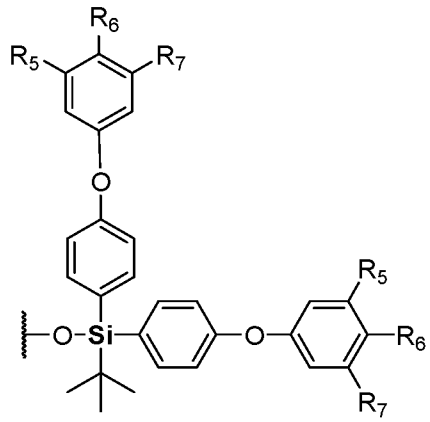

-O-TBMPS ,


-O-DMIPS ,


-O-IPoDPS ,

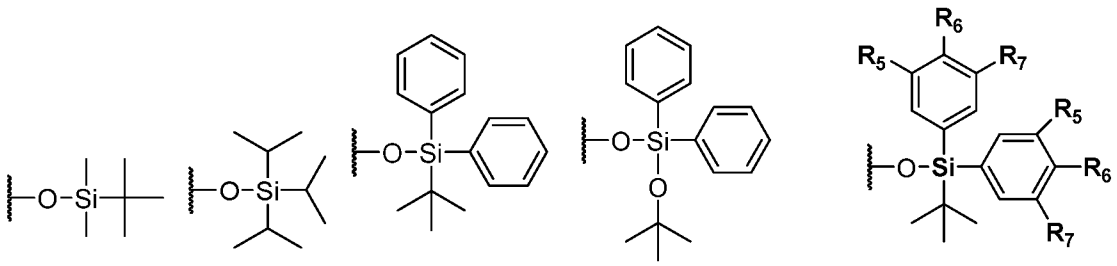

-O-TPS ,


-O-TBDAS-1 ,


and -O-TBDAS-2 ;

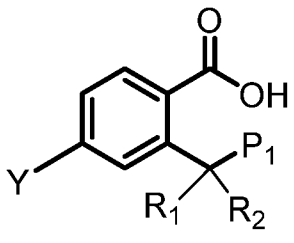
wherein ⊢ represents the point of attachment for P_1; and R_5, R_6 and R_7 are each independently H, C_{1-30}alkyl, or C_{1-30}alkoxy. The remainder of the variables in Formula I' or B are described in any one of the first through sixth embodiments.

[062] In an eighth embodiment, the present disclosure provides a compound of formula I' or B or a salt thereof, wherein P_1 is selected from the group consisting of -O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:

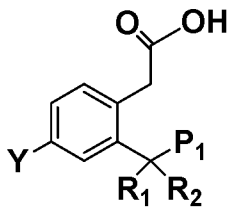


-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , and -O-TBDAS . The remainder of the variables in Formula I' are described in any one of the first through seventh embodiments.

[063] In a ninth embodiment, the present disclosure provides a compound of formula I or Ia:



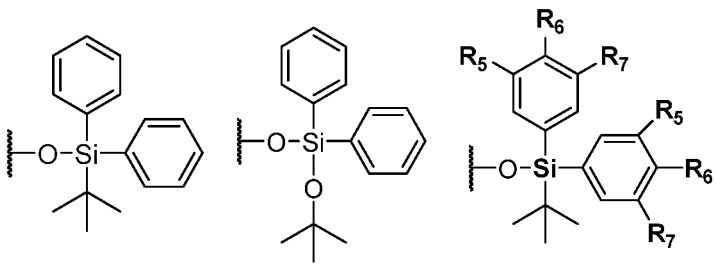
, or



I Ia

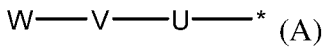
or a salt thereof;

wherein P_1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDPS , -O-TBoDPS , -O-TBDAS . The remainder of the variables in Formula I or Ia are described in the first, second, and/or fifth through eighth embodiments.

[064] In a tenth embodiment, the present disclosure provides a compound of Formulae I', B, or Formula I or a salt thereof, wherein Y is represented by Formula A:

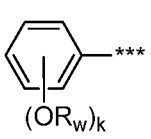
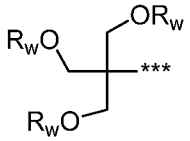
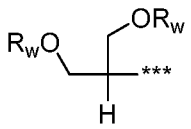


(A)

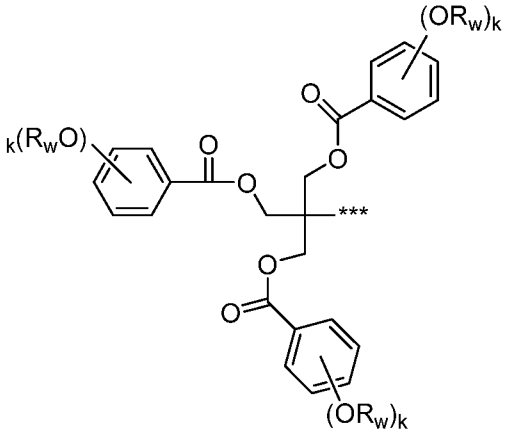
wherein:

—* represents the point of attachment for Y;

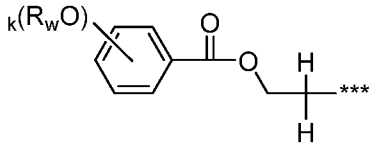
W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:


(A1),

(A2),

(A2-1),

(A2-2),


(A3),

(A3-1),

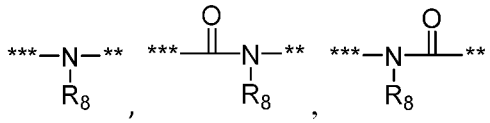
or

(A3-2),

wherein

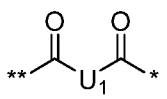
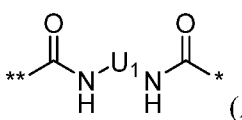
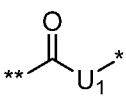
—*** represents the point where W and V connect;

each R_w is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

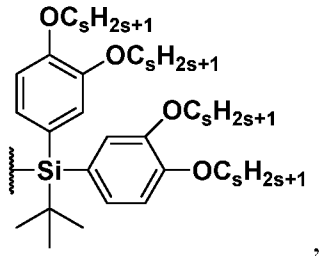
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:


(A4),

(A5), or

(A6),

wherein U_1 is $C_{1\text{-}6}$alkylene, $C_{1\text{-}6}$alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. The remainder of the variables in Formula I, Ia, B, or Formula I' are described in any one of the first through ninth embodiments.

[065] In an eleventh embodiment, the present disclosure provides a compound of Formula I', B, or Formula I or a salt thereof, wherein the TBDAS group is:

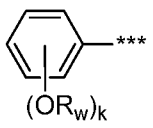


,

wherein s is an integer from 1 to 30. The remainder of the variables in Formula I, Ia, B, or Formula I' are described in any one of the seventh through tenth embodiments.

[066] In a twelfth embodiment, the present disclosure provides a compound of Formula I', B or Formula I or Ia or a salt thereof, wherein P_1 is –O-TBDPS. The remainder of the variables in Formula I, Ia or Formula I' or B are described in any one of the first through eleventh embodiments.

[067] In a thirteenth embodiment, the present disclosure provides a compound of Formula I, Ia, B, or I' or a salt thereof, wherein W is represented by Formula A1:

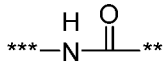


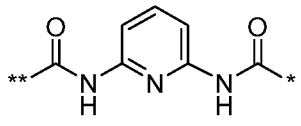
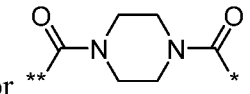
A1,

wherein R_w is C_nH_{2n+1}; and n is an integer from 1 to 30. The remainder of the variables in Formula I, B, or I' are described in the tenth embodiment.

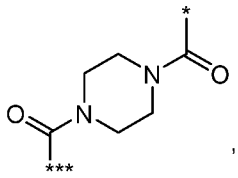
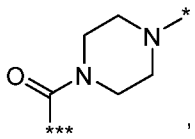
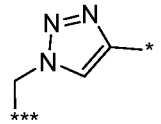
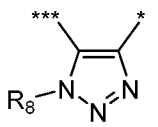
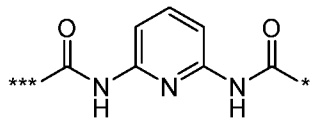
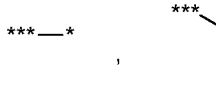
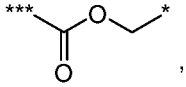
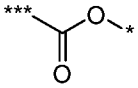
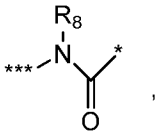
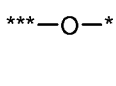
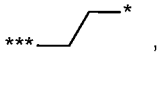
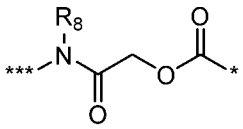
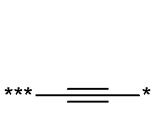
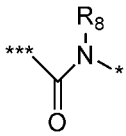
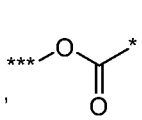
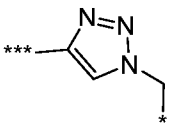
[068] In a fourteenth embodiment, the present disclosure provides a compound of Formula I, Ia, B, or I' or a salt thereof, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and $C_{28}H_{57}$. The remainder of the variables in Formula I, Ia B, or I' are described in any one of the tenth through thirteenth embodiments.

[069] In a fifteenth embodiment, the present disclosure provides a compound of Formula I, Ia, B, or I' or a salt thereof, wherein V is a bond, CH_2, CH_2CH_2, C(=O), ***-C(=O)-O-**, or

 . The remainder of the variables in Formula I, Ia, B, or I' are described in any one of the tenth through fourteenth embodiments.

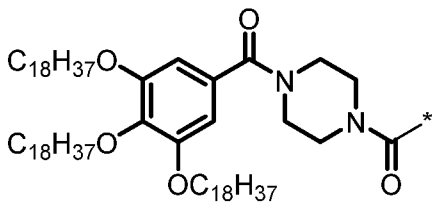
[070] In a sixteenth embodiment, the present disclosure provides a compound of Formula I, Ia, B, or I' or a salt thereof, wherein U is a bond, CH_2, CH_2CH_2, carbonyl, triazolylene, piperazinylene,  , or  . The remainder of the variables in Formula I, Ia, B, or I' are described in any one of the tenth through fifteenth embodiments.

[071] In a seventeenth embodiment, the present disclosure provides a compound of Formula I, Ia, B, or I' or a salt thereof, wherein U-V is selected from the group consisting of:

 ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  ;

wherein R_8 is H or $C_{1\text{-}6}$alkyl. The remainder of the variables in Formula I, B, or I' are described in any one of the tenth through sixteenth embodiments.

[072] In an eighteenth embodiment, the present disclosure provides a compound of Formula I or Ia or Formula I' or B or a salt thereof, wherein Y is selected from the groups consisting of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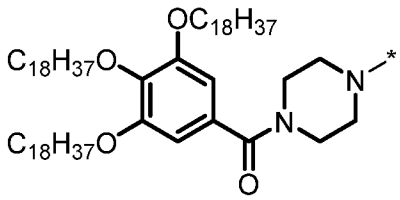
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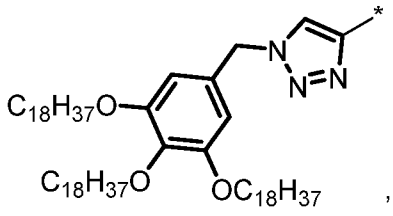
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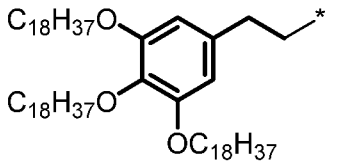
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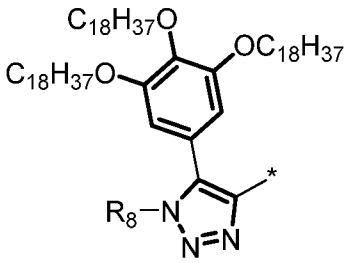
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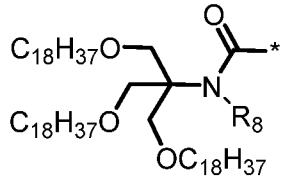
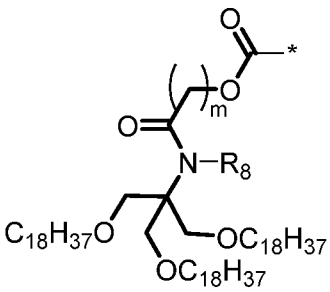
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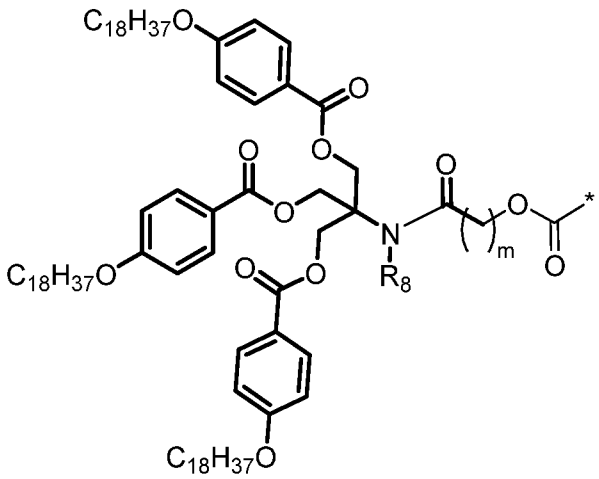

;

wherein

R_8 is H or $C_{1\text{-}6}$alkyl; and

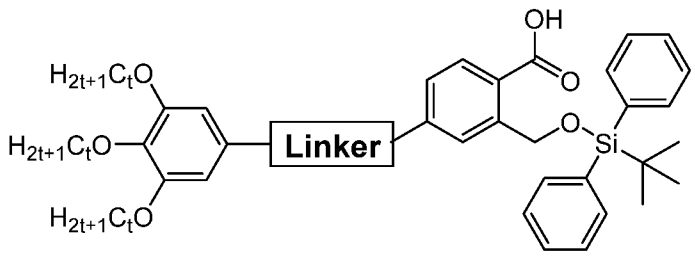
m is an integer from 1 to 5. The remainder of the variables in Formula I', B, or Formula I or Ia are described in any one of the first through twelfth embodiments.

[073] In a nineteenth embodiment, the present disclosure provides a compound of Formula I or Ia or Formula I' or a salt thereof, wherein R_1 and R_2 are independently H or CH_3. The remainder of the variables in Formula I or Ia or Formula I' are described in the first, second, and/or fifth through eighteenth embodiments. In a specific embodiment, R_1 and R_2 are both H. In another specific embodiment, R_1 and R_2 are both CH_3.

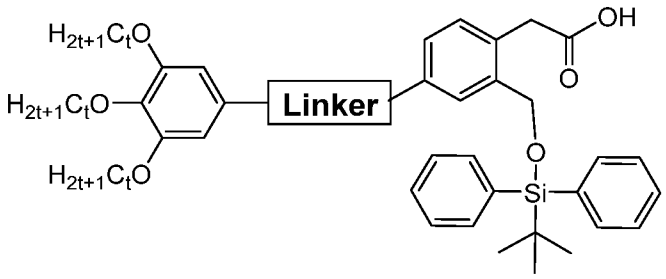
[074] In a twentieth embodiment, the present disclosure provides a compound of Formula I' or Formula B or a salt thereof, wherein e is 0, 1, or 2; and f is 0, 1, or 2. The remainder of the variables in Formula I' are described in the first, second, third, and/or fifth through nineteenth embodiments.

[075] In a twenty-first embodiment, the present disclosure provides a compound of Formula I, Ia, I', or B, or a salt thereof, wherein R_8 is H or C_{1-4}alkyl. The remainder of the variables in Formula I, Ia, I', or B are described in any one of the tenth through twentieth embodiments.

[076] In a twenty-second embodiment, the present disclosure provides a compound of Formula II or IIa:



(II), or

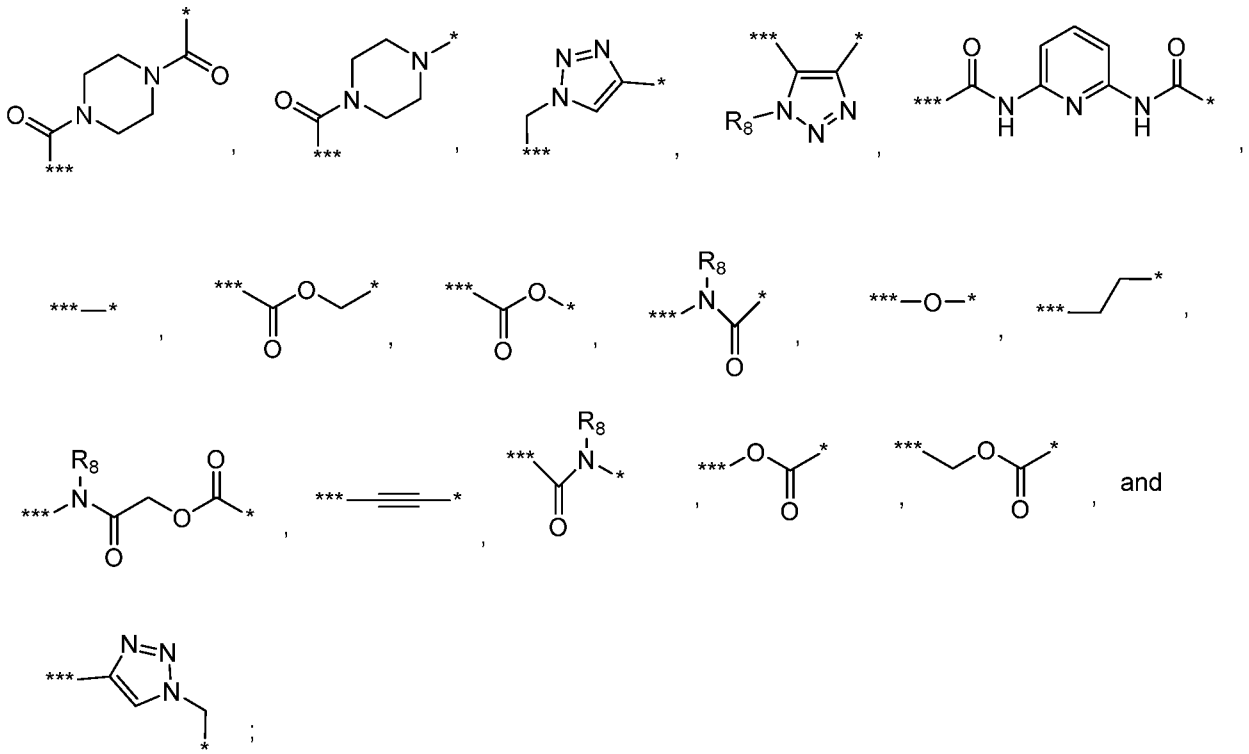


(IIa),

or a salt thereof, wherein:

t is an integer from 10 to 30;

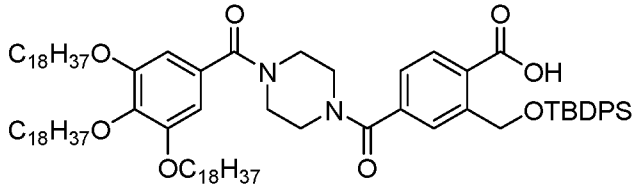
Linker is selected from the group consisting of



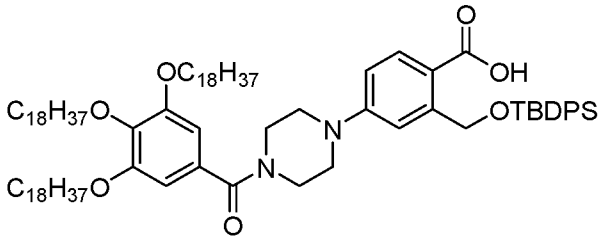
;

wherein R_8 is H or C_{1-6}alkyl.

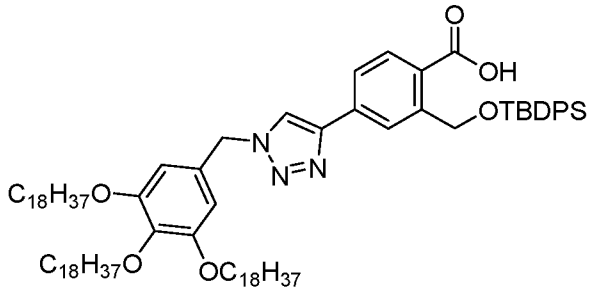
[077] In a twenty-third embodiment, the present disclosure provides a compound of Formula II or a salt thereof that is selected from the group consisting of



,

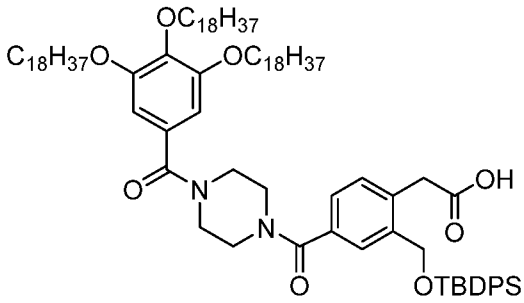


, and



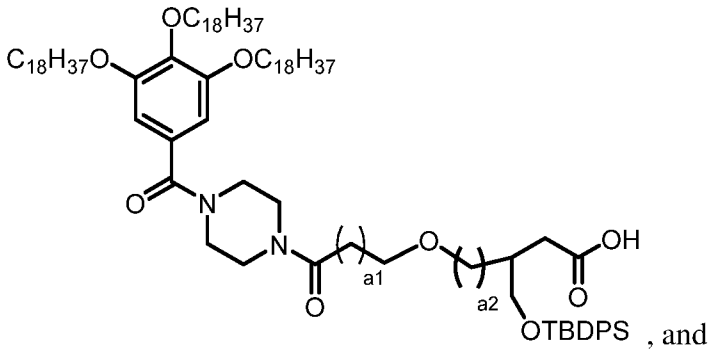
or a salt thereof.

[078] In a twenty-fourth embodiment, the present disclosure provides the following compound:

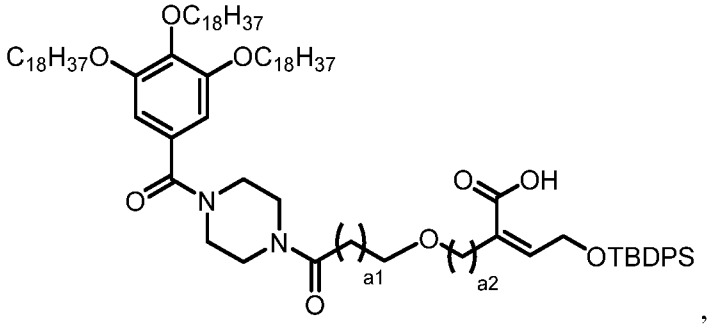


,or a salt thereof.

[079] In a twenty-fifth embodiment, the present disclosure provides a compound selected from one of the following formulae:



, and

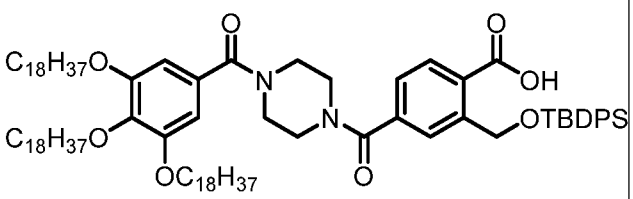
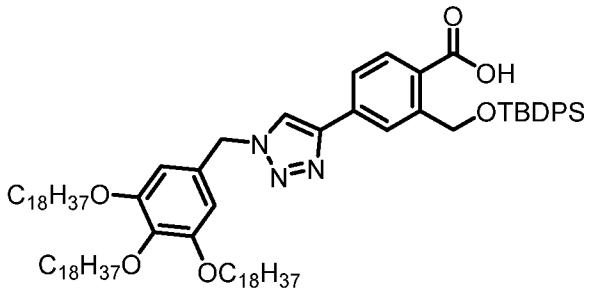


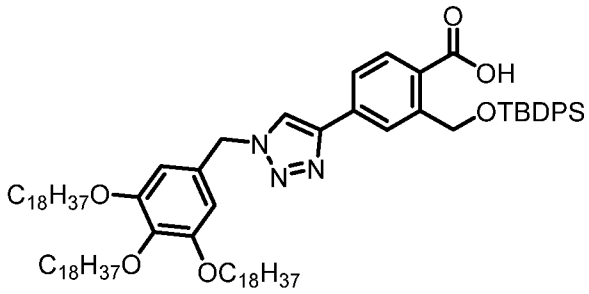
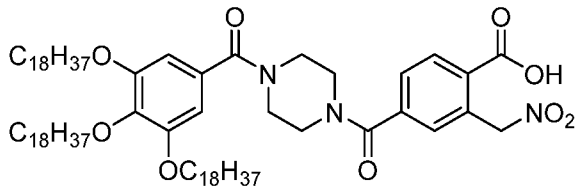
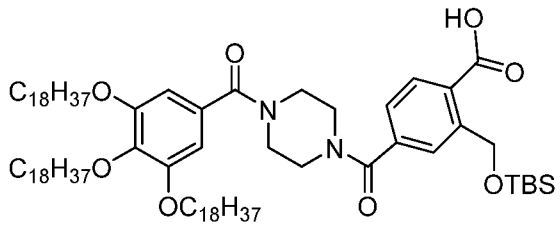
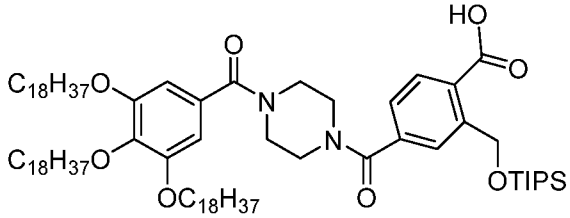
,

or a salt thereof. The remainder of the variables in the above formulae are described in the first embodiment. In some embodiments, a1 and a2 are each an integer from 1 to 6, 1 to 5, or 1 to 4.

[080] In a twenty-sixth embodiment, the present disclosure provides the compounds depicted in Table 1 and prepared in the Exemplification, both the neutral form and salts thereof.

Table 1

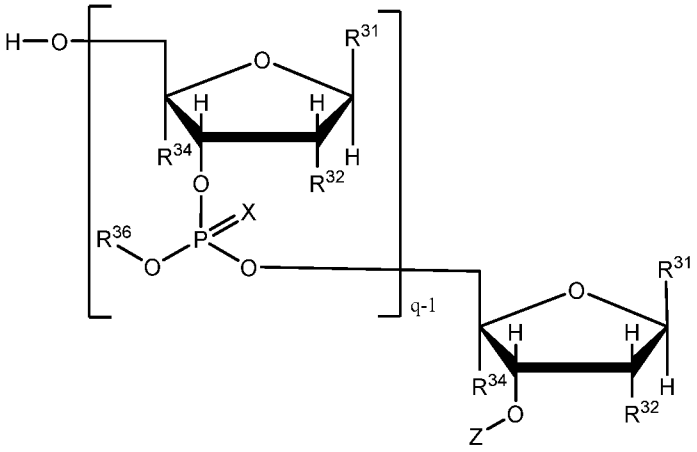
Id	Structure	Chemical Name
M19		2-(((tert-butyldiphenylsilyl)oxy) methyl)-4-(4-(3,4,5-tris(octadecyloxy)benzoyl) piperazine-1-carbonyl)benzoic acid
M22		2-(((tert-butyldiphenylsilyl)oxy)methyl)-4-(1-(3,4,5-tris(octadecyloxy)benzyl)-1H-1,2,3-triazol-4-yl)benzoic acid

M36		2-(((tert-butyldiphenylsilyl)oxy) methyl)-4-(4-(3,4,5-tris(octadecyloxy)benzoyl)piperazin-1-yl)benzoic acid
M40		2-(nitromethyl)-4-(4-(3,4,5-tris(octadecyloxy)benzoyl)piperazine-1-carbonyl)benzoic acid
M50		2-(((tert-butyldimethylsilyl)oxy)methyl)-4-(4-(3,4,5-tris(octadecyloxy)benzoyl)piperazine-1-carbonyl)benzoic acid
M60		2-(((triisopropylsilyl)oxy)methyl)-4-(4-(3,4,5-tris(octadecyloxy)benzoyl)piperazine-1-carbonyl)benzoic acid

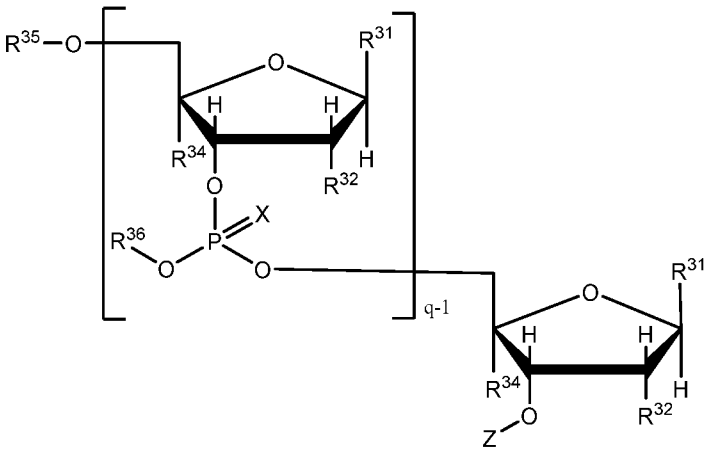
2. 3'-Protected Nucleotides or Oligonucleotides

[081] In a second aspect, the present disclosure describes a nucleotide or an oligonucleotide protected by a 3'-hydroxyl protecting group described herein. In one embodiment, the 3'-hydroxyl protecting group is derived from the Regent described above. In another embodiment, the protected nucleotide or oligonucleotide is separated by selective precipitation. In another embodiment, the protected nucleotide or oligonucleotide is soluble in a non-polar organic solvent such as dichloromethane but precipitate in a polar organic solvent such as acetonitrile.

[082] In a twenty-seventh embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III or IIIP,



(III), or



(IIIP);

or a salt thereof,

wherein

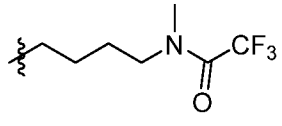
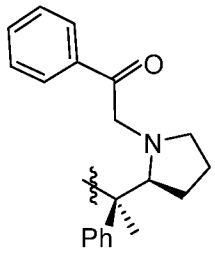
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is optionally protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

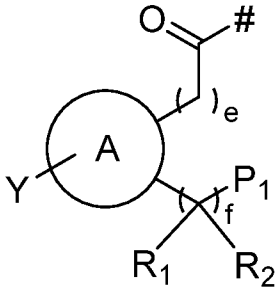
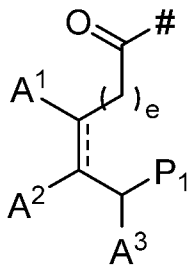
R^{36}, for each occurrence, is independently H, C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is  , or  ;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B*,

 (I^*) or  (B*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

≡ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurance, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

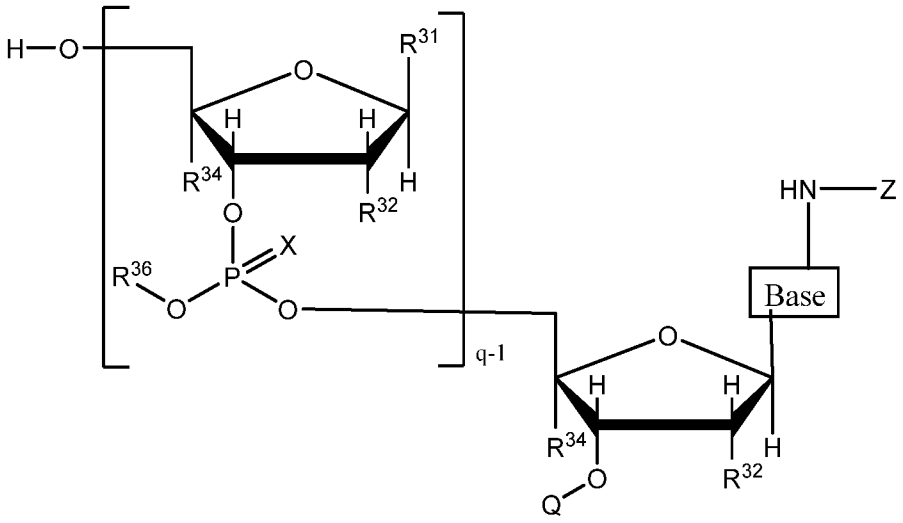
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

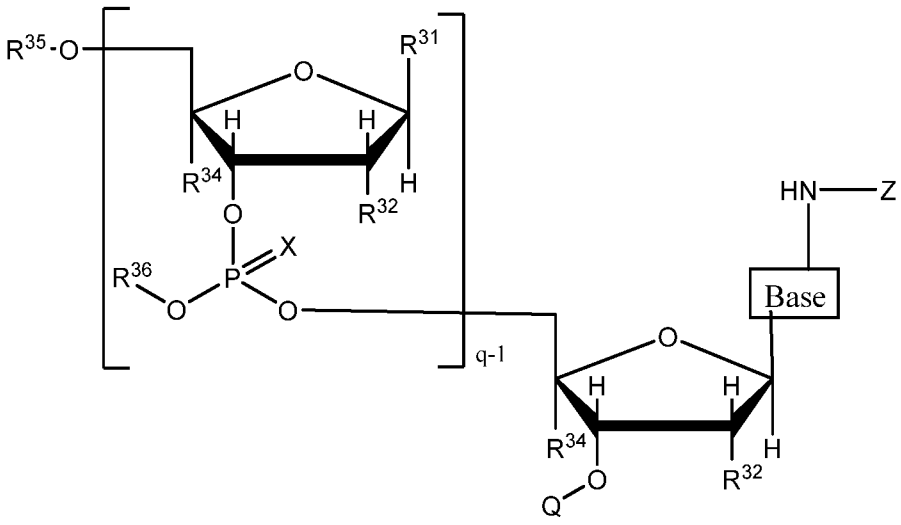
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

[083] In a twenty-eighth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III' or IIIP',



(III'), or



(IIIP');

or a salt thereof, wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

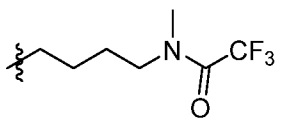
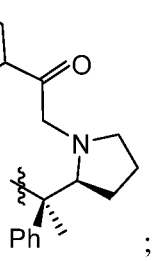
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is optionally protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

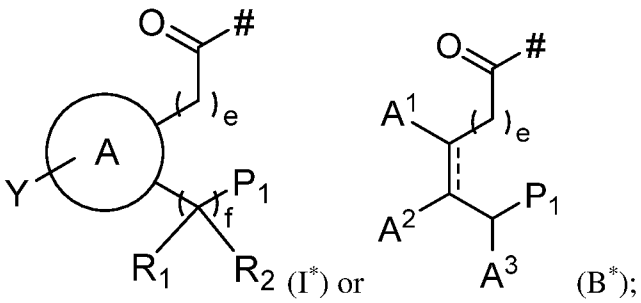
R^{36}, for each occurrence, is independently H, C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is  , or  ;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,



(I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is $Y\text{-}(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, $-OC(O)R^{8A}$, $-C(O)OR^{8A}$, $NR^{8A}R^{9A}$, $-NR^{8A}COR^{9A}$, $-CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

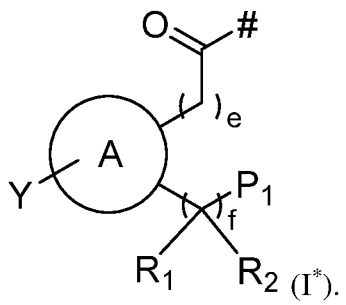
R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

[084] In certain embodiments, the hydroxyl protecting group of R^{32} is a silyl protecting group. In certain embodiments, the silyl protecting group is selected from the group consisting of trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylthexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-t-butylmethylsilyl tri(trimethylsilyl)silyl, t-butylmethoxyphenylsilyl, and t-butoxydiphenylsilyl.

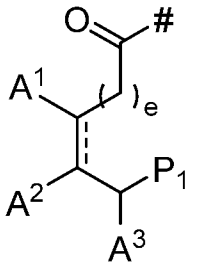
[085] In a twenty-ninth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP', or a salt thereof, wherein Z is a group represented by Formula I^*,



(I^*).

The remainder of the variables in Formula III, III', IIIP, or IIIP', are described in the twenty-seventh and/or twenty-eighth embodiments.

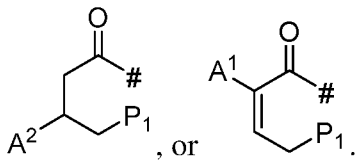
[086] In a thirtieth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is a group represented by Formula B^*,



(B*).

The remainder of the variables in Formula III, III', IIIP, or IIIP', are described in the twenty-seventh and/or twenty-eighth embodiments.

[087] In a thirty-first embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is a group represented by Formula B-1* or B-2*:



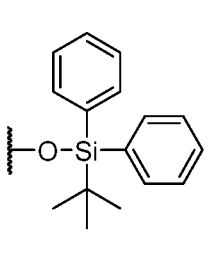
B-1* B-2*

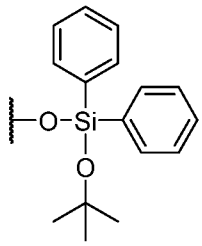
The remainder of the variables in Formula III, III', IIIP, or IIIP', are described in the twenty-seventh and/or twenty-eighth embodiments.

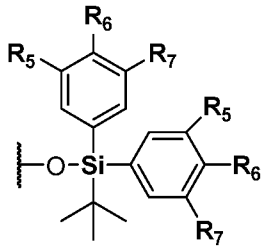
[088] In a thirty-second embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the twenty-seventh and/or twenty-eighth embodiments.

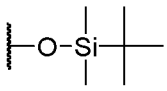
[089] In a thirty-third embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein ring A is phenyl or naphthalenyl. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the twenty-seventh, twenty-eighth, and/or thirty-second embodiments.

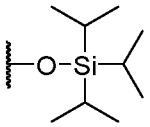
[090] In a thirty-fourth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein P_1 is a silyl hydroxyl protecting group selected from the following:

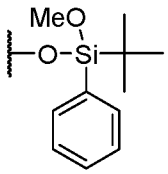

-O-TBDPS ,

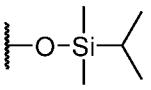

-O-TBoDPS ,

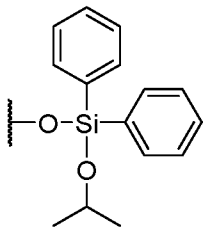

-O-TBDAS ,

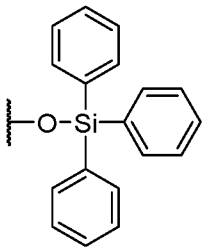

-O-TBDMS ,

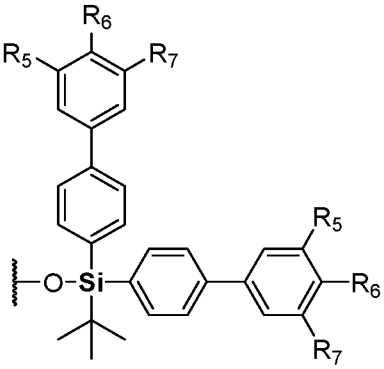

-O-TIPS ,

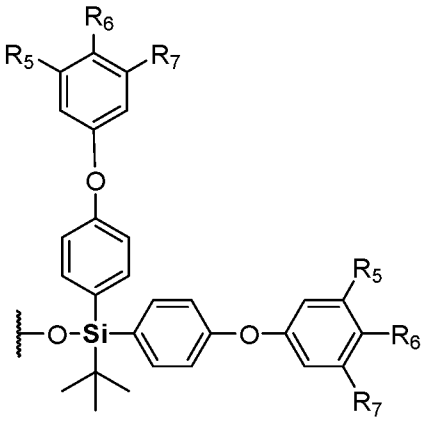

-O-TBMPS ,


-O-DMIPS ,

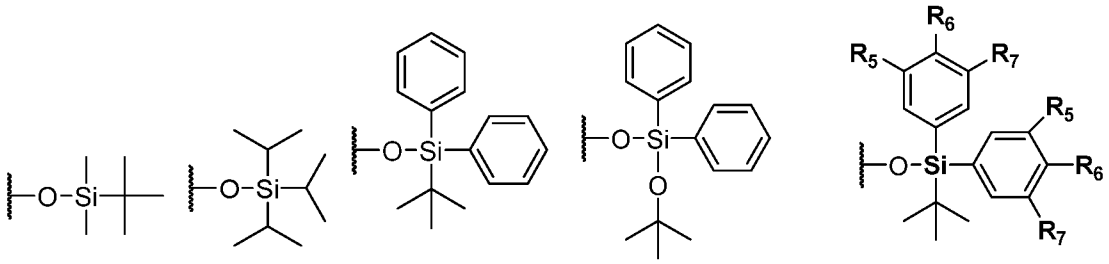

-O-IPoDPS ,


-O-TPS ,


-O-TBDAS-1 ,


and -O-TBDAS-2 ; wherein ⊢ represents the point of attachment for P_1 and R_5, R_6 and R_7 are each independently H, C_{1-30}alkyl, or C_{1-30}alkoxy. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through thirty-third embodiments.

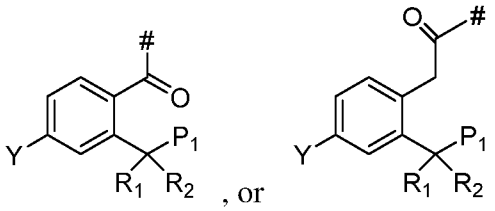
[091] In a thirty-fifth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein P_1 is selected from the group consisting of –O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , and -O-TBDAS .

The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through thirty-fourth embodiments.

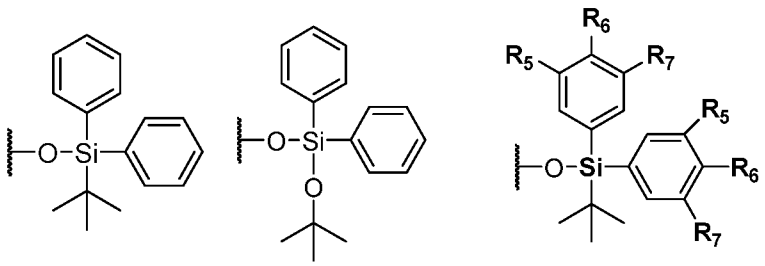
[092] In a thirty-sixth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is a group represented by by Formula I** or Ia**:



, or

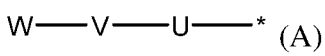
I^{**} Ia^{**}

or salt thereof, wherein P1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDPS , -O-TBoDPS , and -O-TBDAS . The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through thirty-fifth embodiments.

[093] In a thirty-seventh embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Y is represented by Formula A:

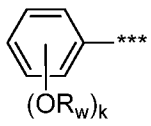
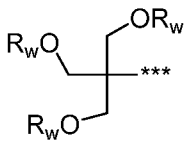
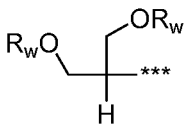
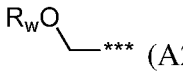


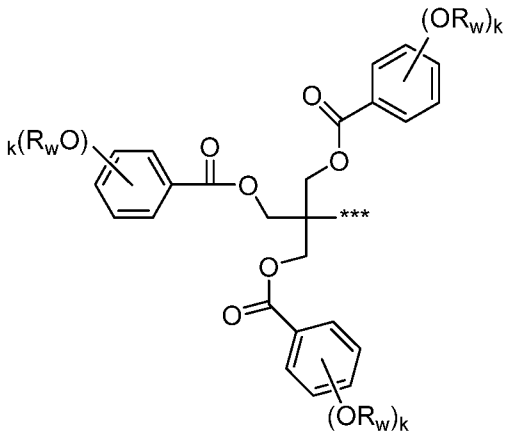
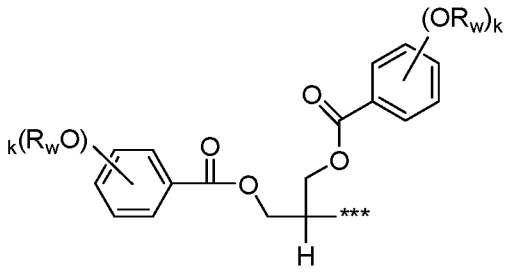
(A)

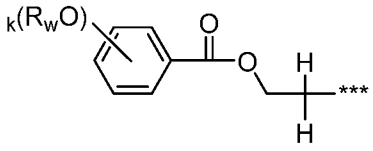
wherein:

—* represents the point of attachment for Y;

W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:


(A1),

(A2),

(A2-1),

(A2-2),


(A3),

(A3-1),

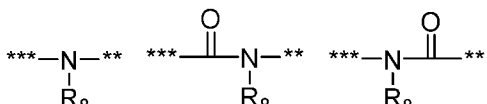
or

(A3-2),

wherein

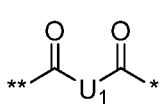
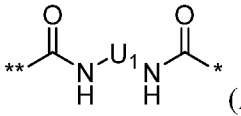
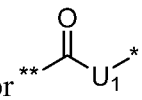
—*** represents the point where W and V connect;

each R_W is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

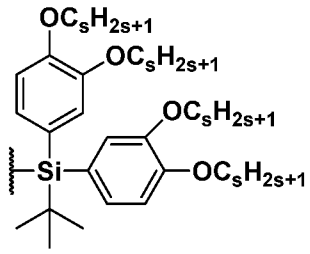
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:


(A4),

(A5), or

(A6),

wherein U_1 is $C_{1\text{-}6}$alkylene, $C_{1\text{-}6}$alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through thirty-sixth embodiments.

[094] In a thirty-eighth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein the TBDAS group is:

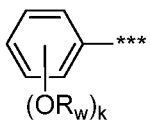


,

wherein s is an integer from 1 to 30. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the thirty-fourth through thirty-seventh embodiments.

[095] In a thirty-ninth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein P_1 is TBDPS. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through thirty-seventh embodiments.

[096] In a fortieth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein W is represented by Formula A1:

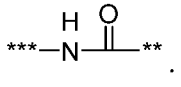


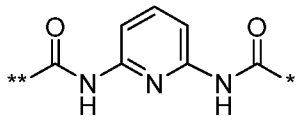
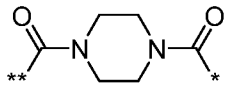
A1,

wherein R_w is C_nH_{2n+1}; and n is an integer from 1 to 30. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the thirty-seventh embodiment.

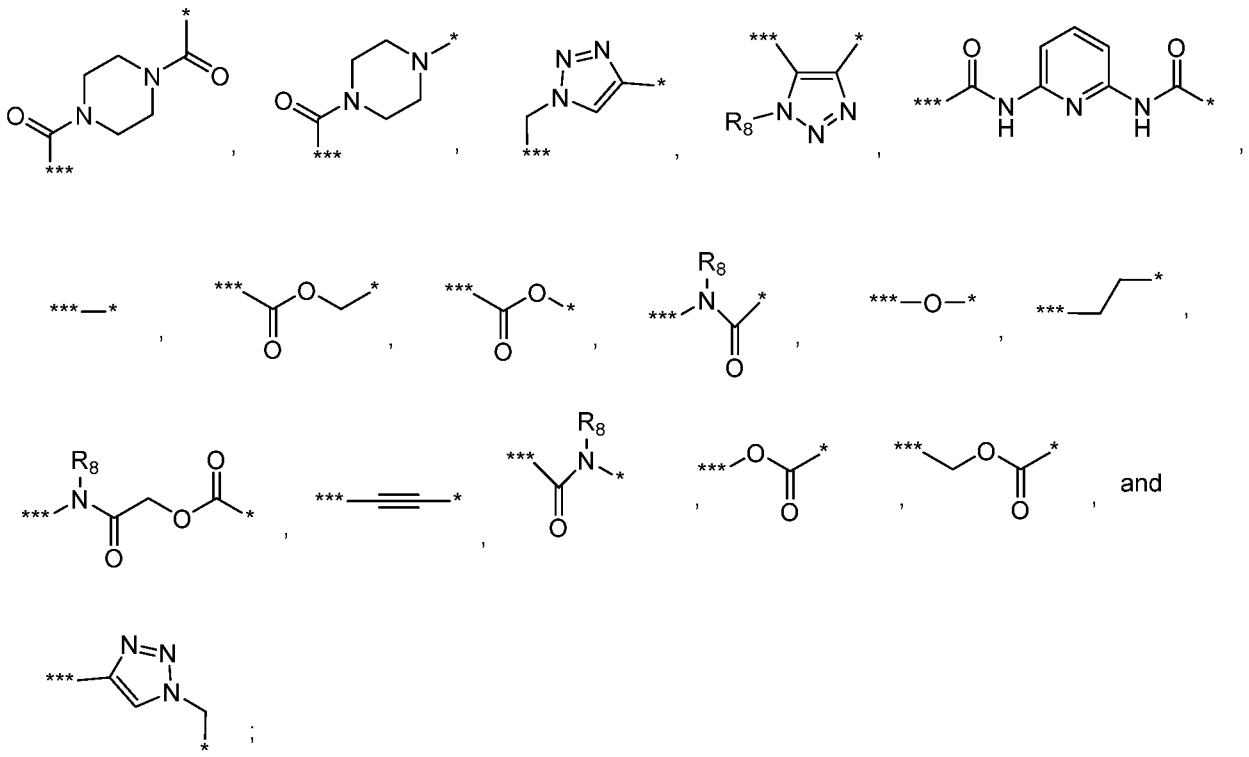
[097] In a forty-first embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and

$C_{28}H_{57}$. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the thirty-seventh and/or fortieth embodiments.

[098] In a forty-second embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein V is a bond, CH_2, CH_2CH_2, C(=O)-, ***-C(=O)-O-**, or  . The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the thirty-seventh through forty-first embodiments.

[099] In a forty-third embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein U is a bond, CH_2, CH_2CH_2, carbonyl, triazolylene, piperazinylene,  , or  . The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the thirty-seventh through forty-second embodiments.

[0100] In a forty-four embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein U-V is selected from the group consisting of

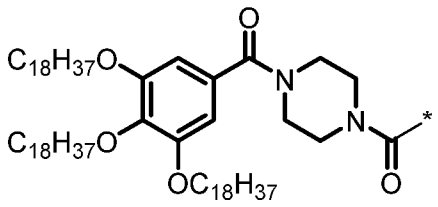


and

;

wherein R_8 is H or C_{1-6}alkyl. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the thirty-seventh through the forty-first embodiments.

[0101] In a forty-fifth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Y is selected from the groups consisting of: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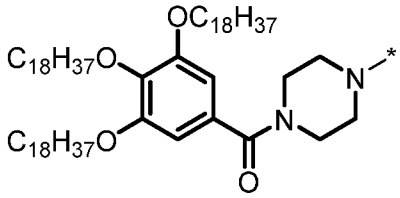
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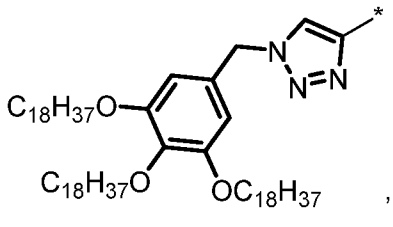
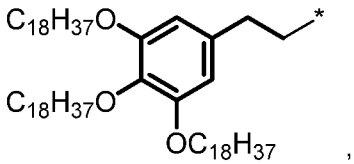

,

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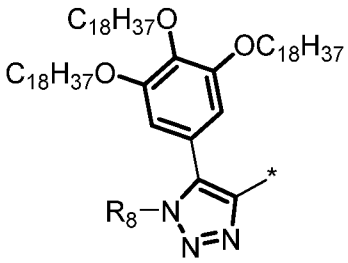
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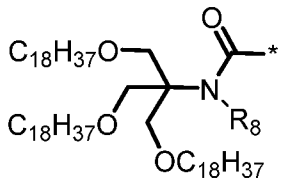
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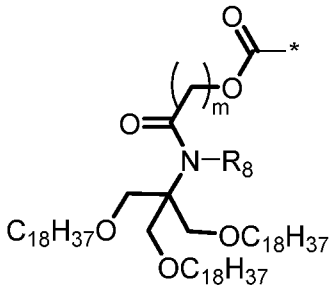
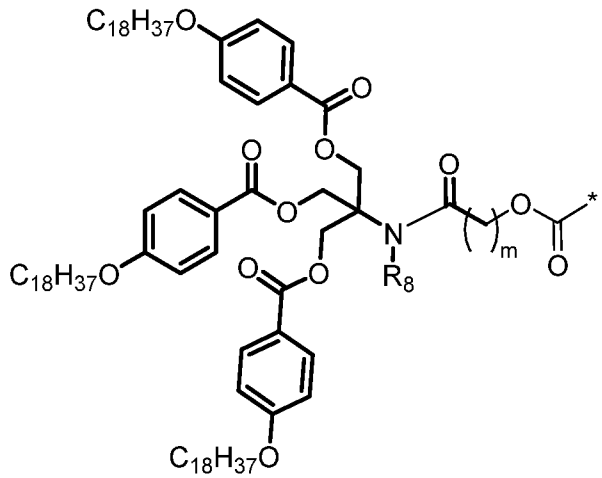

, and

;

wherein

R_8 is H or C_{1-6}alkyl; and

m is an integer from 1 to 5.

The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the thirty-ninth embodiments.

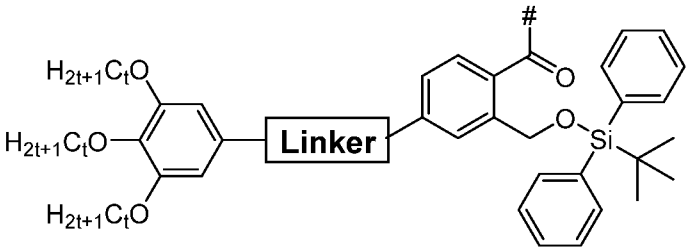
[0102] In a forty-sixth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R_1 and R_2 are independently H or CH_3. The remainder of the variables in Formula III, III', IIIP, or

IIIP' are described in any one of the twenty-seventh through forty-fifth embodiments. In a specific embodiment, R_1 and R_2 are both H. In another specific embodiment, R_1 and R_2 are both CH_3.

[0103] In a forty-seventh embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein e is 0, 1, or 2; and f is 0, 1, or 2. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the forty-sixth embodiments.

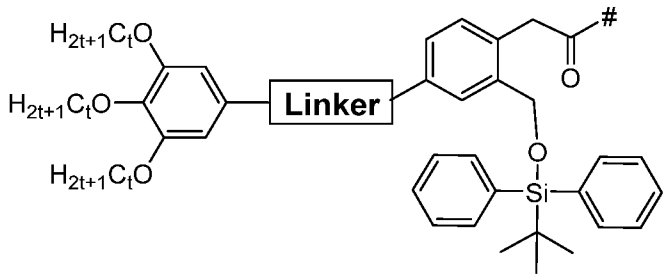
[0104] In a forty-eighth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof. wherein R_8 is H or C_{1-4}alkyl. The remainder of the variables in Formula III or IIIP are described in the thirty-seventh embodiment. In one embodiment, R_8 is H or methyl.

[0105] In a forty-ninth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is represented by Formula II* or IIa*,



, or

II*

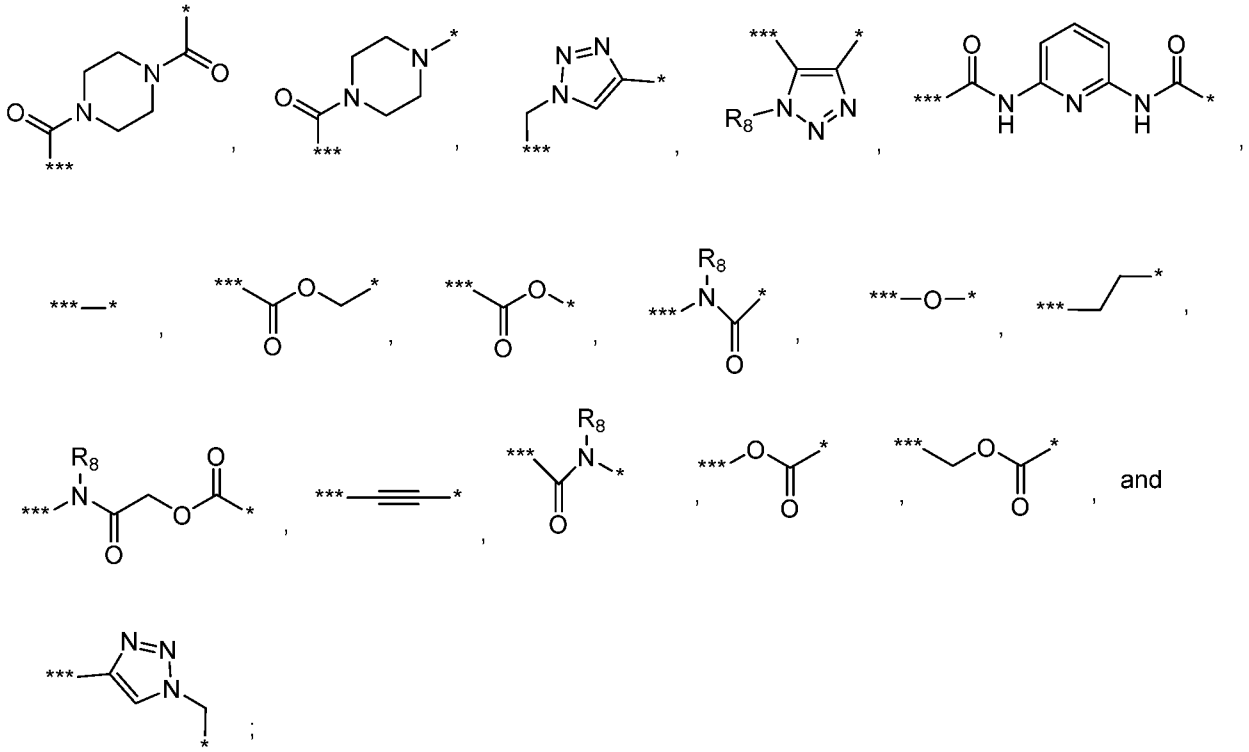


IIa*

Wherein:

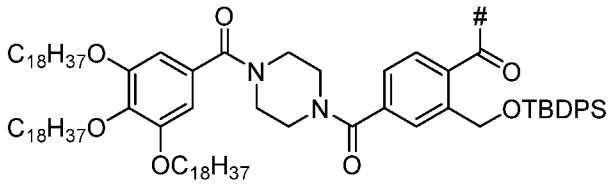
t is an integer from 10 to 30;

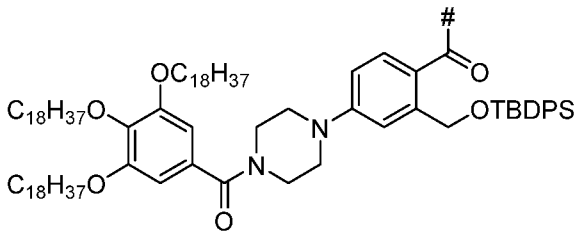
Linker is selected from the group consisting of

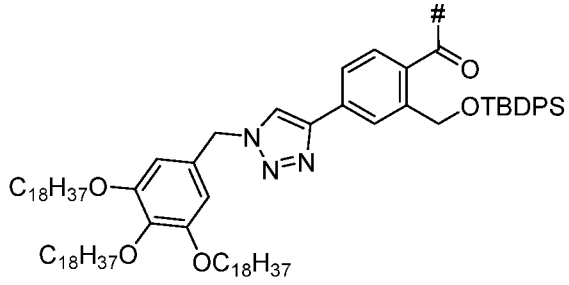


wherein R_8 is H or $C_{1\text{-}6}$alkyl. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the twenty-seventh and/or twenty-eighth embodiments.

[0106] In a fiftieth embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is

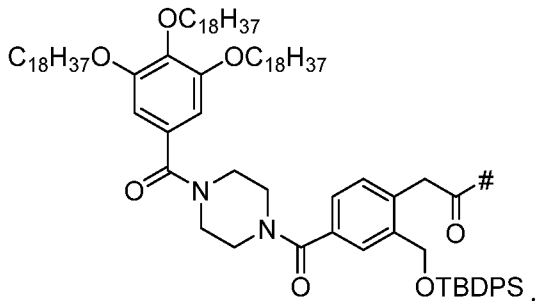






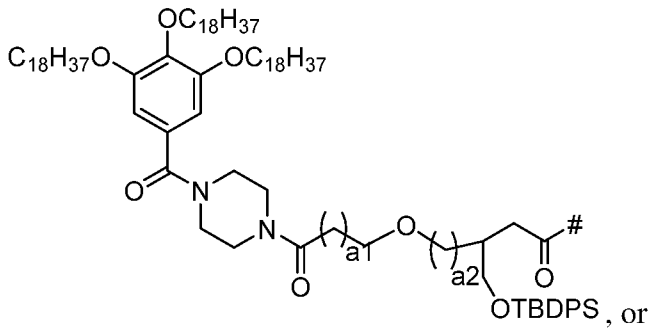
The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the forty-ninth embodiment.

[0107] In a fifty-first embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is

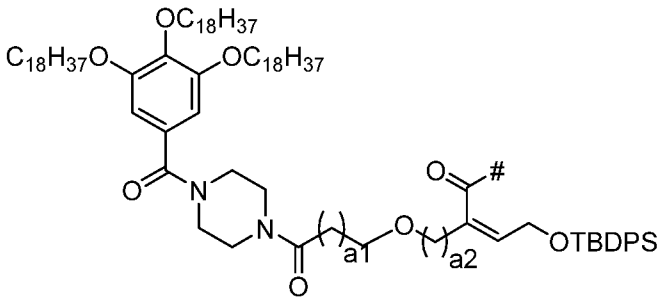


The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the twenty-seventh and/or twenty-eighth embodiments.

[0108] In a fifty-second embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein Z is



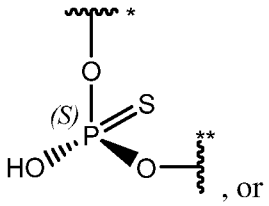
, or



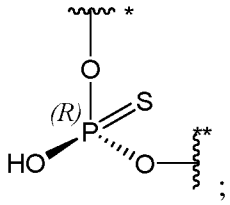
.

The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in the twenty-seventh and/or twenty-eighth embodiments. In some embodiments, a1 and a2 are each an integer from 1 to 6, 1 to 5, or 1 to 4.

[0109] In a fifty-third embodiment, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein when X is S, the phosphorothiolate group has *S*-configuration as shown below:


, or

R-configuration as shown below:


;

wherein ~~~~ * indicates the connection point to 3'-OH group and ⁑ indicates the connection point to 5'-OH group. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-second embodiments.

[0110] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{31}, for each occurrence, is adenine (A), guanine (G), thymine (T), cytosine (C), or uracil (U). The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments.

[0111] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{32}, for each occurrence, is independently H, F, Cl, Br, I, or $-OCH_2CH_2OMe$. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments. In a specific embodiment, R^{32}, for each occurrence, is independently H or $-OCH_2CH_2OMe$.

[0112] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{34}, is H. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments.

[0113] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{35} is 4,4'-dimethoxytrityl. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments.

[0114] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{36} is

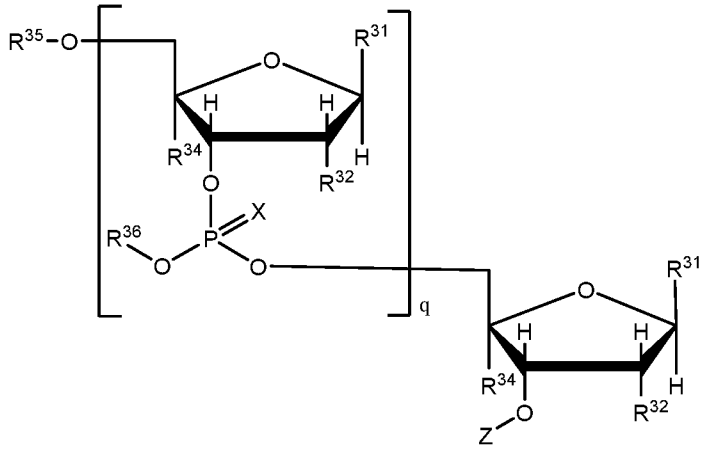
$-CH_2CH_2CN$. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments.

[0115] In certain embodiments, the present disclosure provides a nucleotide or oligonucleotide represented by Formula III, III', IIIP, or IIIP' or a salt thereof, wherein R^{32} is $-OCH_2CH_2OMe$. The remainder of the variables in Formula III, III', IIIP, or IIIP' are described in any one of the twenty-seventh through the fifty-third embodiments.

3. Process for Preparing Oligonucleotide Fragment

[0116] In a third aspect, the present disclosure describes a process of preparing an oligonucleotide fragment bearing a hydroxyl protecting group (*e.g.*, a hydrophobic hydroxyl protecting group) at the 3'-end (when the fragment bears a hydrophobic hydroxyl protecting group, it can be referenced herein as the "3'-fragment") or an amino protecting group at a nucleobase (when the nucleobase comprises a NH_2 group. It can be referenced herein as the "nucleobase SiLHPG fragment"). It is surprisingly discovered that the methods of the present disclosure for synthesizing the 3'-fragment or the nucleobase SiLHPG fragment can be used to prepare an oligonucleotide fragment having 3 to 20 (*e.g.*, 3 to 10, 3 to 8, 3 to 5 or 4 to 5) nucleotides with high purity without chromatographic purification. In some embodiments, a hydrophobic 3'-hydroxyl protecting group is used, which facilitates the separation of the oligonucleotide fragment product by selective precipitation. In some embodiments, a hydrophobic amino protecting group is used, which facilitates the separation of the oligonucleotide fragment product by selective precipitation. In some embodiments, the liquid phase process comprises (1) 5'-OH deprotection step, (2) coupling step, and (3) oxidation or sulfurization step, wherein the steps (1), (2) and (3) are repeated until the desired number of nucleotides are linked together to form the 3'-oligonucleotide fragment.

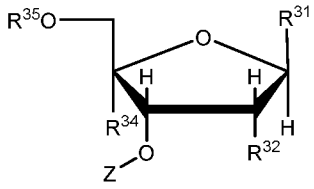
[0117] In a fifty-fourth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V),



(V),

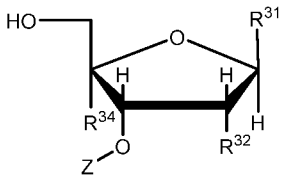
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



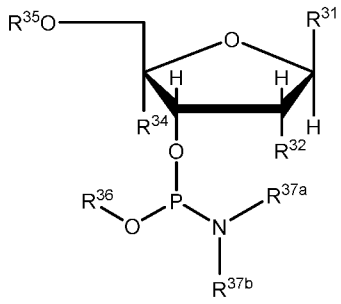
(VA),

or a salt thereof, to form a compound of formula (VB):



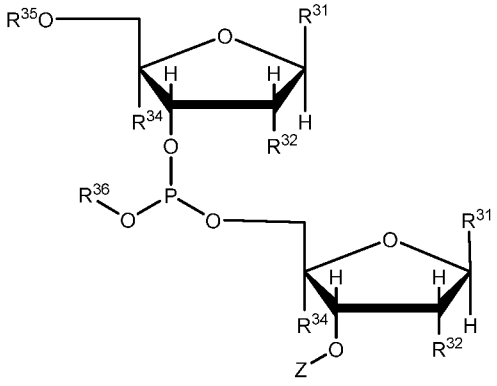
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC):



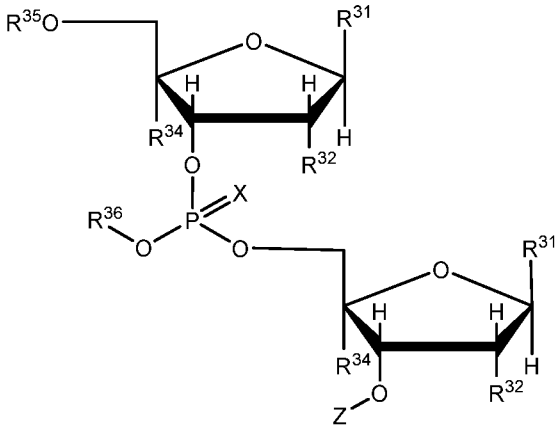
(VC),

or a salt thereof, to form a compound of formula (VD),



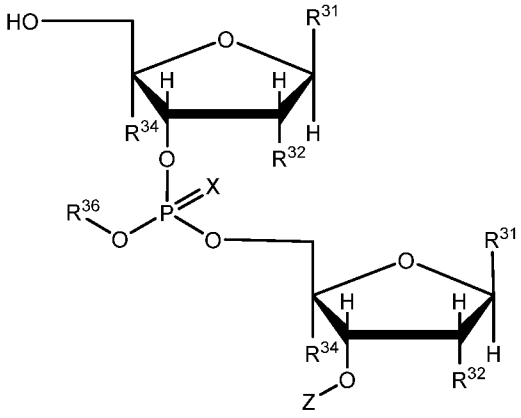
(VD), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE):



(VE), or a salt thereof;

4) deprotecting the compound of formula (VE), or a salt thereof to form a compound of formula (VF):



(VF), or a salt thereof;

5) when q is equal or greater than 2, starting with the compound of formula (VF), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V), or a salt thereof, wherein:

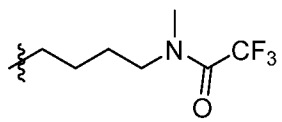
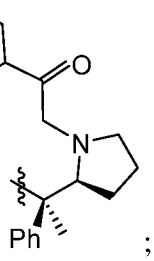
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

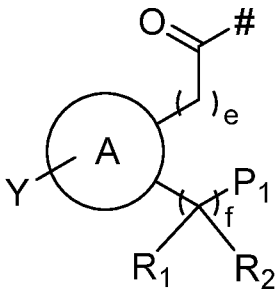
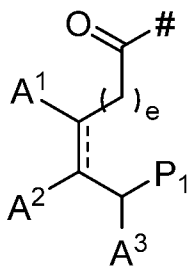
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B*,

 (I^*) or  (B*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, $-OC(O)R^{8A}$, $-C(O)OR^{8A}$, $NR^{8A}R^{9A}$, $-NR^{8A}COR^{9A}$, $-CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

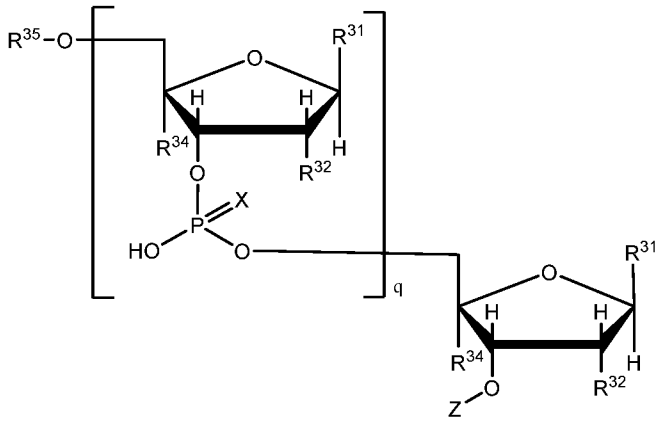
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

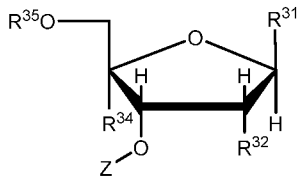
[0118] In a fifty-fifth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V'),



(V'),

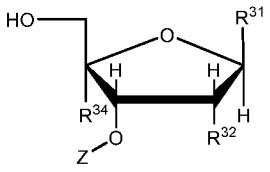
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



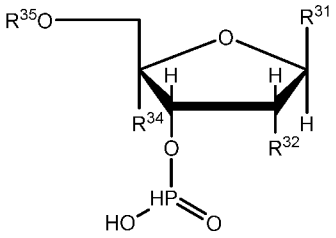
(VA),

or a salt thereof, to form a compound of formula (VB):



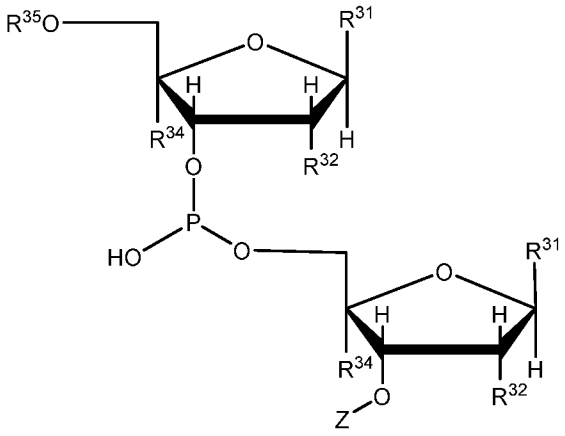
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC'):



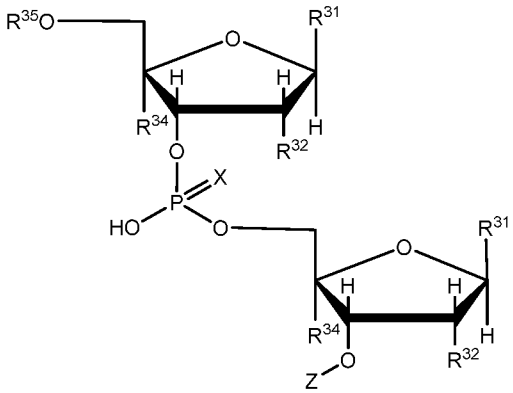
(VC'),

or a salt thereof, to form a compound of formula (VD'),



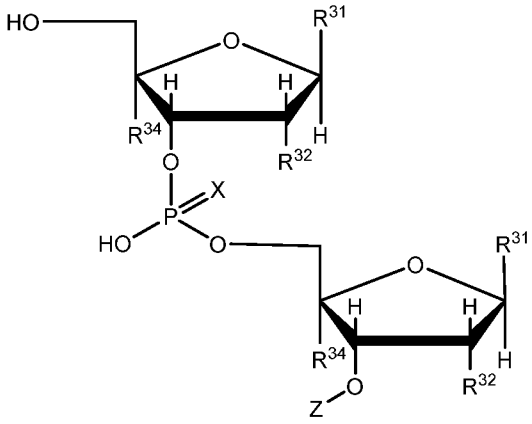
(VD'), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD'), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE'):



(VE'), or a salt thereof;

4) deprotecting the compound of formula (VE'), or a salt thereof to form a compound of formula (VF'):

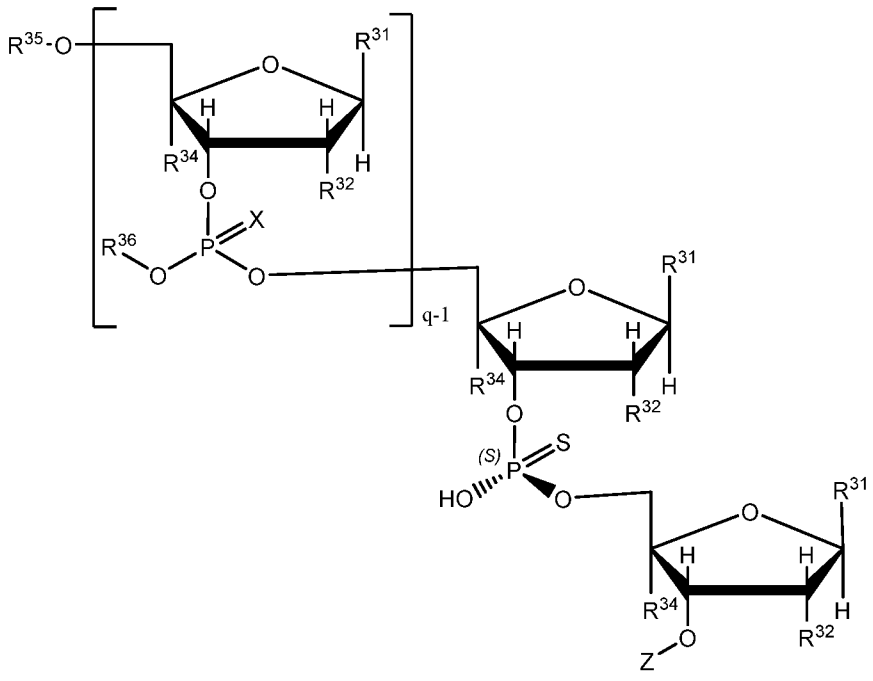


(VF'), or a salt thereof;

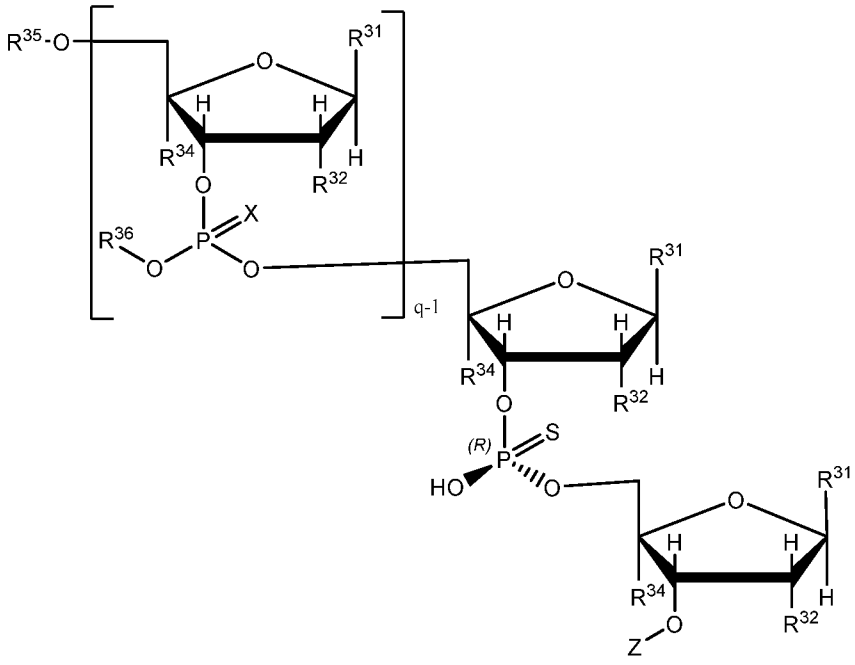
5) when q is equal or greater than 2, starting with the compound of formula (VF'), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V'), or a salt thereof, wherein:

R^{31}, R^{32}, R^{34}, R^{35}, q, X and Z are as described above for formula (V) in the fifty-fourth embodiment.

[0119] In a fifty-sixth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



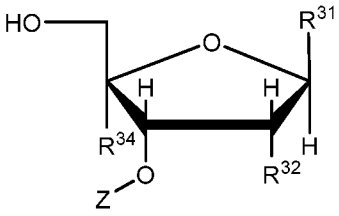
(V-C1), or



(V-C2)

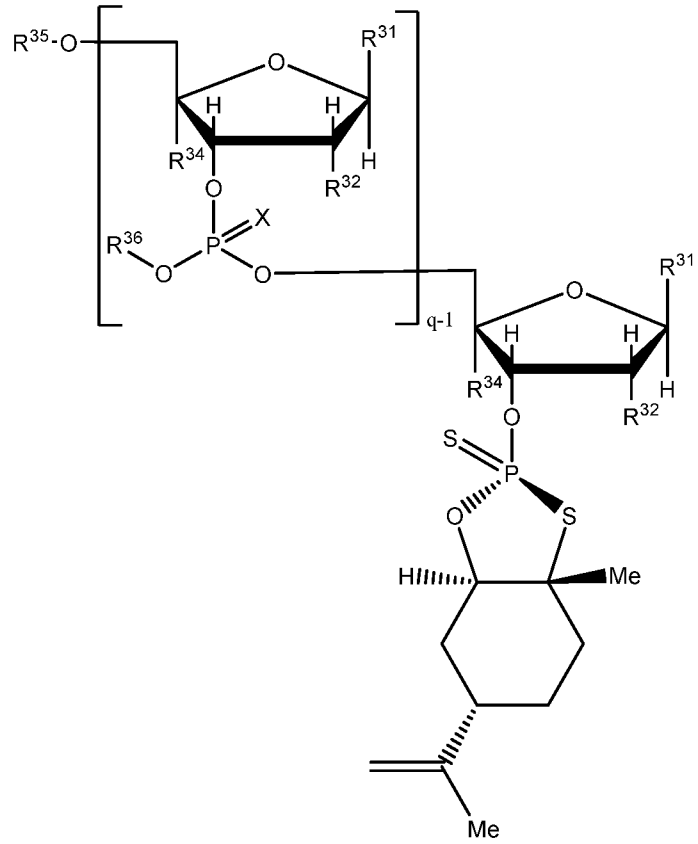
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

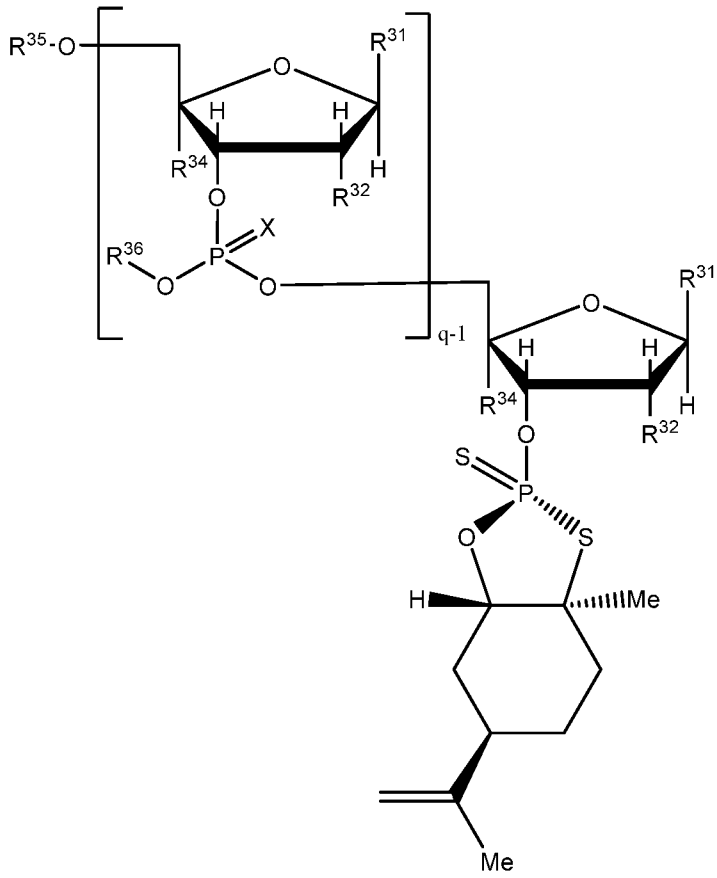


(VB),

or a salt thereof, with a compound of formula (V-CR1) or (V-CR2),



(V-CR1),

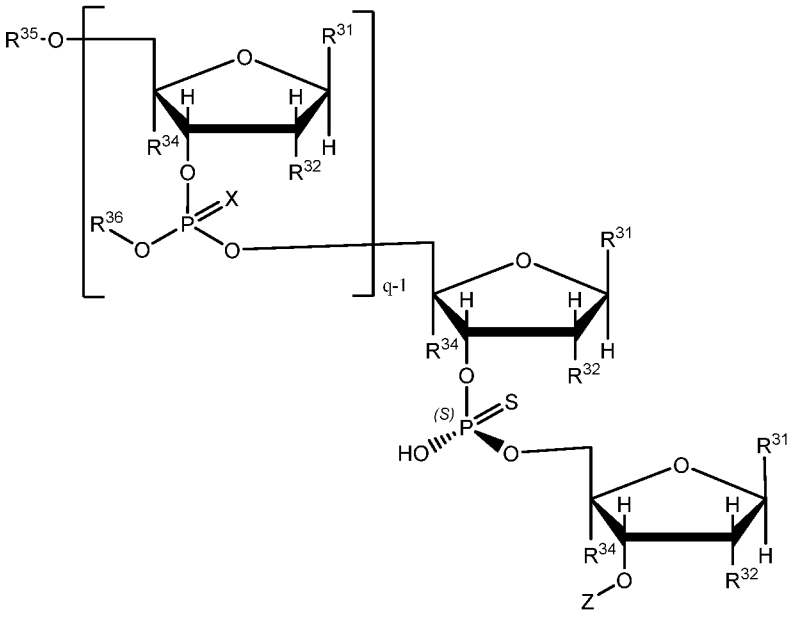


(V-CR2)

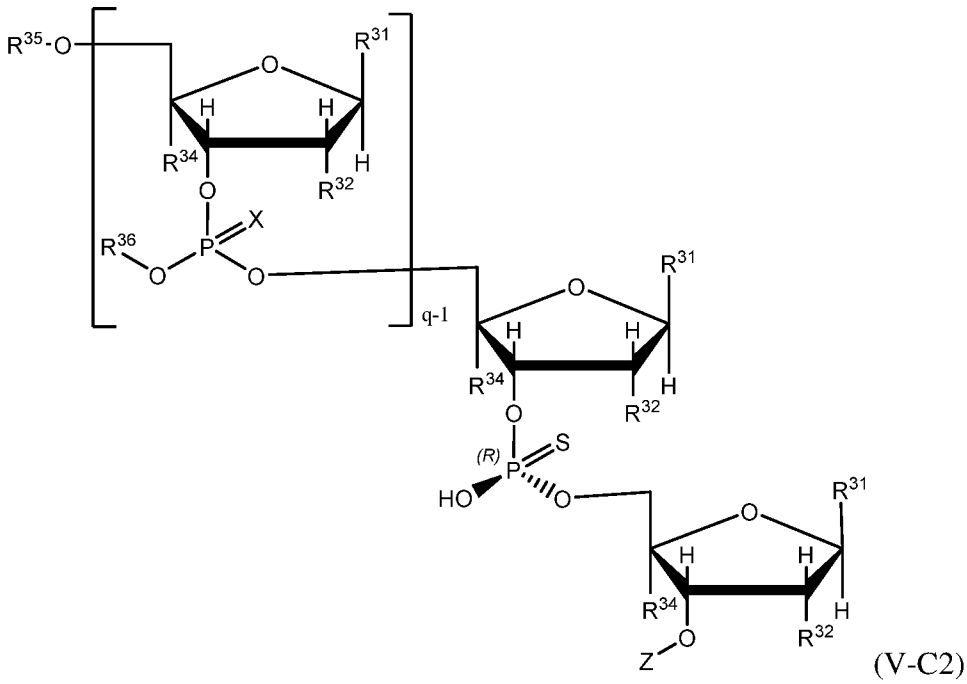
or a salt thereof, and a base, to form the compound of formula (V-C1) or (V-C2), wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X and Z are as described above for formula (V) in the fifty-fourth

embodiment. The reaction of formula (VB) with (V-CR1) forms the compound of formula (V-C1) and the reaction of formula (VB) with (V-CR2) forms the compound of formula (V-C2).

[0120] In a fifty-seventh embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



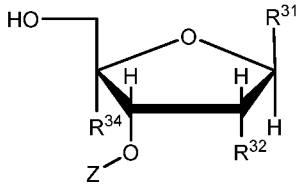
(V-C1), or



(V-C2)

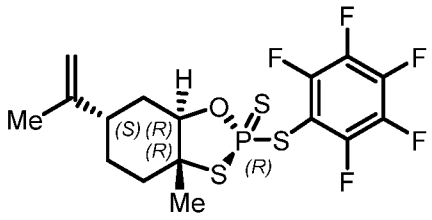
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

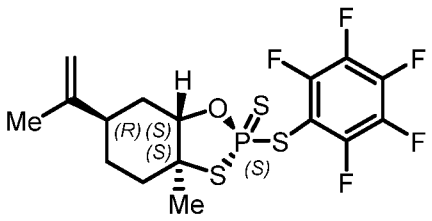


(VB),

or a salt thereof, with a reagent of formula (VR1) or (VR2),

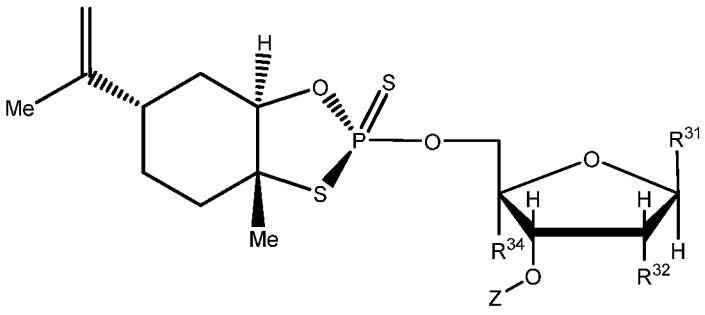


(VR1), or

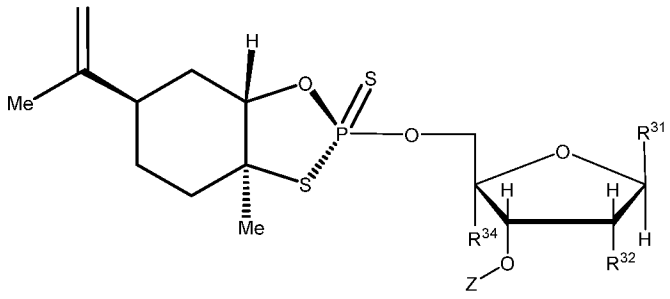


(VR2)

to form a compound of formula (V-CR3) or (V-CR4),



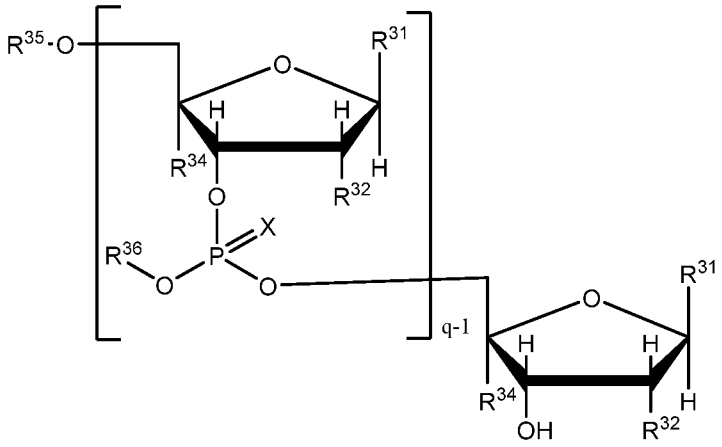
(V-CR3), or



(V-CR4)

or a salt thereof;

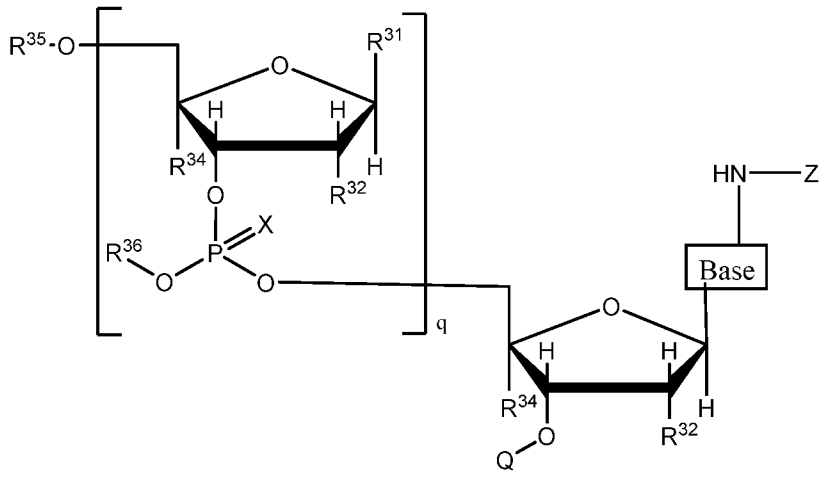
2) reacting the compound of formula (V-CR3) or (V-CR4), or a salt thereof, with a compound of formula (VG):



(VG),

or a salt thereof, and a base, to form a compound of formula (V-C1) or (V-C2), wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, X and Z are as described above for formula (V) in the fifty-fourth embodiment. The reaction of reagent (VR1) with the compound of formula (VB) forms the compound of formula (V-CR3), which reacts with the compound of formula (VG) to form the compound of formula (V-C1). The reaction of reagent (VR2) with the compound of formula (VB) forms the compound of formula (V-CR4), which reacts with the compound of formula (VG) to form the compound of formula (V-C2).

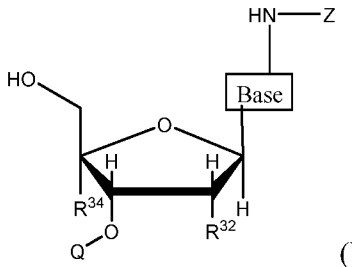
[0121] In a fifty-eighth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (VBZ),



(VBZ),

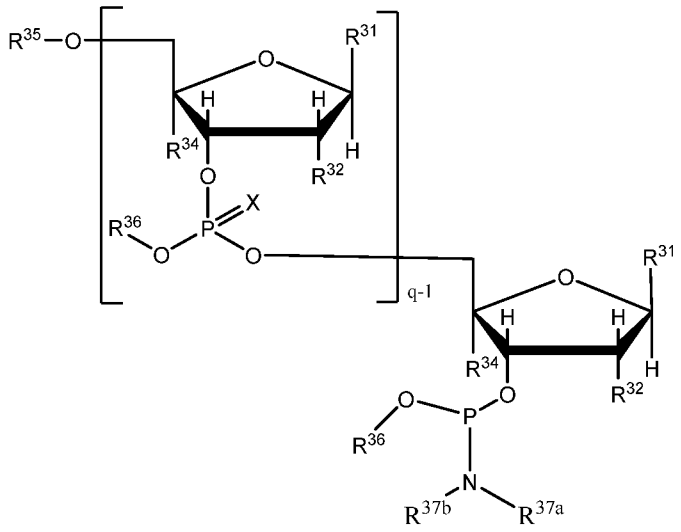
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VBZ-1),



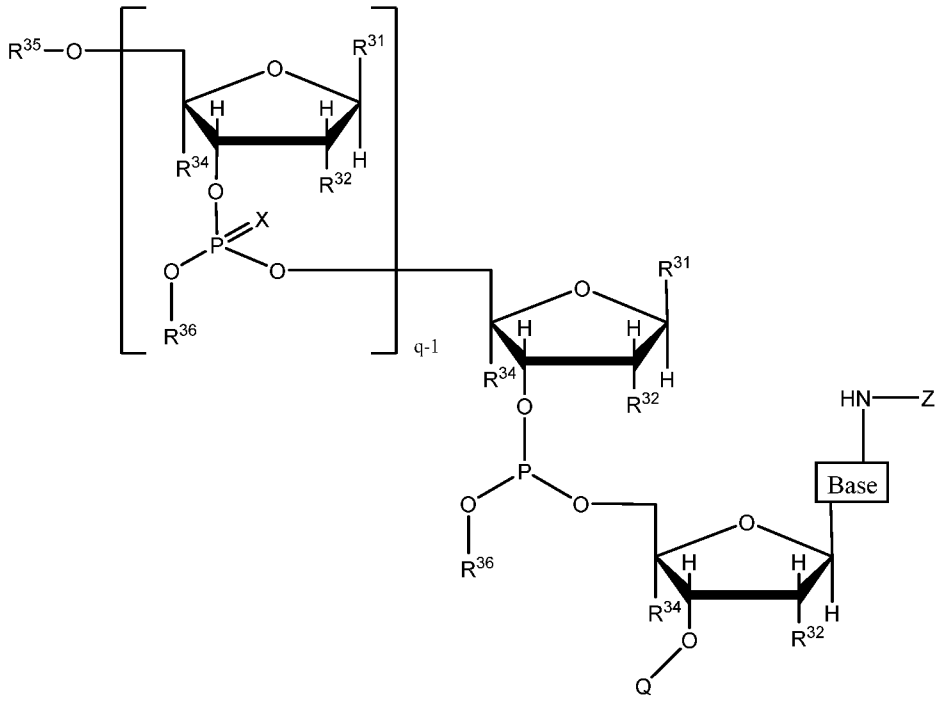
(VBZ-1),

or a salt thereof, with a compound of formula (VBZ-2):



(VBZ-2),

or a salt thereof, to form a compound of formula (VBZ-3),



(VBZ-3), or a salt thereof;

2) sulfurizing or oxidizing the compound of formula (VBZ-3), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VBZ), or a salt thereof;

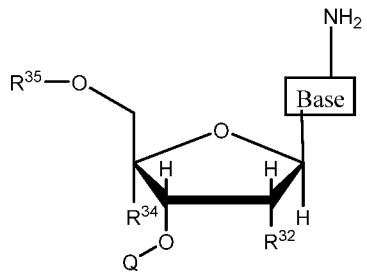
wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z; and R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a}, R^{37b}, q, X and Z are as described above for formula (V) in the fifty-fourth embodiment.

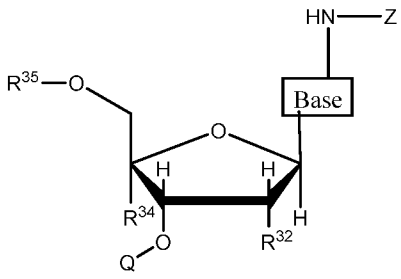
[0122] In a fifty-ninth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (VBZ) or a salt thereof described in the fifty-eighth embodiment, wherein the compound of formula VBZ-1 is prepared by

1) reacting the compound of formula (VBZ-4),



(VBZ-4),

or a salt thereof, with Z-OH to form a compound of formula VBZ-5,



(VBZ-5),

or a salt thereof;

2) deprotecting the compound of formula (VBZ-5) to form the compound of formula (VBZ-1).

[0123] In a sixty embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V), (V'), (V-C1), (V-C2), or (VBZ) or a salt thereof described in the fifty-fourth through fifty-ninth embodiment, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms. The remainder of the variables in Formula (V), (V'), (V-C1), (V-C2), or (VBZ) are described in any one of the fifty-fourth through the fifty-ninth embodiments.

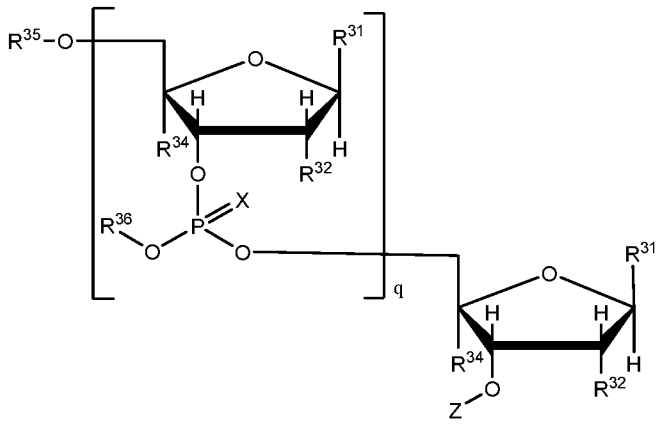
[0124] In a sixty-first embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V), (V'), (V-C1), (V-C2), or (VBZ) or a salt thereof described in the fifty-fourth through fifty-ninth embodiment, wherein no chromatography is used for purifying the reaction product of any one of steps 1), 2), 3) and 4).

[0125] In a sixty-second embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V), (V'), (V-C1), (V-C2), or (VBZ) or a salt thereof described in the fifty-fourth through fifty-ninth embodiment, wherein the reaction product of any one of steps 1), 2), 3) and 4) is purified by selective precipitation. In certain embodiments, the selective precipitation of the reaction product of any one of steps 1), 2), 3)

and 4) or a salt thereof can be achieved by adding acetonitrile to a solution of the crude product in DCM. Alternatively, the solution of the crude product can be added to acetonitrile to precipitate out the desired product.

[0126] In certain embodiments, the reaction product of any one of steps 1), 2), 3) and 4) or a salt thereof is purified by extracting a solution comprising the reaction product of any one of steps 1), 2), 3) and 4) or a salt thereof in an organic solvent (MBTE, EtOAc, heptane/MBTE mixture, DCM, etc.) with an aqueous solution (*e.g.*, $NaHCO_3/H_2O$ or $NaCl/H_2O$) in addition to selective precipitation. In certain embodiments, the extraction is carried out before selective precipitation. Alternatively, the extraction is carried out after selective precipitation. In certain embodiments, the selective precipitation of the reaction product of any one of steps 1), 2), 3) and 4) or a salt thereof can be achieved by adding heptane or a heptane/MBTE mixture to a solution of the crude product in DCM or EtOAc. Alternatively, the solution of the crude product can be added to heptane or a heptane/MBTE mixture to precipitate out the desired product. A heptane/MBTE mixture with a suitale volume ratio (*e.g.*, a volume ratio described herein) can be used.

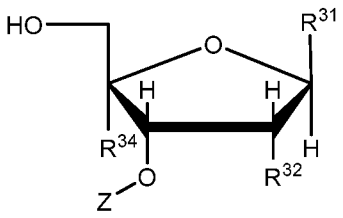
[0127] In a sixty-third embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V)



(V),

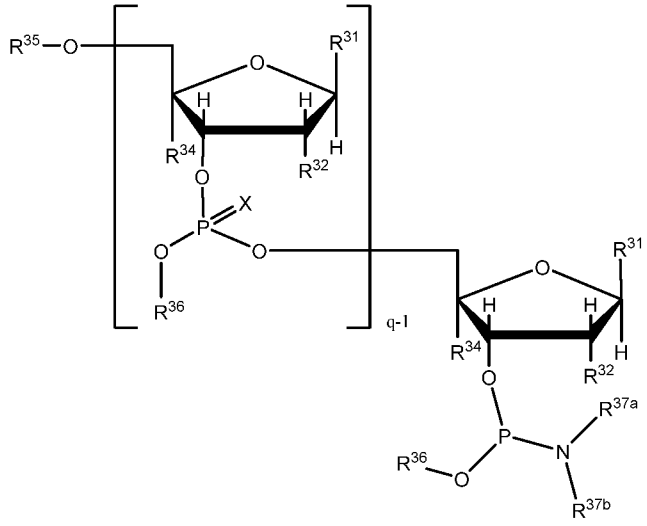
or a salt thereof, comprising the steps of:

a) coupling a nucleotide of formula (V-1):



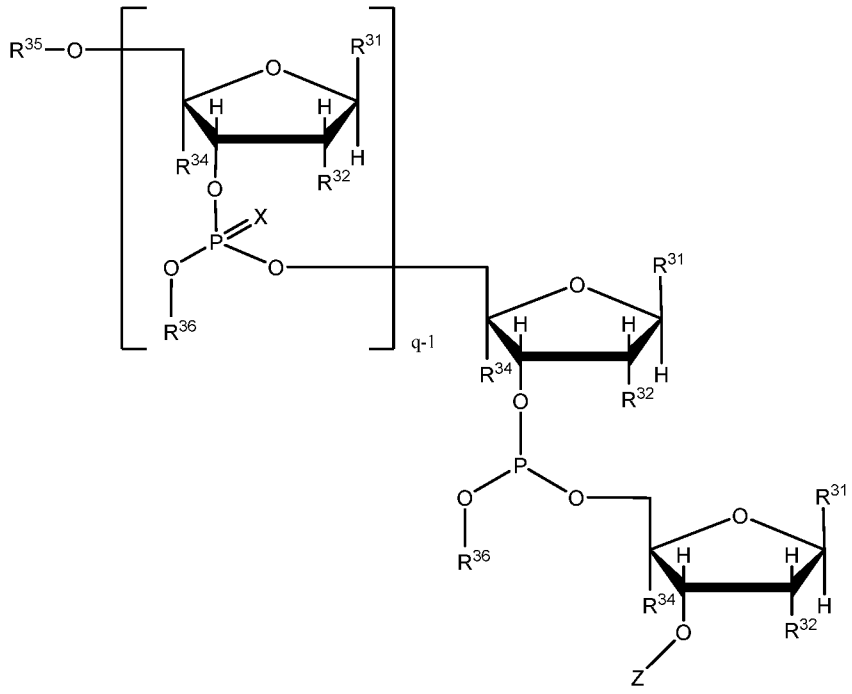
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2):



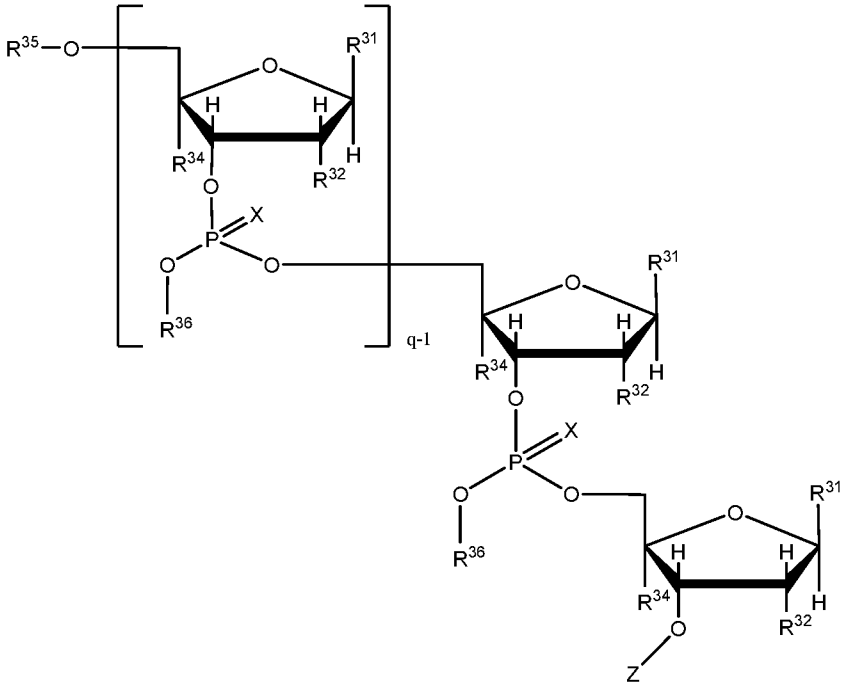
(V-2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3),



(V-3) or a salt thereof; and

b) sulfurizing or oxidizing the oligonucleotide of formula (V-3), or a salt thereof, to form an oligonucleotide of formula (V):



(V) or a salt thereof;

wherein:

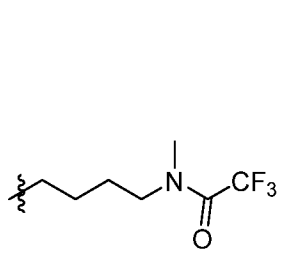
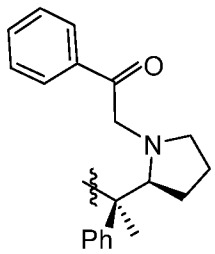
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

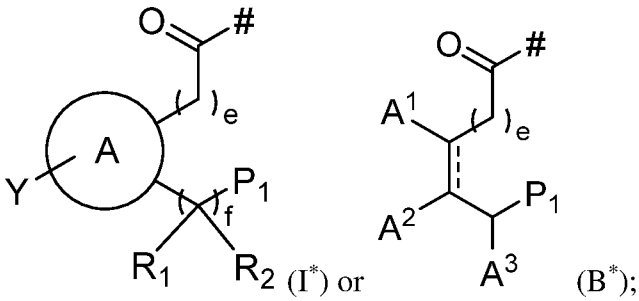
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,



wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

═══ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} is independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurance, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

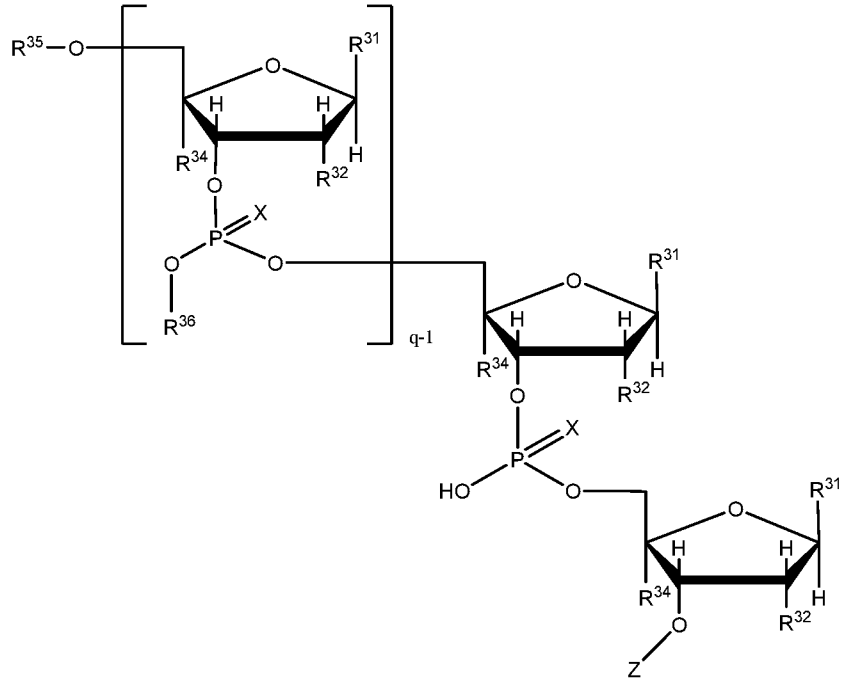
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

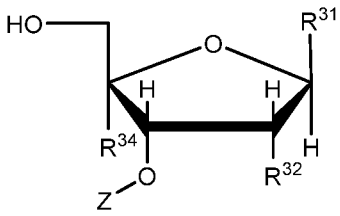
[0128] In a sixty-fourth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V*),



(V*),

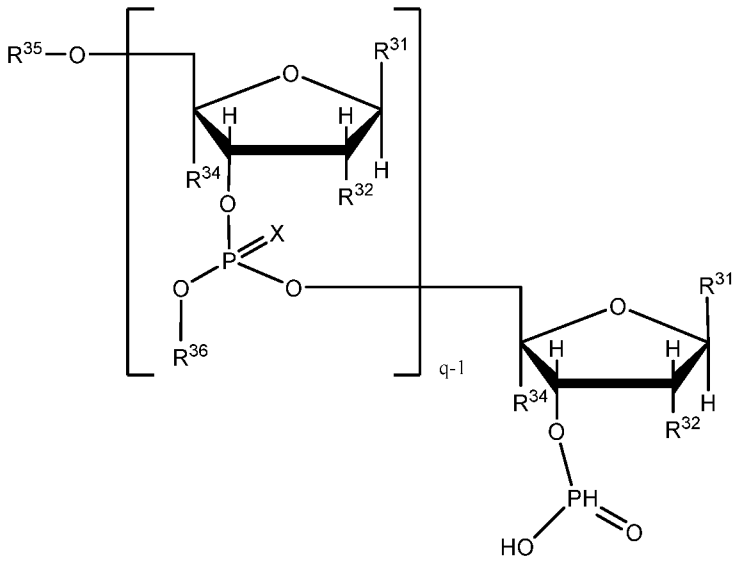
or a salt thereof, comprising the steps of:

a) coupling a nucleotide of formula (V-1):



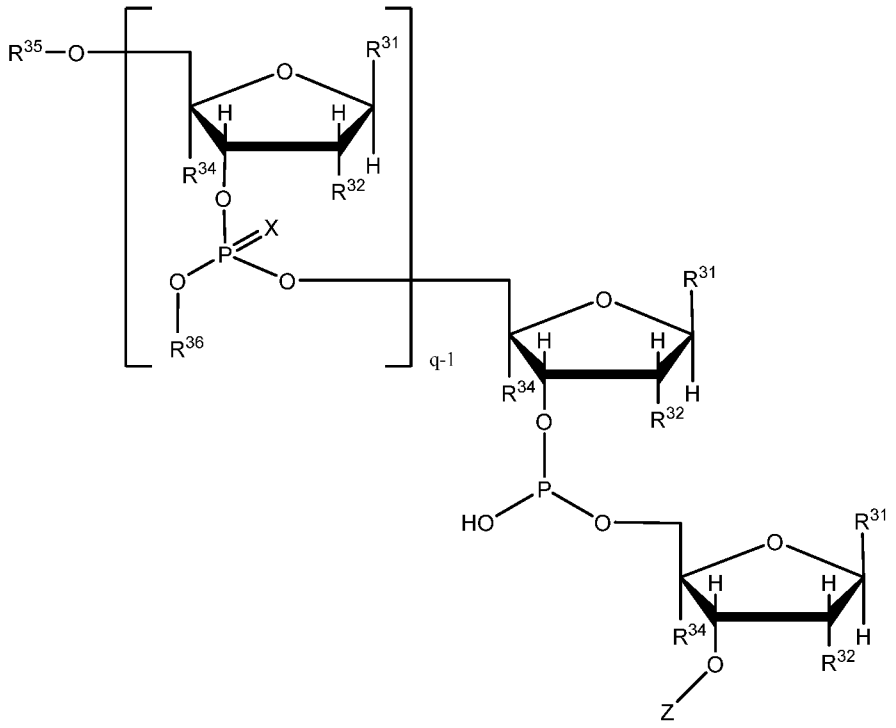
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2'):



(V-2'), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3'),



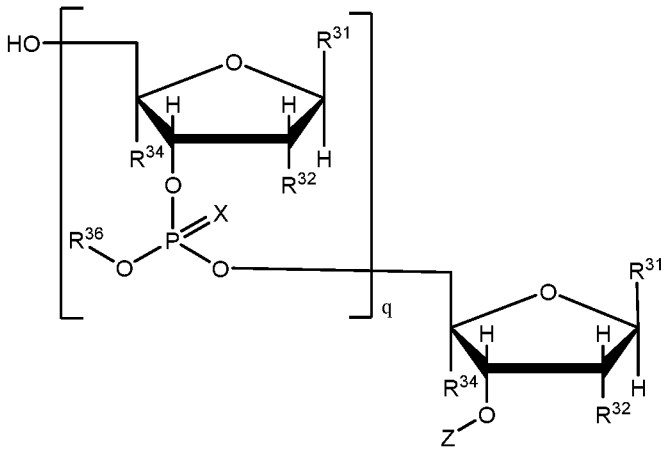
(V-3') or a salt thereof;

and

b) sulfurizing or oxidizing the oligonucleotide of formula (V-3'), or a salt thereof, to form the oligonucleotide of formula (V*) or a salt thereof; wherein R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a}, R^{37b}, q, X and Z are as described above for formula (V) in the sixty-third embodiment.

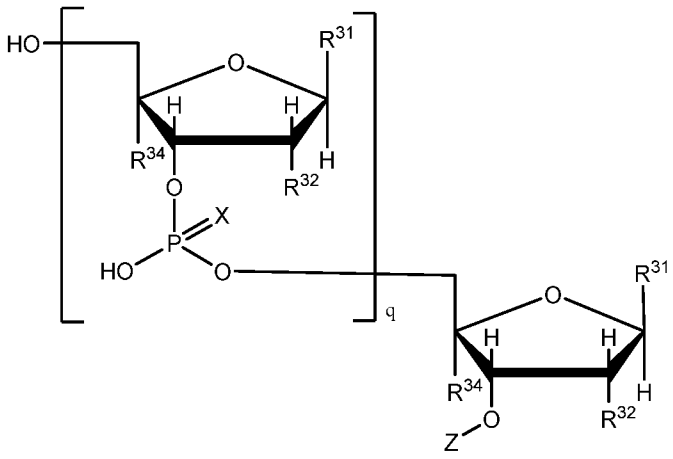
[0129] In a sixty-fifth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V) or (V*) or a salt thereof described in the sixty-third or sixty-fourth embodiment, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

[0130] In a sixty-sixth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V) or a salt thereof described in the fifty-fourth or the sixty-third embodiments, further comprising deprotecting the fragment of formula (V) to form deprotected fragment of formula (VH):



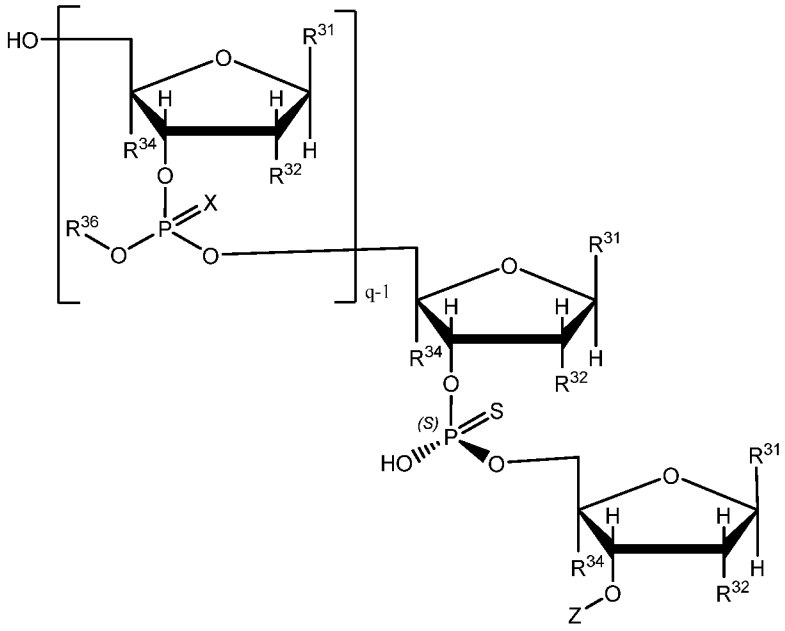
(VH), or a salt thereof.

[0131] In a sixty-seventh embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V') or a salt thereof described in the fifty-fifth embodiment, further comprising deprotecting the fragment of formula (V') to form deprotected fragment of formula (VH'):

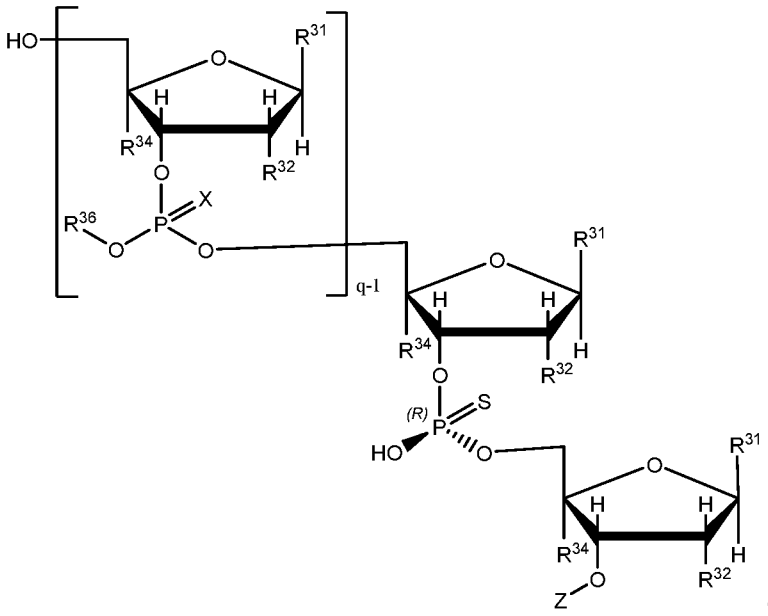


(VH'), or a salt thereof.

[0132] In a sixty-eighth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2), or a salt thereof described in the fifty-sixth or the fifty-seventh embodiment, further comprising deprotecting the fragment of formula (V-C1) or (V-C2) to form a deprotected fragment of formula (V-C3) or (V-C4):

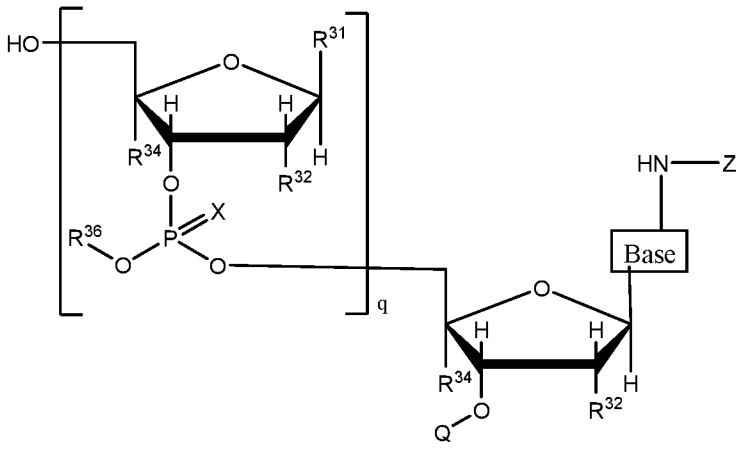


(V-C3), or a salt thereof, or



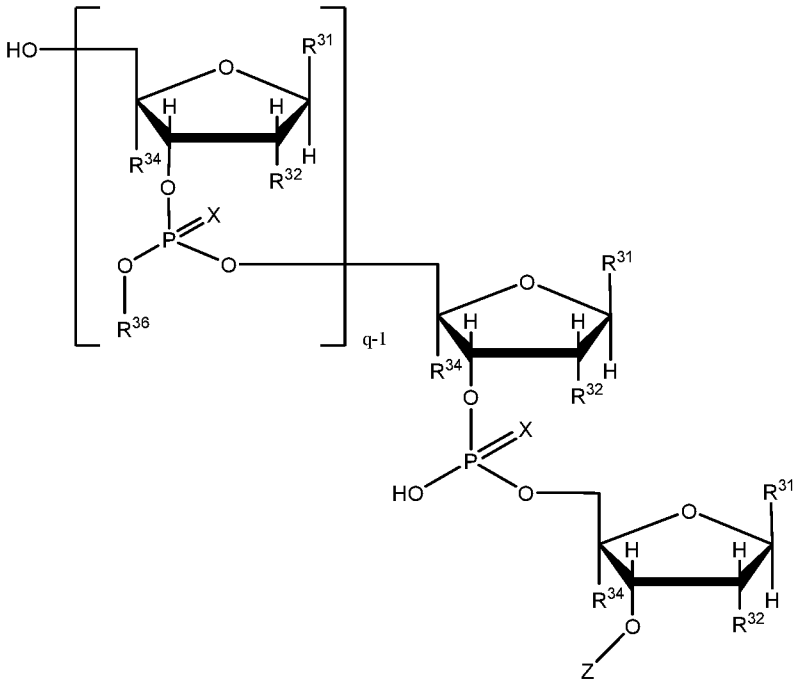
(V-C4), or a salt thereof.

[0133] In a sixty-ninth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (VBZ), or a salt thereof described in the fifty-eighth embodiment, further comprising deprotecting the fragment of formula (VBZ) to form deprotected fragment of formula (VBZ-6):



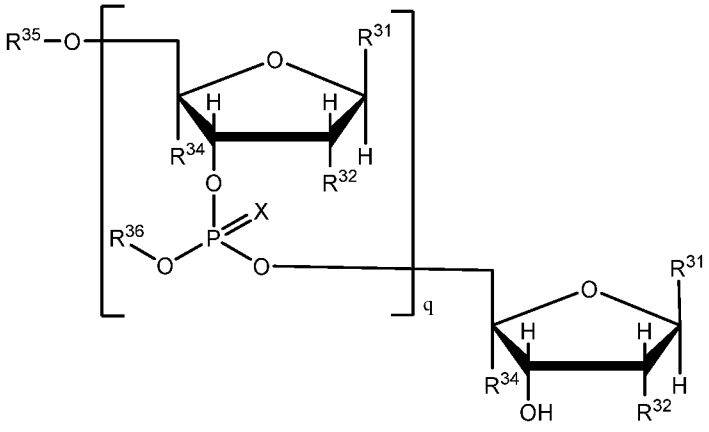
(VBZ-6), or a salt thereof.

[0134] In a seventieth embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V*), or a salt thereof described in the sixty-fourth embodiment, further comprising deprotecting the fragment of formula (V*) to form a deprotected fragment of formula (V*-1):

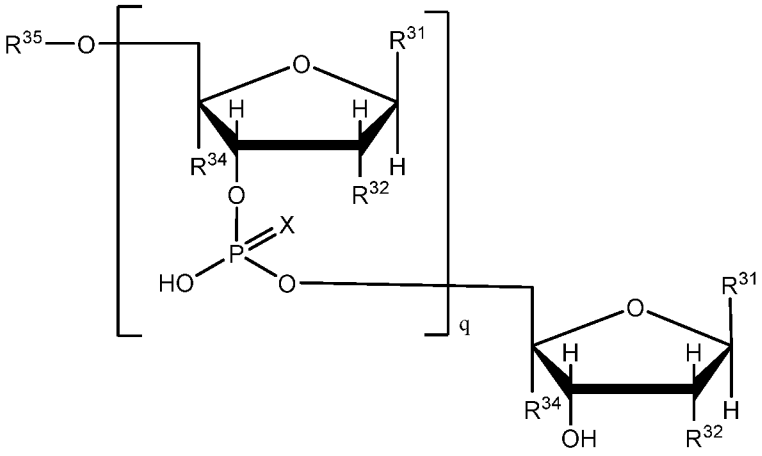


(V*-1), or a salt thereof.

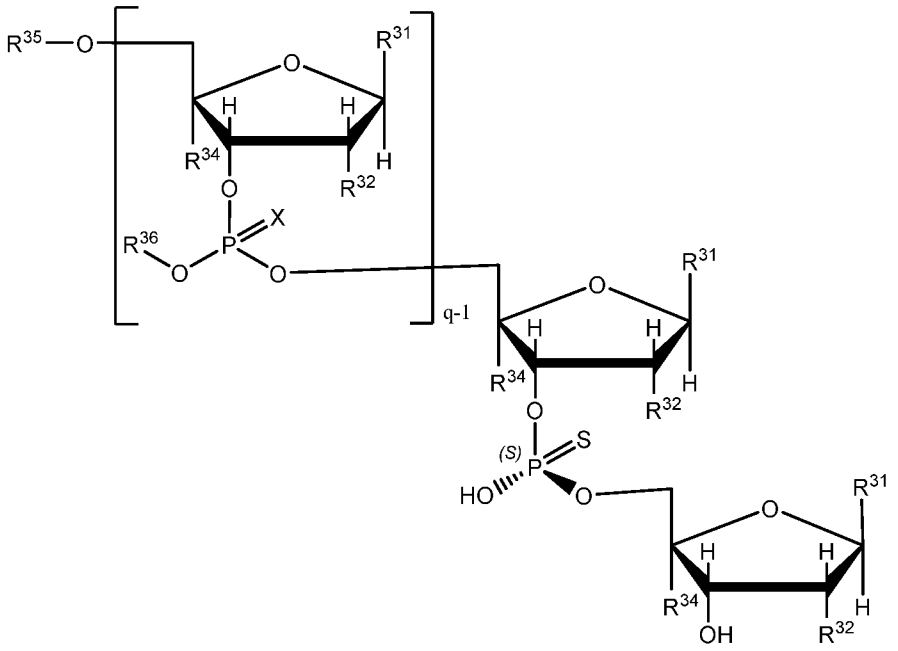
[0135] In a seventy-first embodiment, the present disclosure provides a process for preparing an oligonucleotide fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*), or a salt thereof described in the fifty-fourth through sixty-fourth embodiment, further comprising desilylation of the fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) to form the fragment of formula (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), or (V*-2), respectively:



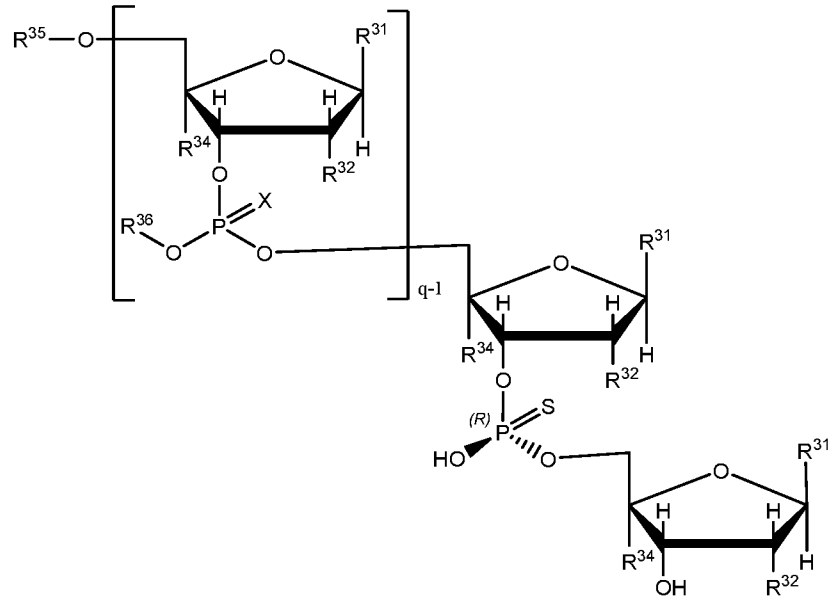
(VJ), or a salt thereof,



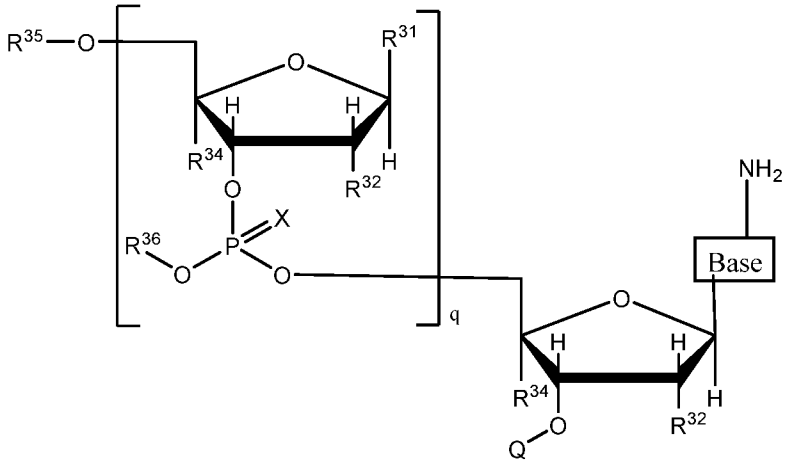
(VJ'), or a salt thereof,



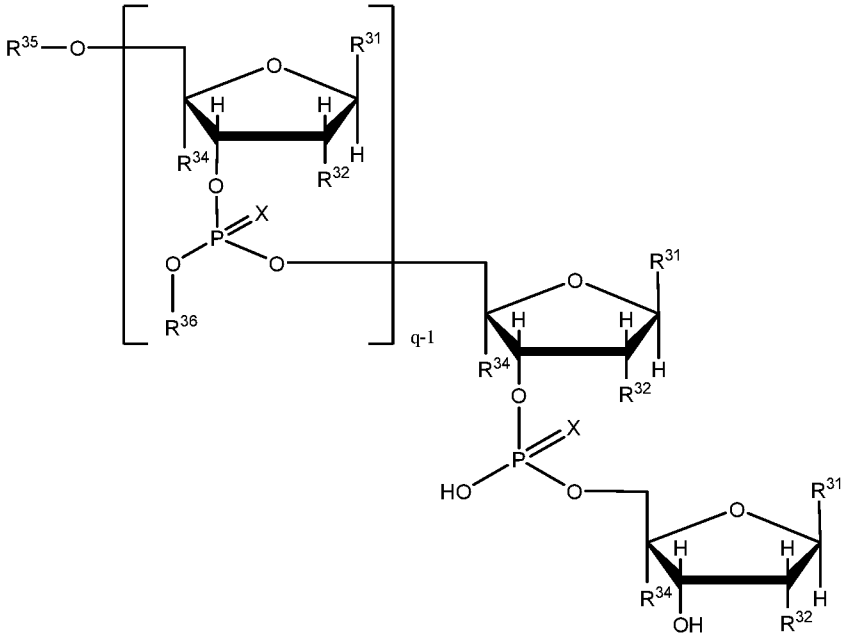
(V-C5), or a salt thereof,



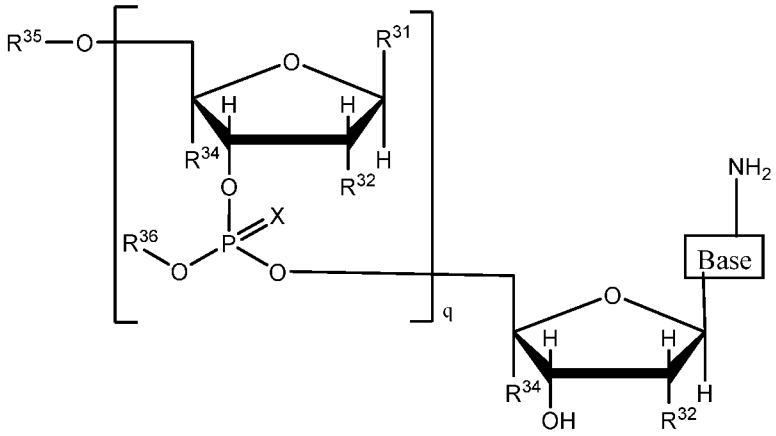
(V-C6), or a salt thereof,



(VBZ-7), or a salt thereof, or



(V*-2), or a salt thereof. In one embodiment, when Q and P_1 in formula (VBZ) are the same, the desilylation reaction forms a compound of formula (VBZ-7'):



(VBZ-7').

[0136] In a seventy-second embodiment, the present disclosure provides a process for preparing the fragment of formula (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), or (V*-2) described in any one of the seventy-first embodiment, wherein the desilylation reaction is carried out by reacting the compound of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) with HF in the presence of a base.

[0137] In a seventy-third embodiment, the present disclosure provides a process described in the seventy-second embodiment, wherein the base is imidazole or pyridine, wherein the imidazole or pyridine are optionally substituted. In certain embodiments, the pyridine and/or imidazole is each independently substituted with one to three substituents selected from halogen, C_{1-6}alkyl, C_{1-6}alkoxy, -OH, and C_{1-6}haloalkyl.

[0138] In a seventy-fourth embodiment, the present disclosure provides a process described in the seventy-third embodiment, wherein the desilylation reaction is carried out by reacting

the compound of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) with HF in the presence of pyridine and imidazole.

[0139] In a seventy-fifth embodiment, the present disclosure provides a process described in the seventy-fourth embodiment, wherein the molar ratio of imidazole to HF is in the range of 0.5:1 to 10:1.

[0140] In a seventy-sixth embodiment, the present disclosure provides a process described in the seventy-fifth embodiment, wherein the molar ratio of imidazole to HF is in the range of 1.1:1 to 5:1.

[0141] In a seventy-seventh embodiment, the present disclosure provides a process described in the seventy-sixth embodiment, wherein the molar ratio of imidazole to HF is in the range of 2:1.

[0142] In a seventy-eighth embodiment, the present disclosure provides a process described in the seventy-fourth through seventy-seventh embodiments, wherein the molar ratio of pyridine to HF is in the range of 100:1 to 1:1.

[0143] In a seventy-ninth embodiment, the present disclosure provides a process described in the seventy-fourth through seventy-seventh embodiments, wherein the molar ratio of pyridine to HF is in the range of 1:1.

[0144] In an eightieth embodiment, the present disclosure provides a process described in any one of the fifty-fourth through seventy-first embodiments, wherein the fragment for formula (V), (V'), (V-C1), (V-C2), (VBZ), (V*), (VH), (VH'), (V-C3), (V-C4), (VBZ-6), (V*-1), (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), (VBZ-7') or (V*-2) is not purified by chromatography.

[0145] In an eighty-first embodiment, the present disclosure provides a process described in the eightieth embodiments, wherein the fragment for formula (V), (V'), (V-C1), (V-C2), (VBZ), (V*), (VH), (VH'), (V-C3), (V-C4), (VBZ-6), (V*-1), (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), (VBZ-7') or (V*-2) is purified by selective precipitation and/or extraction.

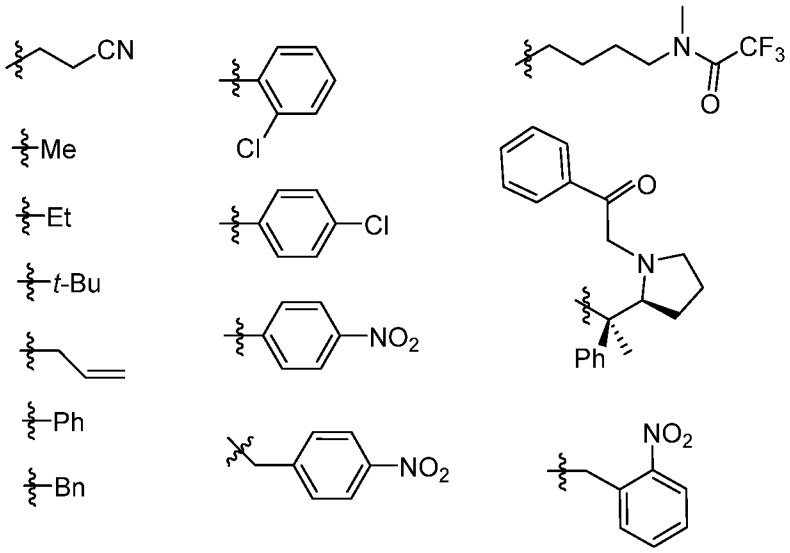
[0146] In an eighty-second embodiment, the present disclosure provides a process described in any one of the fifty-fourth through eighty-first embodiments, wherein q is 2 to 5.

[0147] In an eighty-third embodiment, the present disclosure provides a process described in any one the eighty-second embodiment, wherein q is 4.

[0148] In certain embodiments, for the process described in the third aspect or any embodiments describe therein (*e.g.*, the sixty-fifth to seventy-fifth embodiments), the variables R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, and/or Z are described in the second aspect or any one of embodiments described therein (*e.g.*, the twenty-seventh to thirty-third embodiments).

[0149] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the 5'-OH deprotection step is a detritylation method for removing a 5'-trityl group. It is discovered that when the detritylation reaction is carried out under anhydrous or substantially anhydrous conditions, significant reduction of side reactions (*e.g.*, deamination of nucleobase cytosine or 5-methylcytosine or their derivatives commonly used for oligonucleotide synthesis) can be achieved. The present detritylation method also involves the addition of a cation scavenger to facilitate the completion of the reaction. As a result, the product with high purity can be obtained without the need of chromatography (*e.g.*, column chromatography). Water level of the detritylation reaction can be controlled by the use of drying agent (*e.g.*, molecular sieves), azeoptropic distillation or other suitable methods known in the art. Alternatively, solvents, acids, and other reagents used in the detritylation reaction, substrates to be subjected to detritylation reaction, and the reaction vessels can be dried to meet the residual water levels prior to use for the detritylation reaction.

[0150] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), R^{36} is one of the following:



See *Nat Biotechnol.* 2017 Sep;35(9):845-851; *J. Org. Chem.* 1999, 64, 7515-7522; *Biopolymers (Peptide Science)*, 2001, 60, 3, each of which is incorporated herein by reference.

[0151] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the 5'-OH deprotection (or detritylation) reaction is carried out in the presence of a drying agent. Any

suitable drying agents can be used in the deprotection reaction. In some embodiments, the drying agent is selected from calcium chloride, potassium chloride, sodium sulfate, calcium sulfate, magnesium sulfate and molecular sieves.

[0152] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the drying agent is molecular sieves.

[0153] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the size of molecular sieves is 3Å or 4Å. In one embodiment, the size of molecular sieves is 3Å.

[0154] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the anhydrous or substantially anhydrous solution for the deprotection reaction is obtained by removing water using azeotropic distillation prior to the deprotection reaction.

[0155] Alternatively, solvents, acids or acid solutions, and other reagents or solutions comprising the reagents to be used in the detritylation reaction, substrates or substrate solutions to be subjected to detritylation reaction, and the reaction vessels can be dried individually or combined prior to the detritylation reaction.

[0156] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the deprotection reaction is carried out in the presence of a scavenger selected from a cation scavenger comprising a –SH group, a silane scanveger (such as $HSiPh_3$, $HSiBu_3$, triisopropylsilane etc.), siloxane, polystyrene, furan, pyrrole and indole.

[0157] In certain embodiments, the deprotection reaction is carried out in the presence of a scavenger selected from 1-dodecanethiol, cyclohexanethiol, 1-octanethiol, triisopropylsilane, indole, 2,3-dimethylfuran, diphenylsilane, 2-mercaptoimidazole, diphenylmethylsilane, phenylsilane, 5-methoxyindole, methylphenylsilane, chlorodimethylsilane, 1,1,3,3-tetramethyldisiloxane, 1-thioglycerol, triphenylsilane, *tert*-butyldimethylsilane, butylsilane, methyldiethoxysilane, 1,1,3,3,5,5-hexamethyltrisiloxane, hexylsilane, (mercaptomethyl)polystyrene, or dimethylphenylsilane.

[0158] In certain embodiments, the cation scavenger is a compound of formula RSH, wherein R is an alkyl, a cycloalkyl, a heterocycloalkyl, an aryl or a heteroaryl group, each of which is optionally substituted.

[0159] In certain embodiments, the cation scavenger is $CH_3(CH_2)_5SH$, $CH_3(CH_2)_{11}SH$, cyclohexanethiol (CySH), or $CH_3CH_2OC(=O)CH_2CH_2SH$.

[0160] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), R^{35} is a 4,4'-dimethoxytrityl (DMT) group.

[0161] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the deprotection reaction is carried out by reacting the compound of formula (VA) with a detritylation reagent. Any suitable detritylation reagent can be used.

[0162] In certain embodiments, the detritylation reagent is a strong organic acid.

[0163] In certain embodiments, the detritylation reagent is selected from CF_3COOH, CCl_3COOH, $CHCl_2COOH$, $CH_2ClCOOH$, H_3PO_4, methanesulfonic acid (MSA), benzenesulfonic acid (BSA), $CClF_2COOH$, CHF_2COOH, $PhSO_2H$ (phenylsulfinic acid) etc. In a preferred embodiment, the detritylation reagent is $CH_2ClCOOH$. In another specific embodiment, the detritylation reagent is CF_3COOH. In yet another specific embodiment, the detritylation reagent is $CHCl_2COOH$.

[0164] In certain embodiments, the detritylation reagent is citric acid. In certain embodiments, the detritylation reagent is saturated citric acid solution.

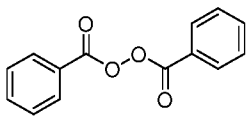
[0165] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the coupling reaction of step 2) can be carried out in the presence of an activator described herein (*e.g.* activators described in the thirty-ninth embodiment). In certain embodiments, the activator is 4,5-dicyanoimidazole (DCI) or 5-ethylthio-1H-tetrazole (ETT).

[0166] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the sulfurization reaction of step 3) is carried out using a sulfurizing agent, such as 3-amino-1,2,4-dithiazole-5-thione (xanthane hydride or ADTT), 3-(N,N-dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole (DDTT), phenylacetyl disulfide (PADS), 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage Reagent), or phenyl-3H-1,2,4-dithiazol-3-one (POS). In a specific embodimet, the sulfurizing agent is DDTT. In a specific embodiment, the sulfurizing agent is xanthane hydride. In certain embodiments, the sulfurization reaction is carried out in the presence of a base as described herein. In certain embodiments, the base is pyridine or imidazole. In certain embodiments, the sulfurization reaction of step 3) is carried out in the presence of DDTT and 4,5-dicyanoimidazole (DCI).

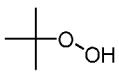
[0167] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the

oxidation reaction of step 3) is carried out by using standard oxidizing agents known in the literature. Exemplary oxidizing agent include, but are not limited to, tert-butylhydroperoxide (t-BuOOH), (1S)-(+)-(10-camphorsulfonyl)oxaziridine (CSO), (1R)-(−)-(10-camphorsulfonyl)oxaziridine (enantiomer of CSO), I_2, and iodine-pyridine-water oxidizer solution. In a specific embodimet, the oxidizing agent is t-BuOOH.

[0168] In certain embodiments, for the process described in the third aspect or any one of embodiments describe therein (*e.g.*, the fifty-fourth to eighty-third embodiments), the coupling/oxidation/detritylation steps are carried out in a one pot reaction. In certain embodiments, the oxidation reagents in the one pot reaction is BPO or tBuOOH:



Benzoyl peroxide (BPO) or



tBuOOH .

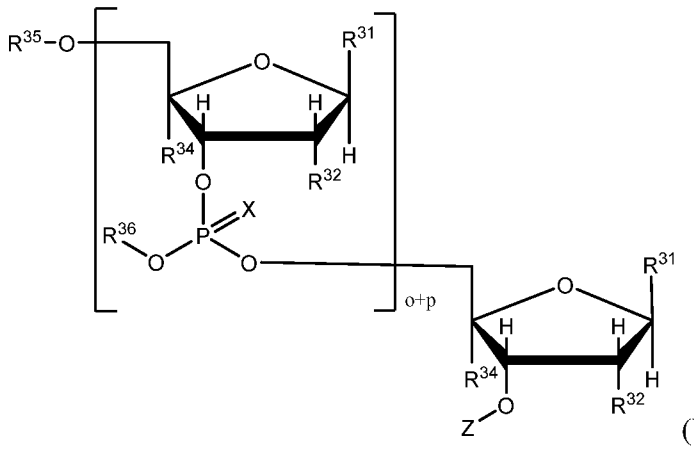
4. Process to Prepare Target Oligonucleotides

[0169] In a fourth aspect, the present disclosure describes a process for preparing target oligonucleotides, wherein the target oligonucleotide is assembled in the direction of the 3'-terminal to the 5'-terminal (3'-5' direction). It has been demonstrated that the process of the present disclosure is successfully used to synthesize target oligonucleotides in large quantities. In addition, high purity protected target oligonucleotide can be obtained by the methods of the present disclosure without chromatographic purification.

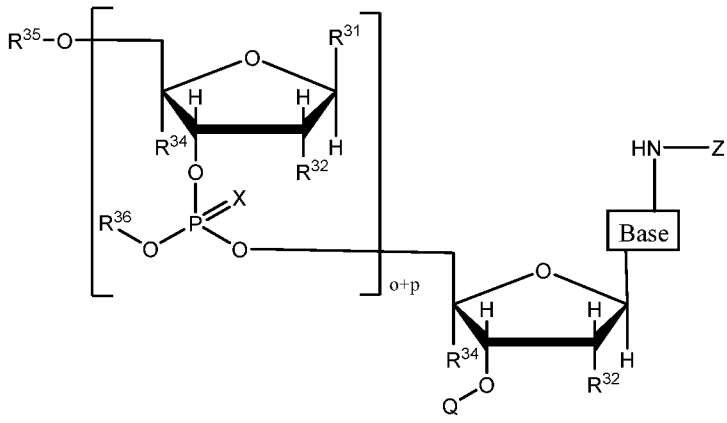
[0170] In certain embodiments, the process described herein involves step by step addition of oligonucleotide fragments in liquid (solution) phase to synthesize the target oligonucleotide. For example, 5-mer and 4-mer fragments are coupled first to synthesize a 9-mer fragment which is further reacted with another 5-mer fragment to synthesize 14-mer oligonucleotide. The 14-mer oligonucleotide can be further coupled with another fragment until the desired length of the target oligonucleotide is obtained. In certain embodiments, a 5-mer fragment having a 3'-hydrophobic hydroxyl protecting group (3'-LHPG) (3'-end fragment) or an amino protecting group at a nucleobase (when the nucleobase comprises a NH_2 group. It can be referenced herein as the "nucleobase LHPG fragment") is first coupled with 5-mer fragment to form a 10-mer fragment having 3'-LHPG group or nucleobase LHPG group, which is then further reacted with a 4-mer fragment to form a 14-mer fragment, which is in turn coupled with another 4-mer fragment to form the target 18-mer oligonucleotide. In certain embodiments, the 3'-end fragment having n nucleotides (*e.g.* 5-mer fragment) is

synthesized by coupling a single nucleotide having the 3'-LHPG group with a fragment having n-1 nucleotides (*e.g.*, 4-mer fragment). In certain embodiments, the nucleobase LHPG fragment having n nucleotides (*e.g.* 5-mer fragment) is synthesized by coupling a single nucleotide having the LHPG group at the nucleobase with a fragment having n-1 nucleotides (*e.g.*, 4-mer fragment).

[0171] In a eighty-fourth embodiment, the present disclosure provides a process for preparing an oligonucleotide of formula (VI) or (VI-1),



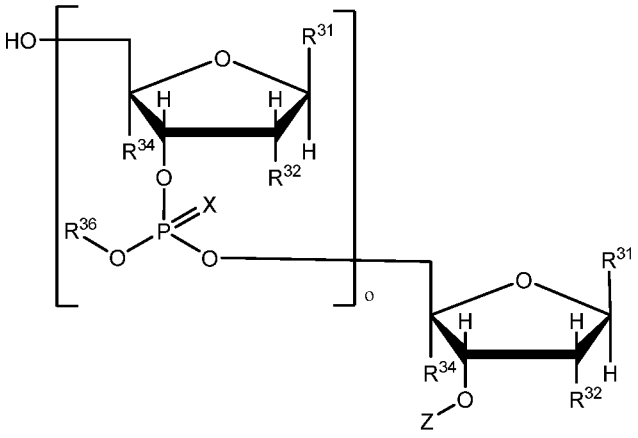
(VI),



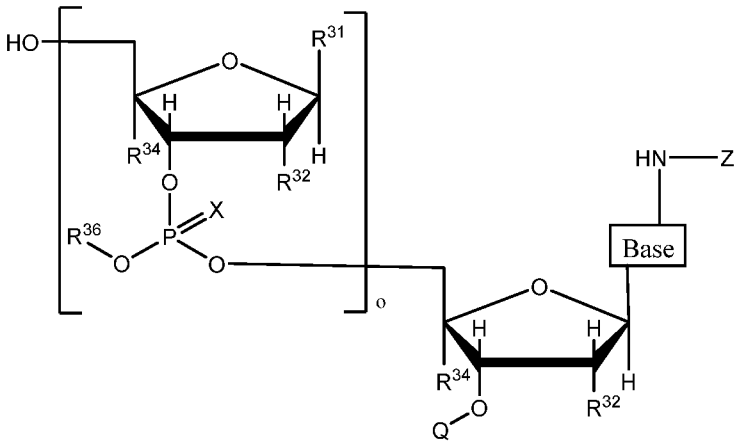
(VI-1)

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):



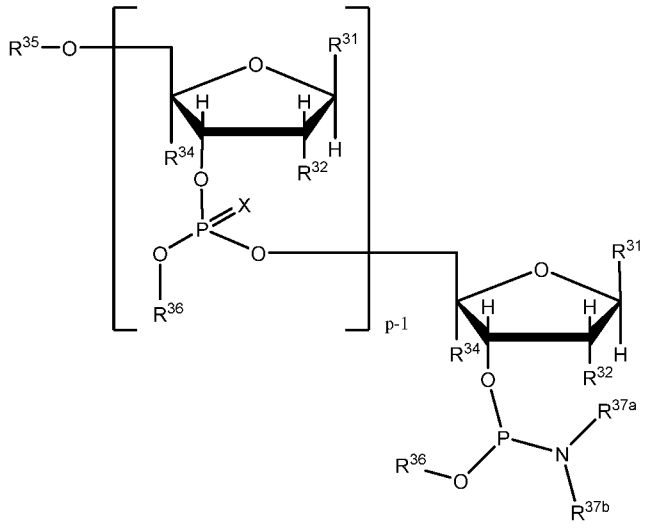
(F1),



(F1-1),

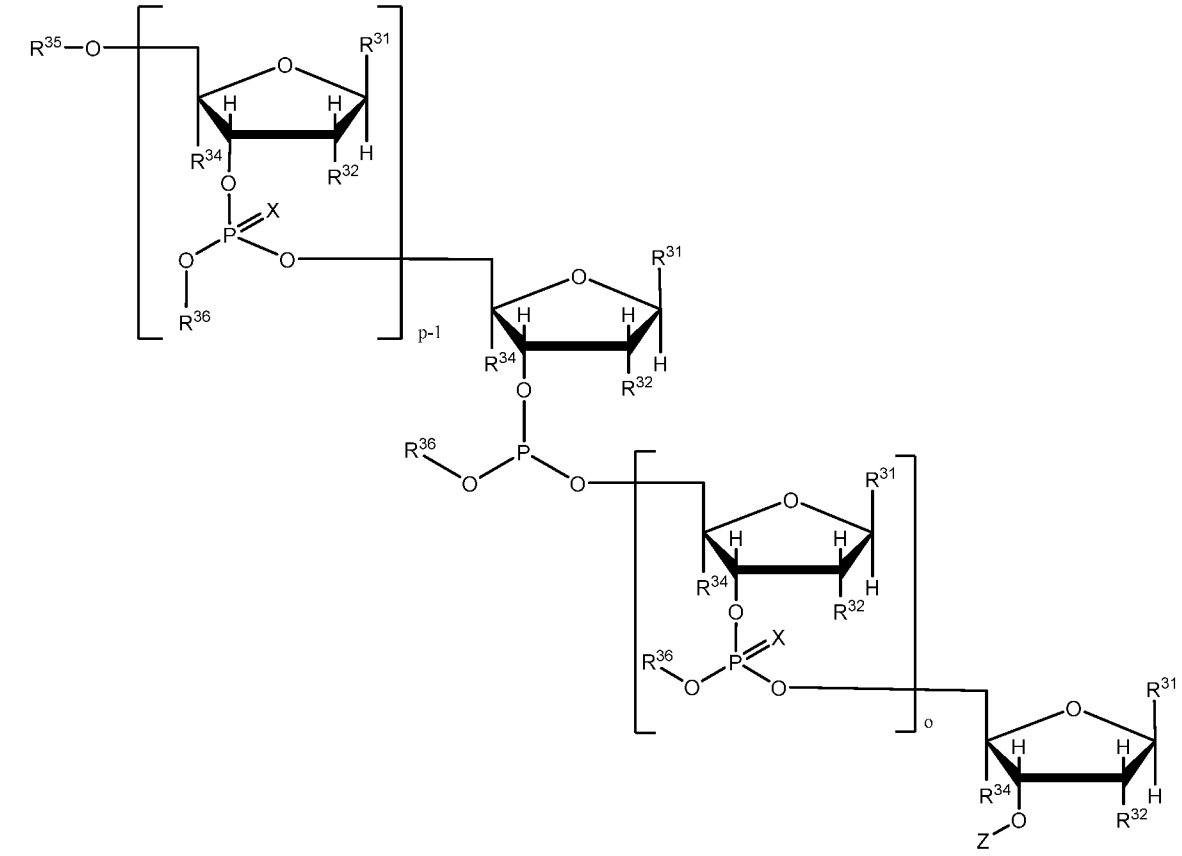
or a salt thereof,

with an oligonucleotide fragment of formula (F2):

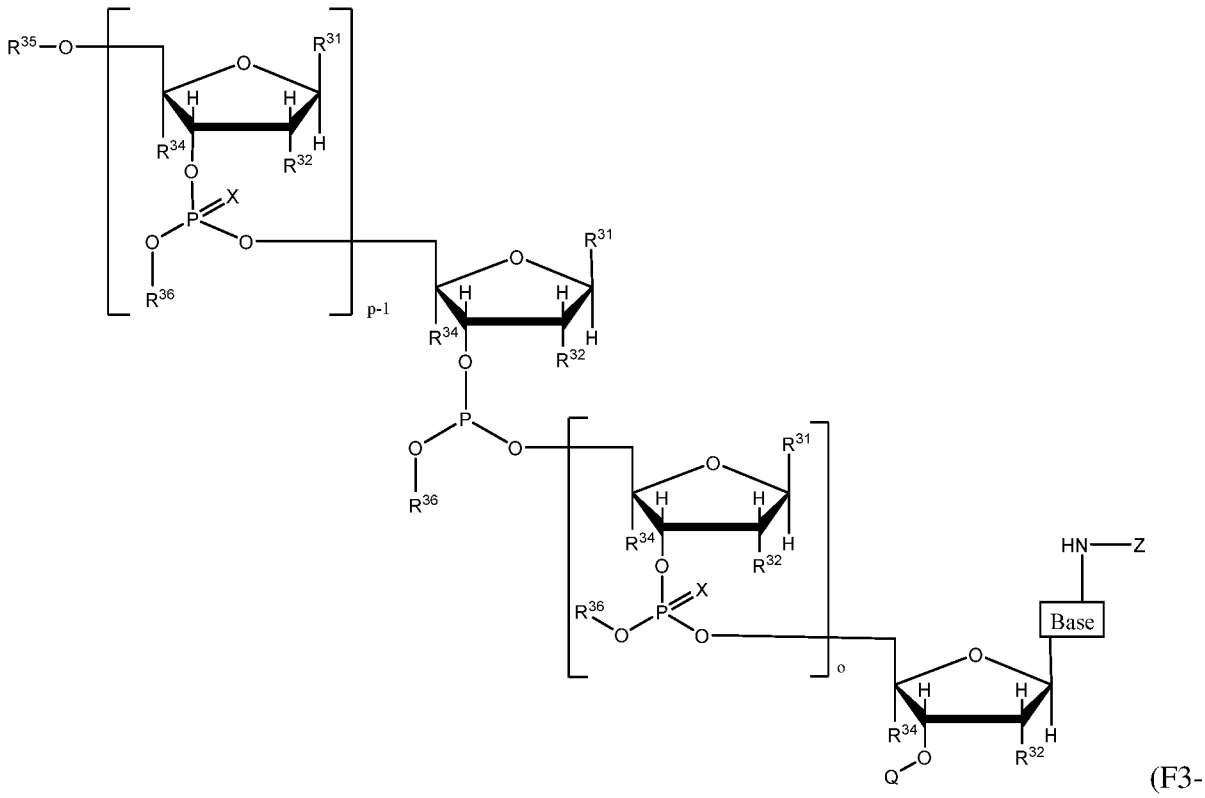


(F2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (F3) or (F3-1),



(F3),



(F3-1),

or a salt thereof; and

b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3) or (F3-1), or a salt thereof, to form the oligonucleotide of formula (VI) or (VI-1) or a salt thereof, wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

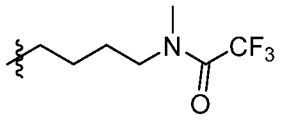
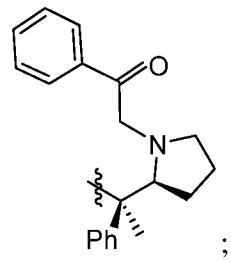
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is  , or  ;

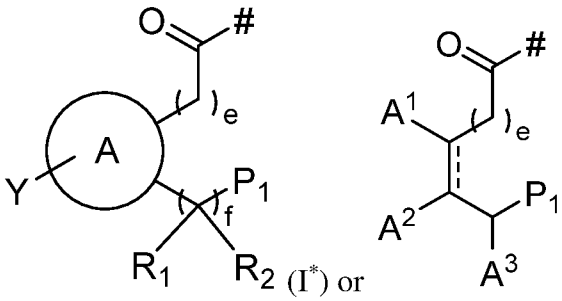
R^{37a} and R^{37b} are independently C_{1-6}alkyl;

p is an integer from 2 to 20;

o is an integer from 1 to 200;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,



(I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

≡ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

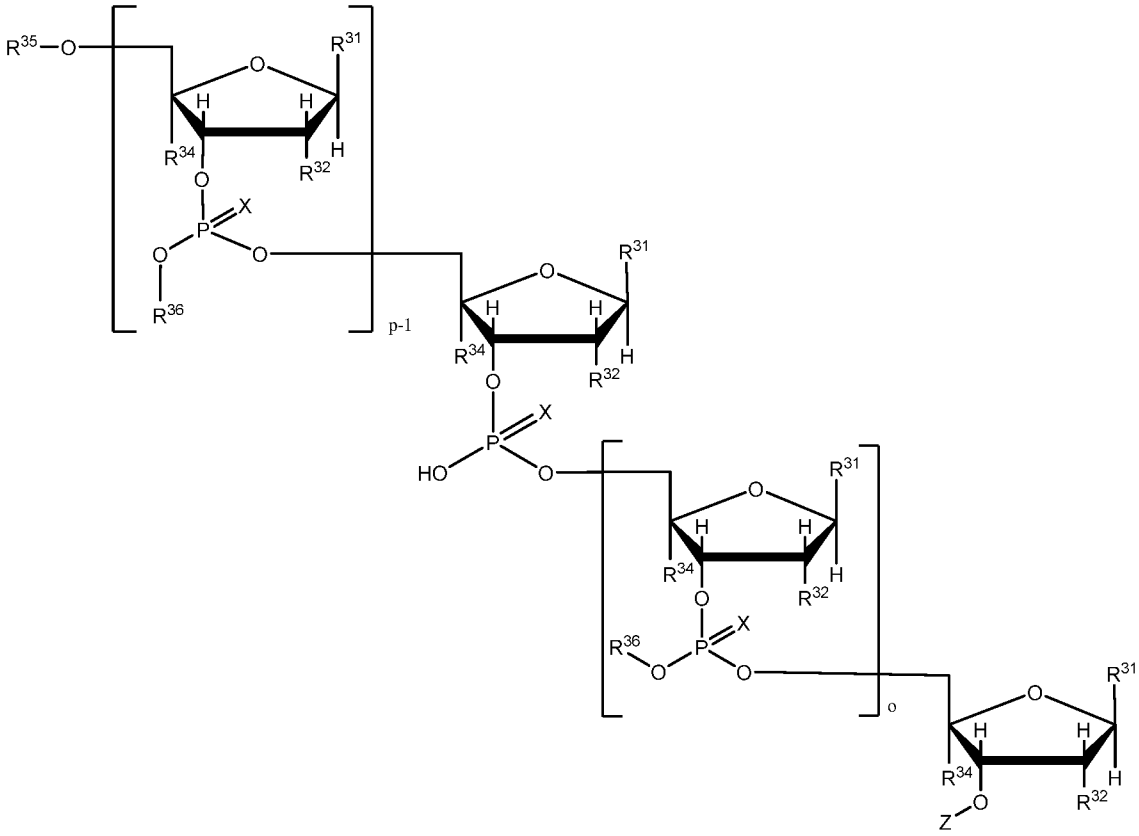
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

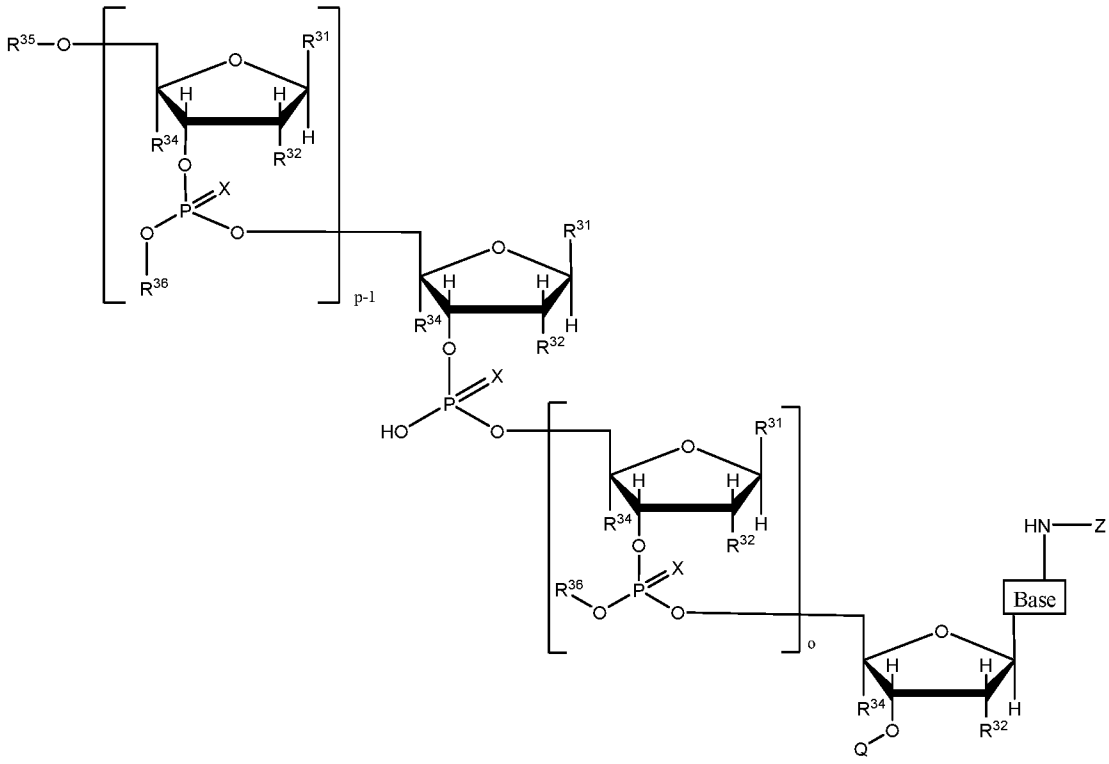
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

[0172] In a eighty-fifth embodiment, the present disclosure provides a process for preparing an oligonucleotide of formula (VI') or (VI'-1),



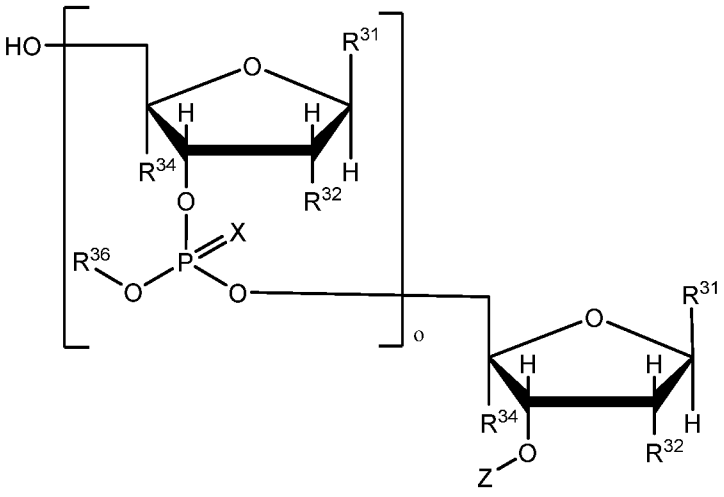
(VI'),



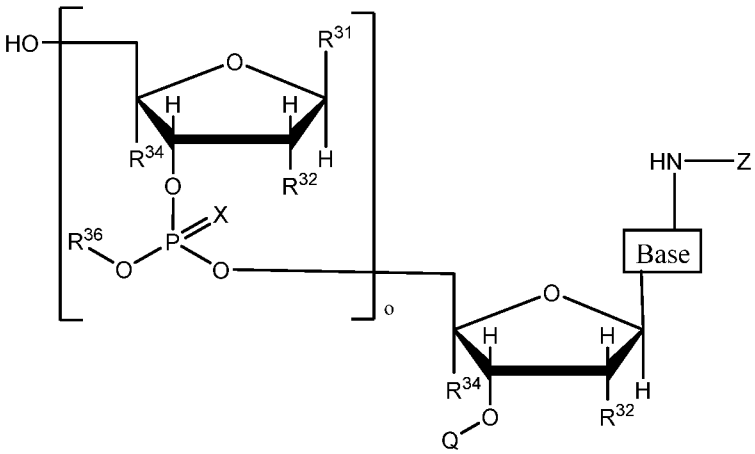
(VI'-1)

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):

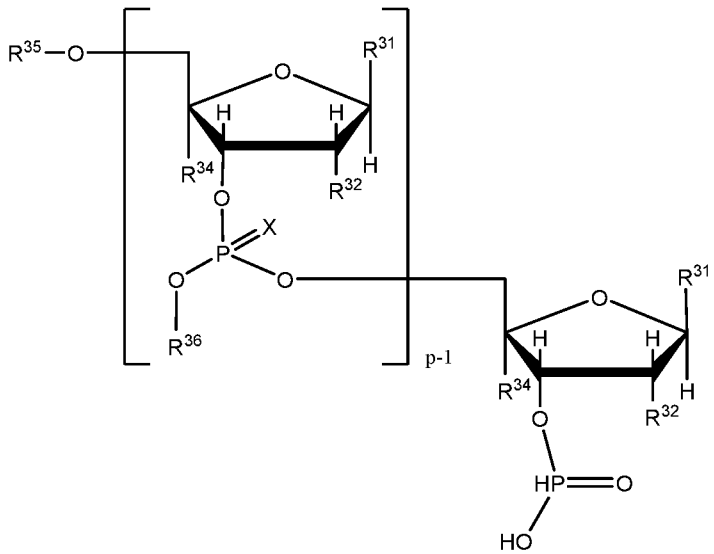


(F1),



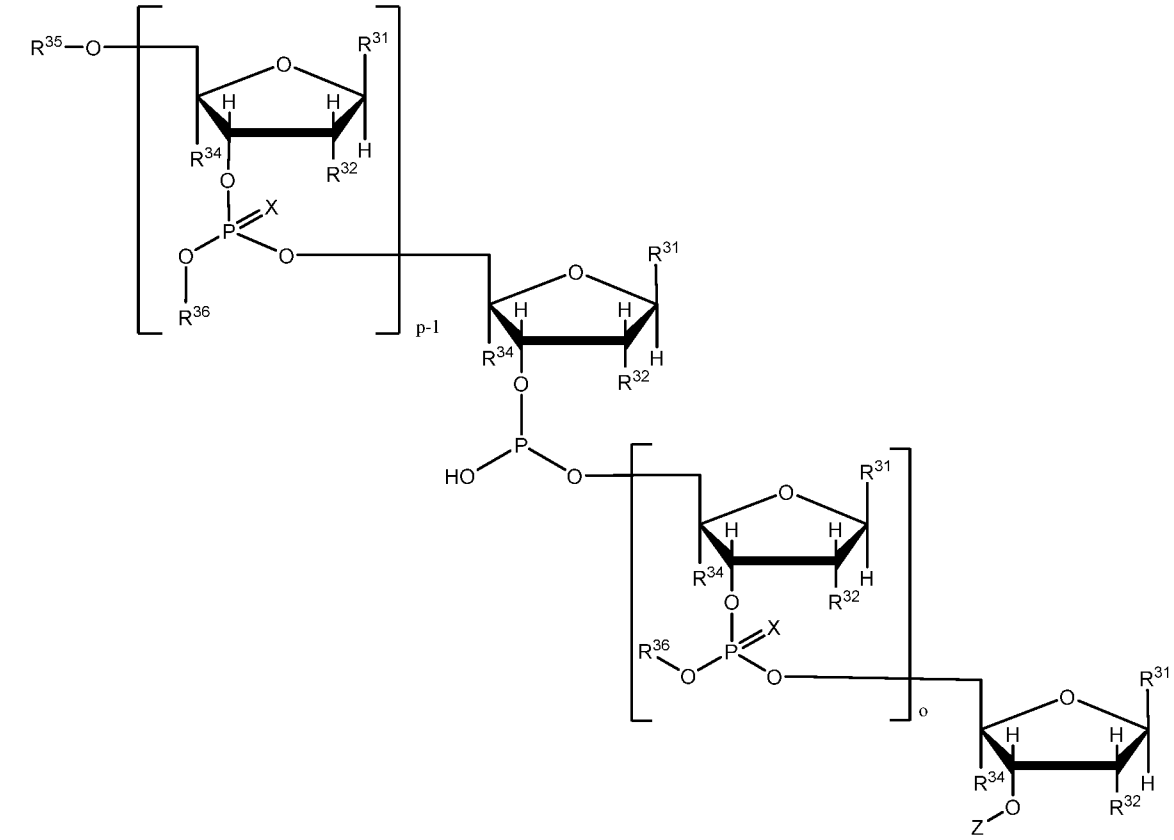
(F1-1),

or a salt thereof, with an oligonucleotide fragment of formula (F2'):

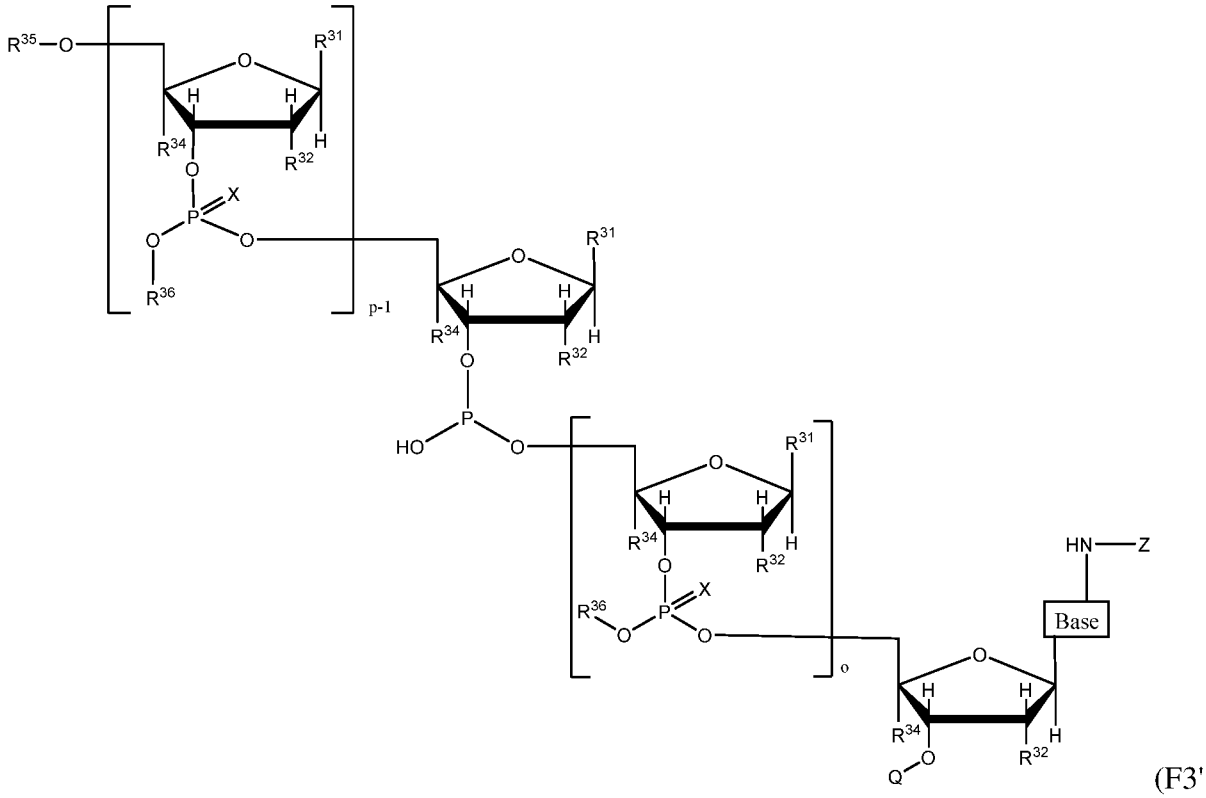


(F2'),

or a salt thereof, in a solution to form an oligonucleotide fragment of formula (F3') or (F3'-1),



(F3'),



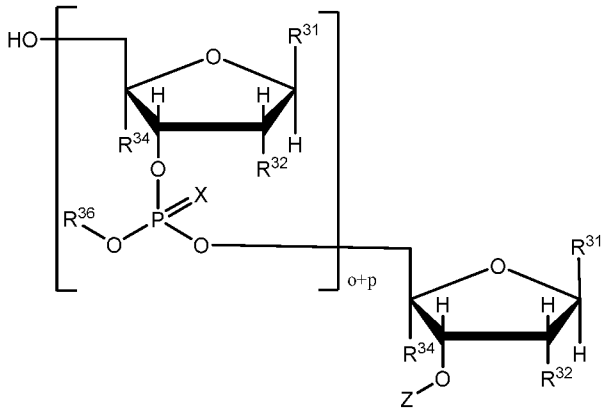
(F3'-1),

or a salt thereof; and

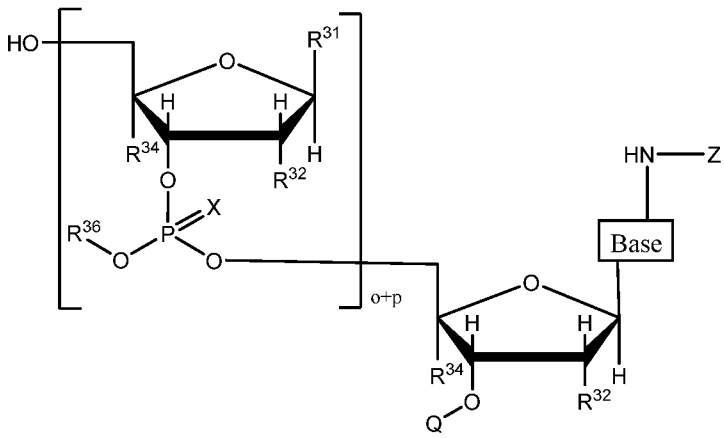
b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3') or (F3'-1), or a salt thereof, to form the oligonucleotide of formula (VI') or (VI'-1) or a salt thereof, wherein Q, Base, R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, R^{37a} and R^{37b}, p, o, X and Z are as described above for Formula (VI) or (VI-1) in the eighty-fourth embodiment.

[0173] In a eighty-sixth embodiment, the present disclosure provides a process for preparing an oligonucleotide of formula (VI), (VI'), (VI-1), or (VI'-1) described in the eighty-fourth or eighty-fifth embodiment, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

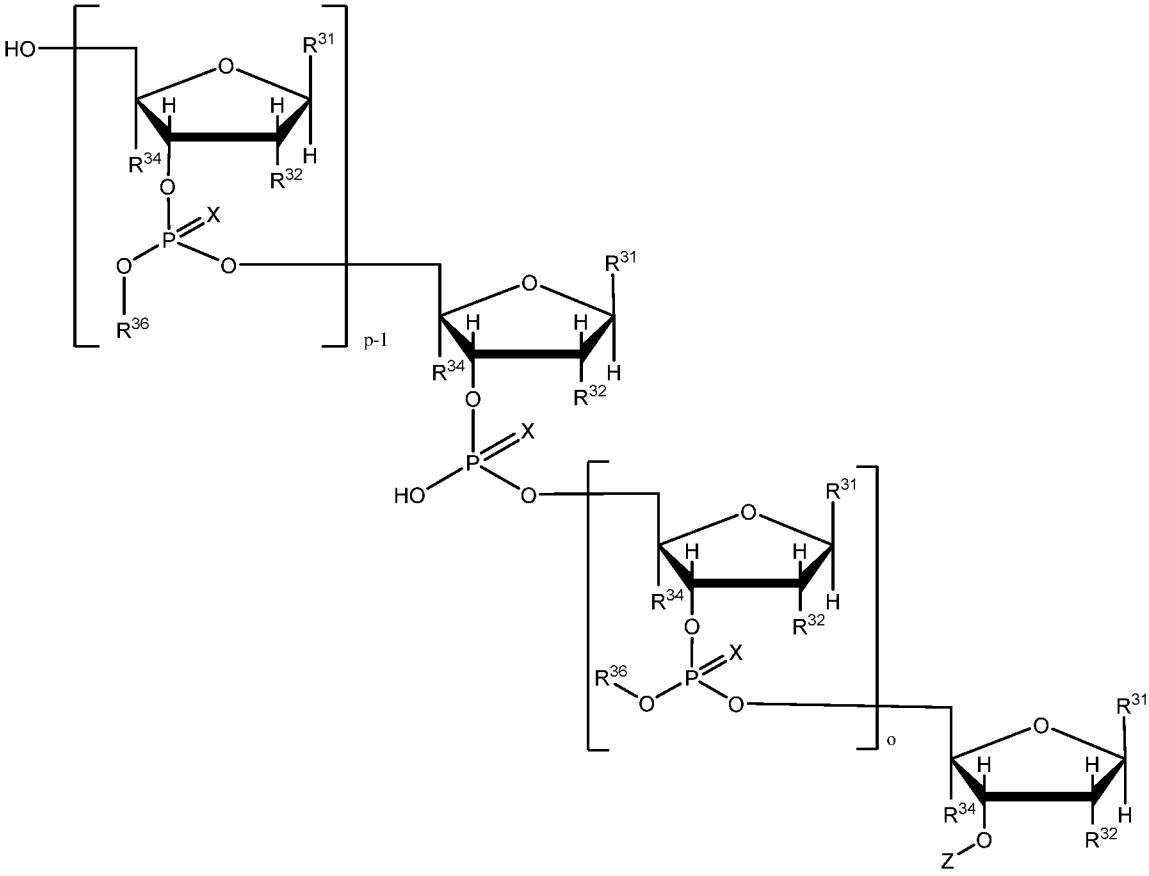
[0174] In a eighty-seventh embodiment, the present disclosure provides a process for preparing an oligonucleotide of formula (VI), (VI'), (VI-1), or (VI'-1) described in the eighty-fourth or eighty-fifth embodiment, further comprising step c) deprotecting the oligonucleotide of formula (VI), (VI'), (VI-1), or (VI'-1) to form an oligonucleotide of formula (VII), (VII-1), (VII'), or (VII'-1) :



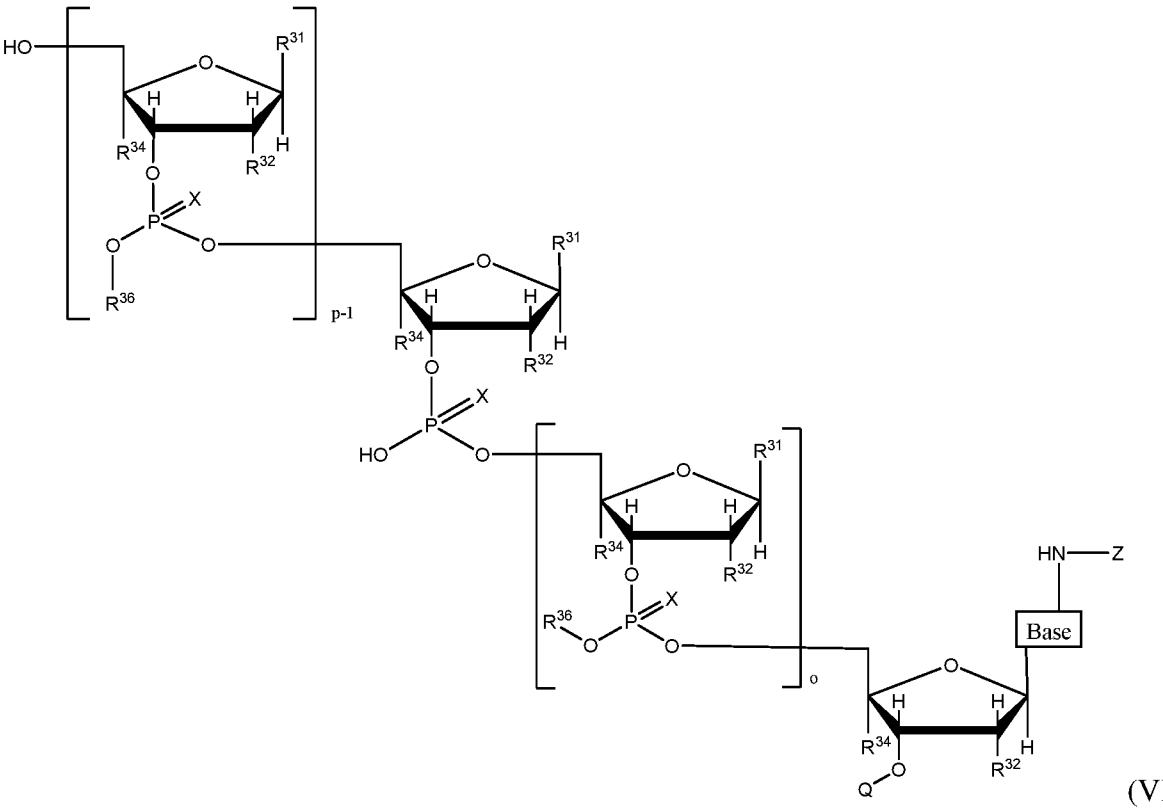
(VII),



(VII-1),



(VII'),



(VII'-1)

or a salt thereof.

[0175] In an eighty-eighth embodiment, the present disclosure provides a process for preparing an oligonucleotide of formula (VII), (VII-1), (VII'), or (VII'-1) described in the eighty-seventh embodiment, wherein starting from oligonucleotide of formula (VII), (VII-1), (VII'), or (VII'-1), the process further comprises repeating steps a), b) and c) for 1 to 10 times, followed by steps a) and b) to form the target oligonucleotide with desired length.

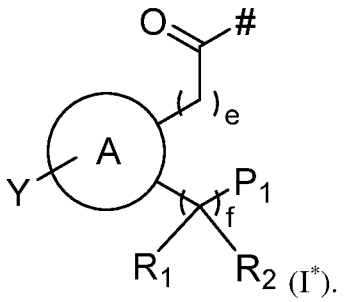
[0176] In an eighty-ninth embodiment, the present disclosure provides a process described in the eighty-eighth embodiment, wherein the process further comprises repeating steps a), b) and c) for 1 to 3 times followed by steps a) and b) to form the target oligonucleotide with desired length.

[0177] In a ninetieth embodiment, the present disclosure provides a process described in the eighty-fourth through eighty-ninth embodiments, wherein o is an integer from 2 to 20.

[0178] In a ninety-first embodiment, the present disclosure provides a process described in the ninetieth embodiment, wherein o is an integer from 2 to 5.

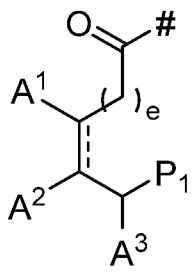
[0179] In a ninety-second embodiment, the present disclosure provides a process described in the ninety-first embodiment, wherein o is 4.

[0180] In a ninety-third embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-second embodiments), wherein Z is a group represented by Formula I*,



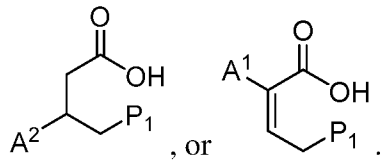
(I*).

[0181] In a ninety-fourth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-second embodiments), wherein Z is a group represented by Formula B*,



(B*).

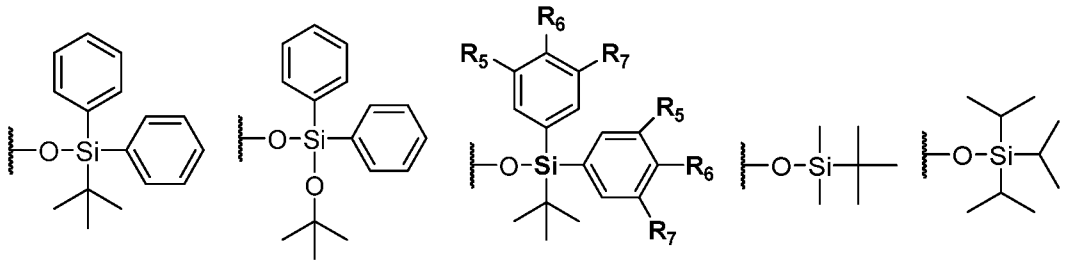
[0182] In a ninety-fifth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-second embodiments), wherein Z is a group represented by Formula B-1* or B-2*:



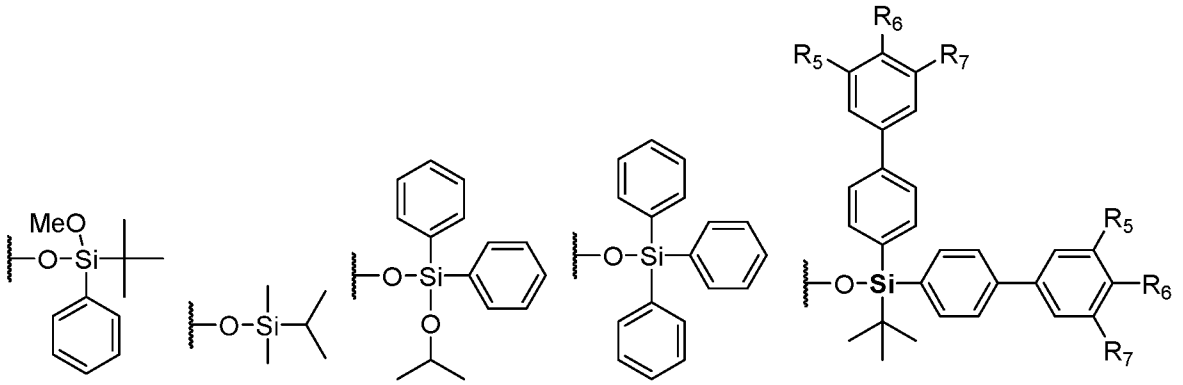
B-1* B-2*

[0183] In a ninety-sixth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-second embodiments), wherein ring A is phenyl or naphthalenyl.

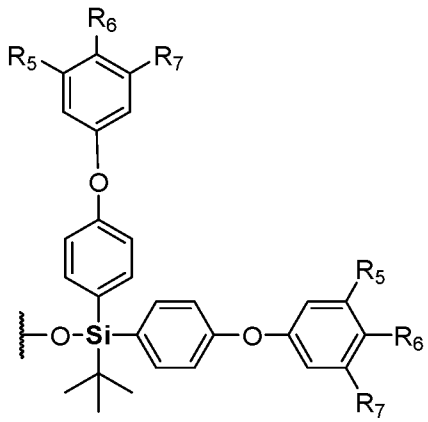
[0184] In a ninety-seventh embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-six embodiments), wherein P_1 is a silyl hydroxyl protecting group selected from the following:



-O-TBDPS , -O-TBoDPS , -O-TBDAS , -O-TBDMS , -O-TIPS ,

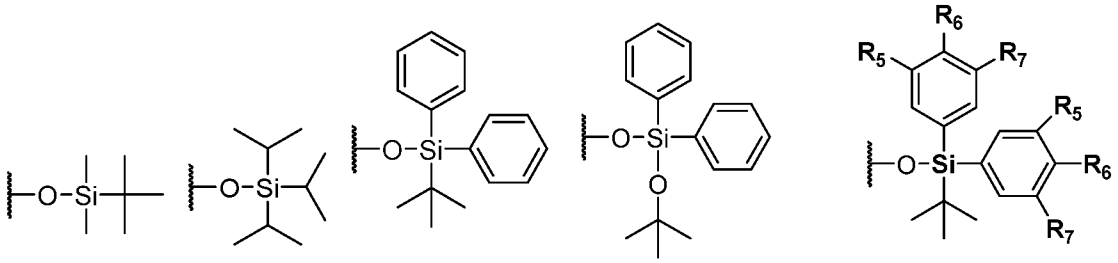


-O-TBMPS , **-O-DMIPS** , **-O-IPoDPS** , **-O-TPS** , -O-TBDAS-1 ,



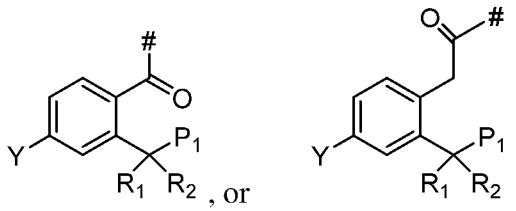
and -O-TBDAS-2 ; wherein represents the point of attachment for P_1 and R_5, R_6 and R_7 are each independently H, C_{1-30}alkyl, or C_{1-30}alkoxy.

[0185] In a ninety-eighth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-seventh embodiments), wherein P_1 is selected from the group consisting of –O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , and -O-TBDAS .

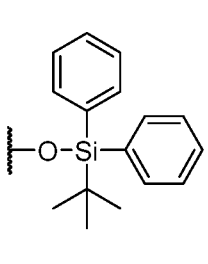
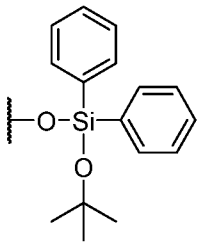
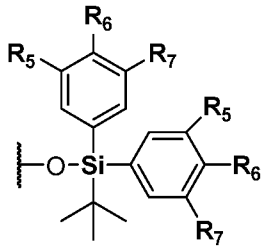
[0186] In a ninety-nineth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-third embodiments), wherein Z is a group represented by Formula I^{**} or Ia^{**}:



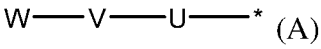
I^{**} , or Ia^{**}

or a salt thereof;

wherein P_1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:


-O-TBDPS ,

-O-TBoDPS ,

-O-TBDAS ; and R_5, R_6 and R_7 are each independently H, $C_{1\text{-}30}$alkyl, or $C_{1\text{-}30}$alkoxy.

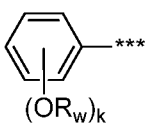
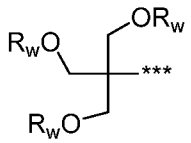
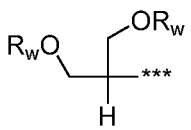
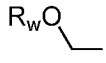
[0187] In a hundredth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, any one of the fifty-fourth through ninety-ninth embodiments), wherein Y is represented by Formula A:

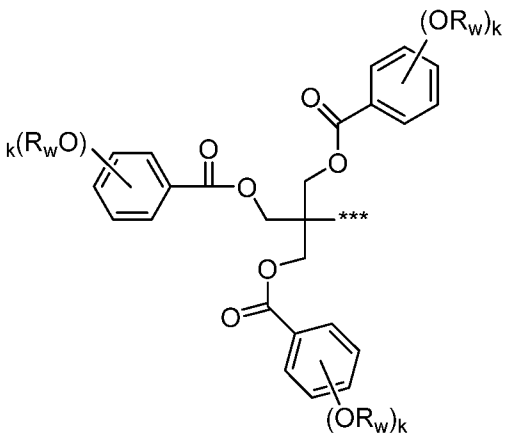
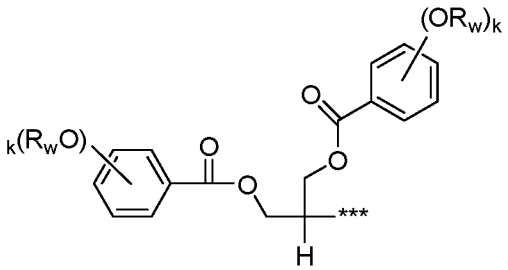

(A)

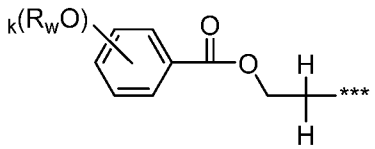
wherein:

—* represents the point of attachment for Y;

W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:


(A1),

(A2),

(A2-1),

(A2-2),


(A3),

(A3-1),

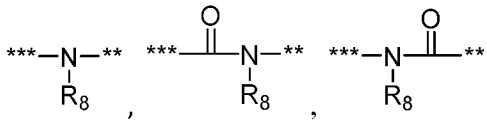
or

(A3-2),

wherein

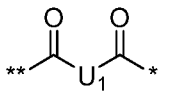
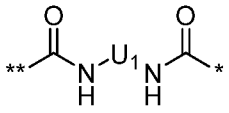
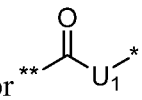
—*** represents the point where W and V connect;

each R_w is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

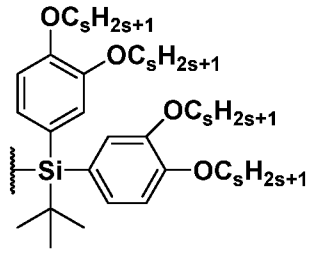
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:

 (A4),  (A5), or  (A6),

wherein U_1 is C_{1-6}alkylene, C_{1-6}alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

[0188] In a one hundred-first embodiment, the present disclosure provides a process described in any one of the ninety-seventh through hundredth embodiments, wherein the TBDAS group is:

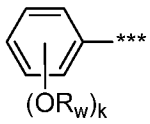


,

wherein s is an integer from 1 to 30.

[0189] In a one hundred-second embodiment, the present disclosure provides a process described in the fifty-fourth through hundredth embodiments, wherein P_1 is TBDPS.

[0190] In a one hundred-third embodiment, the present disclosure provides a process described in the hundredth through one hundred-second embodiments, wherein W is represented by Formula A1:



A1,

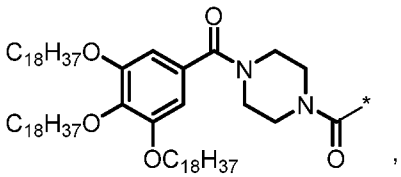
wherein R_w is C_nH_{2n+1};

n is an integer from 1 to 30.

[0191] In a one hundred-fourth embodiment, the present disclosure provides a process described in the one hundredth through one hundred-third embodiments, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and $C_{28}H_{57}$.

[0192] In a one hundred-fifth embodiment, the present disclosure provides a process described in the hundredth through one hundred-fourth embodiments, wherein V is a bond, CH_2, CH_2CH_2, C(=O)-, ***-C(=O)-O-**, or .

[0193] In a one hundred-sixth embodiment, the present disclosure provides a process described in the fifty-fourth through hundredth embodiments, wherein Y is selected from the groups consisting of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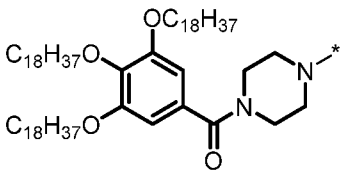
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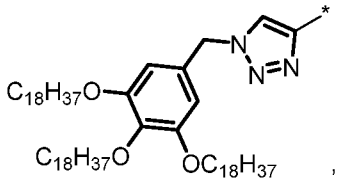
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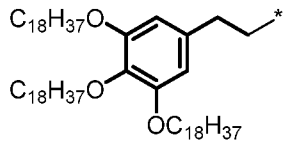
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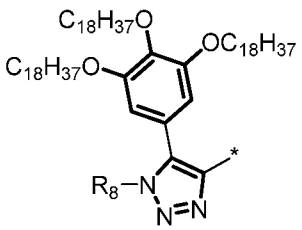
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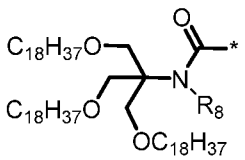
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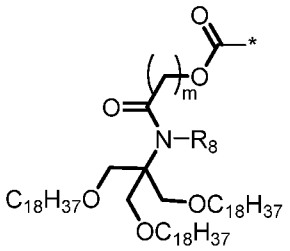
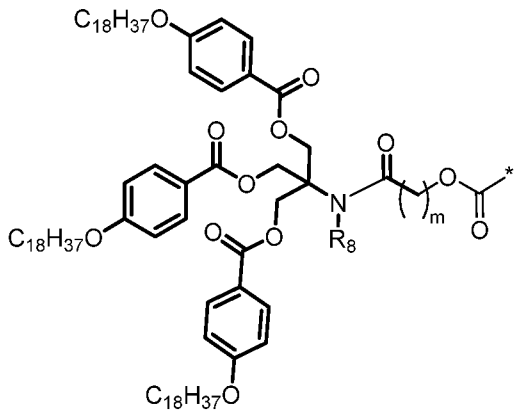

, and

;

wherein

R_8 is H or C_{1-6}alkyl; and

m is an integer from 1 to 5.

[0194] In a one hundred-seventh embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-sixth embodiments), wherein R_1 and R_2 are independently H or CH_3. In a specific embodiment, R_1 and R_2 are both H. In another specific embodiment, R_1 and R_2 are both CH_3.

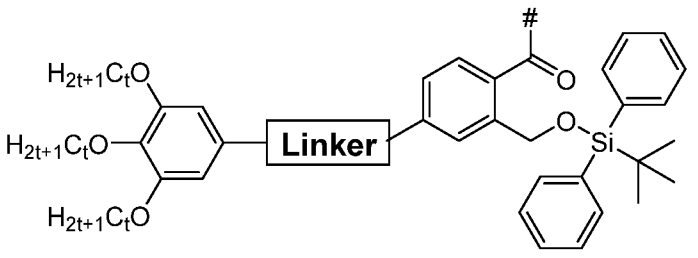
[0195] In a one hundred-eighth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-seventh embodiments), wherein e is 0, 1, or 2; and f is 0, 1, or 2.

[0196] In a one hundred-ninth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-eighth embodiments), wherein e is 1; and f is 1.

[0197] In a one hundred-tenth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-eighth embodiments), wherein e is 0; and f is 1 or e is 1; and f is 0.

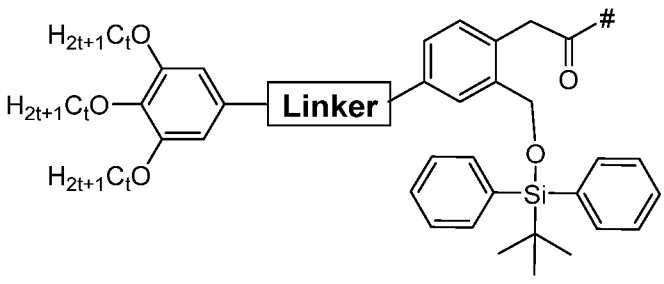
[0198] In a one hundred-eleventh embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-tenth embodiments), wherein R_8 is H or C_{1-4}alkyl.

[0199] In a one hundred-twelfth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-eleventh embodiments), wherein Z is represented by Formula II* or IIa*,



, or

II*

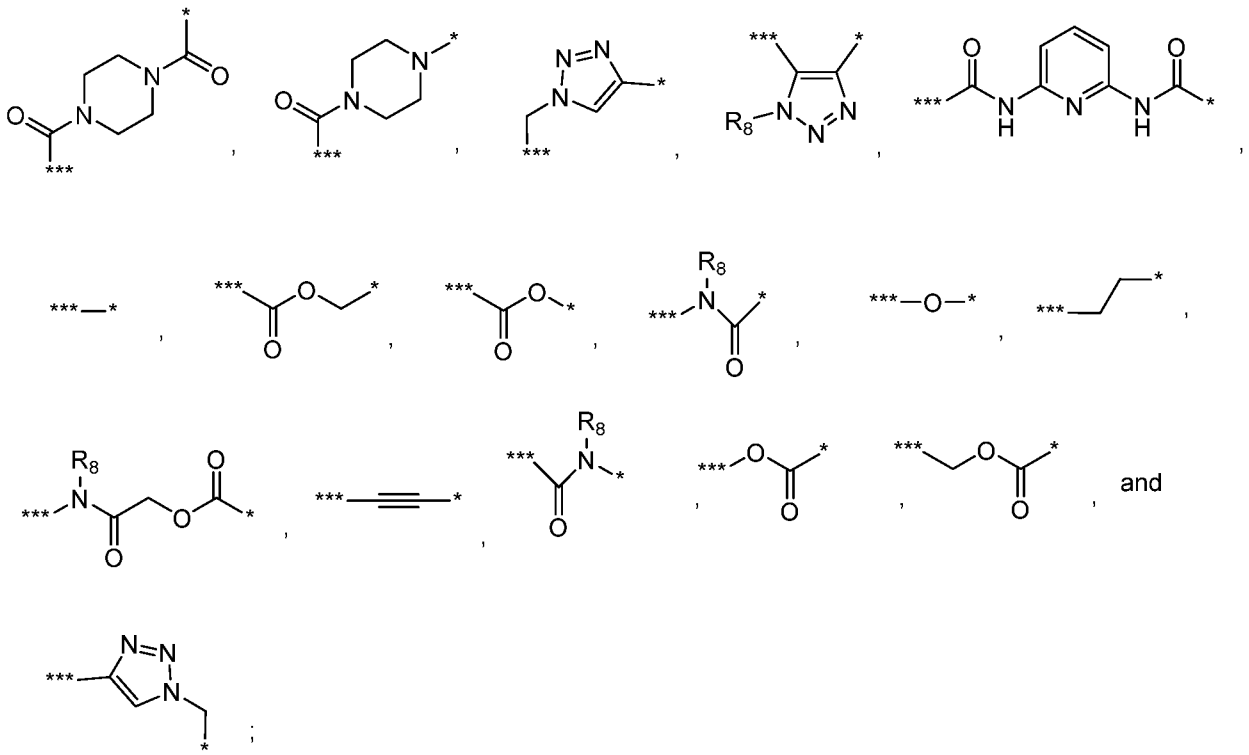


IIa*

wherein

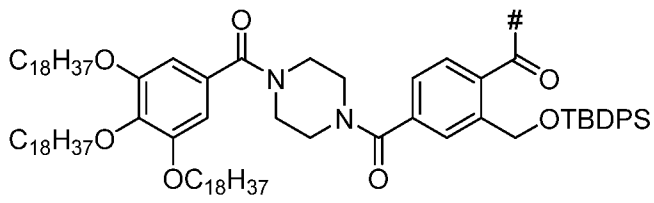
t is an integer from 10 to 30;

Linker is selected from the group consisting of



wherein R_8 is H or $C_{1\text{-}6}$alkyl.

[0200] In a one hundred-thirteenth embodiment, the present disclosure provides a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through one hundred-twelfth embodiments), wherein Z is:



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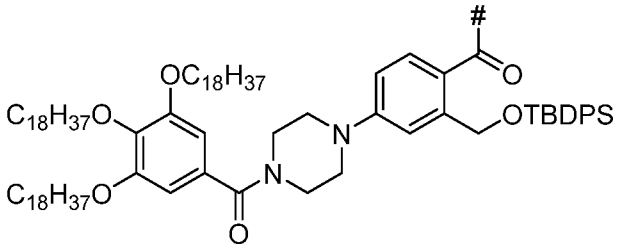
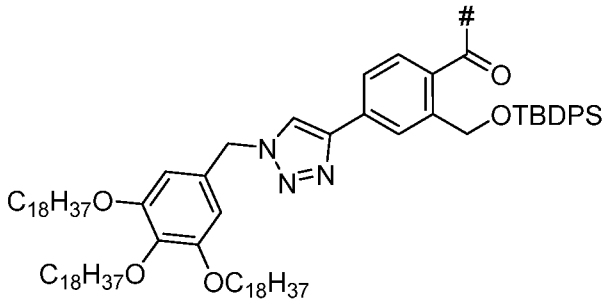
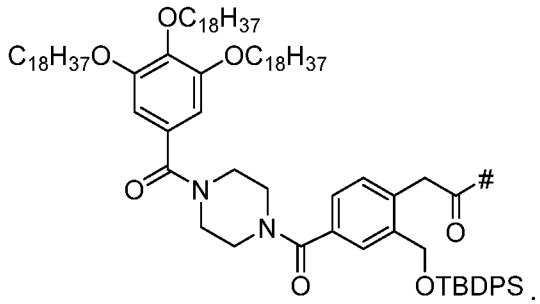


, or



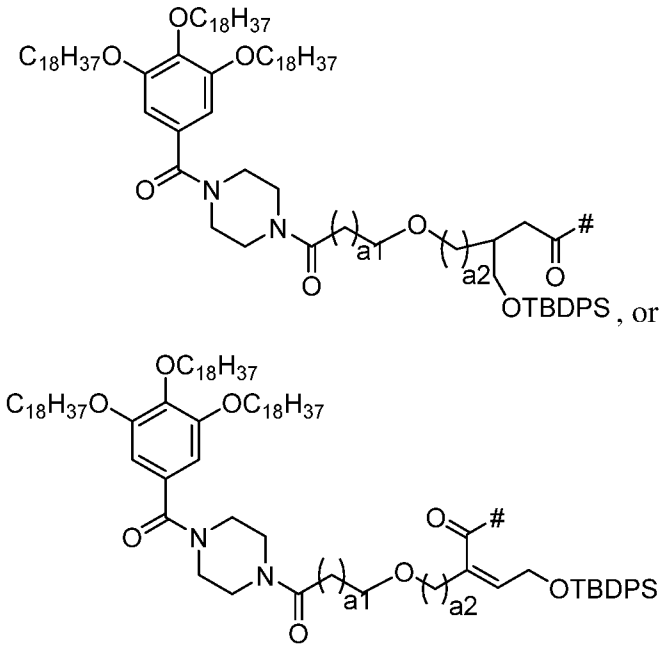
.

[0201] In a one hundred-fourteenth embodiment, the present disclosure a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through ninety-third embodiments), wherein Z is



.

[0202] In a one hundred-fifteenth embodiment, the present disclosure a process described in the third or fourth aspect (*e.g.*, the fifty-fourth through ninety-third embodiments), wherein Z is



[0203] In a one hundred-sixteenth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-fifteenth embodiments, wherein all of the P=X groups in the nucleotide or oligonucleotide are P=S.

[0204] In a one hundred-seventeenth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-fifteenth embodiments, wherein all of the P=X groups in the nucleotide or oligonucleotide are P=O.

[0205] In a one hundred-eighteenth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-fifteenth embodiments, wherein greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the P=X groups in the compound or oligonucleotide are P=S.

[0206] In a one hundred-nineteenth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-fifteenth embodiments, wherein 10-90%, 20-80%, 30-70% or 40-60% of the P=X groups in the compound or oligonucleotide are P=S.

[0207] In a one hundred-twenty embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-sixteenth embodiments, wherein the

nucleobase is selected from the group consisting of cytosine, guanine, adenine, thymine, uracil, hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethylcytosine, wherein the NH_2 group of the nucleobase, if present, is protected by PhCO-, CH_3CO-, *i*PrCO-, Me_2N-CH=, or Me_2N-CMe=.

[0208] In a one hundred-twenty first embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-fifteenth embodiments, wherein the nucleobase is selected from the group consisting of cytosine, guanine, adenine, thymine, uracil, and 5-methylcytosine, wherein the NH_2 group of the nucleobase, if present, is protected by PhCO-, CH_3CO-, *i*PrCO-, Me_2N-CH=, or Me_2N-CMe=.

[0209] In a one hundred-twenty second embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-twenty first embodiments, wherein

each R^{32} is independently selected from the group consisting of H, F, and C_{1-4}alkoxy optionally substituted with C_{1-4}alkoxy;

each R^{34} is independently H or forms a ring with the alkoxy group of R^2, wherein the ring is a 5 or 6-membered ring optionally substituted with 1 to 3 C_{1-4} alkyl groups;

each R^{35} is a 4,4'-dimethoxytirtyl group;

R^{36} is $–CH_2CH_2CN$; and

R^{37a} and R^{37b} are independently C_{1-4}alkyl.

[0210] In a one hundred-twenty third embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-twenty first embodiments, wherein

each R^{32} is independently selected from the group consisting of H, F, $-OCH_3$, $–OCH_2CH_2OCH_3$,and -OTBDMS; and

each R^{34} is independently H or forms a ring with the alkoxy group of R^{32}, wherein the ring is a 5-membered ring.

[0211] In a one hundred-twenty fourth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-twenty first

embodiments, wherein each R^{34} is independently H or together with the alkoxy group of R^{32} form $–CH_2-O-$.

[0212] In a one hundred-twenty fifth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the twenty-seventh through fifty-third embodiments or a process described in the fifty-fourth through one hundred-twenty first embodiments, wherein

each R^{32} is independently selected from H or $–OCH_2CH_2OMe$;

each R^{34} is H;

each R^{35} is a 4,4'-dimethoxytirtyl group;

R^{36} is $–CH_2CH_2CN$; and

R^{37a} and R^{37b} are both $-CH(CH_3)_2$.

[0213] In a one hundred-twenty sixth embodiment, the present disclosure provides a process described in the fifty-fifth, sixty-fourth, or eighty-fifth embodiment, wherein the salt of the compound of formula (VD'), (V-2'), or (F2') is selected from trimethyl amine salt, triethyl amine salt, and triisopropyl amine salt.

[0214] In a one hundred-twenty seventh embodiment, the present disclosure provides a process described in one hundred-twenty sixth the embodiment, wherein the salt of the compound of formula (VD'), (V-2'), or (F2') is triethyl amine salt.

[0215] In a one hundred-twenty eighth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the second aspect (e.g., the twenty-eighth embodiment) or a process described in the third or fourth aspect (e.g., any one of the fifty-eighth, fifty-ninth, sixty-ninth, and seventy-first through ninety-second embodiments), wherein the is adenine, cytosine, or guanine.

[0216] In a one hundred-twenty ninth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the second aspect (e.g., the twenty-eighth embodiment) or a process described in the third or fourth aspect (e.g., any one of the fifty-eighth, fifty-ninth, sixty-ninth, and seventy-first through ninety-second embodiments), wherein the Q is a silyl protecting group.

[0217] In a one hundred-thirtieth embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the second aspect (e.g., the twenty-eighth embodiment) or a process described in the third or fourth aspect (e.g., any one of the fifty-eighth, fifty-ninth, sixty-ninth, and seventy-first through ninety-second embodiments), wherein the Q is selected from the group consisting of trimethylsilyl, triethylsilyl, triisopropylsilyl,

dimethylisopropylsilyl, diethylisopropylsilyl, dimethylthexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-t-butylmethylsilyl tri(trimethylsilyl)silyl, t-butylmethoxyphenylsilyl, and t-butoxydiphenylsilyl.

[0218] In a one hundred thirty-first embodiment, the present disclosure provides a nucleotide or oligonucleotide described in the second aspect (e.g. twenty-eighth embodiment) or a process described in the third or fourth aspect (e.g., any one of the fifty-eighth, fifty-ninth, sixty-ninth, and seventy-first through ninety-second embodiments), wherein the Q is t-butyldiphenylsilyl.

[0219] In certain embodiments, for the process described in the fourth aspect or any embodiments describe therein (*e.g.*, the eighty-fourth to one hundred-fifteenth embodiments), the variables R^{31}, R^{32}, R^{34}, R^{35}, R^{36}, q, and/or Z are described in the second aspect or any one of embodiments described therein (*e.g.*, the twenty-seventh to fifty-third embodiments).

[0220] In certain embodiments, for the process described in the fourth aspect or any embodiments describe therein (*e.g.*, the eighty-fourth to one hundred-fifteenth embodiments), the 5'-OH deprotection (or detritylation) step, the coupling step, and the oxidation or sulfurization step are carried out under conditions described in the third aspect or any one of embodiments described therein (*e.g.*, the fifty-fourth to eighty-third embodiments).

[0221] In certain embodiments, for a nucleotide or oligonucleotide described in the second aspect or any embodiments described therein or a process described in the third or fourth aspect or any embodiments described therein, when X is S, the phosphorothiolate group can have S-configuration, R-configuration or a mixture thereof (e.g., a racemic mixture).

EXEMPLIFICATION

Abbreviation

ACN = acetonitrile

Calcd = calculated

DBU = 8-diazabicyclo[5.4. 0]undec-7-ene

DCA = $CHCl_2COOH$ or dichloroacetic acid

DCM = dichloromethane

DDTT = 3-(N,N-dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole

DCI = 4,5-dicyanoimidazole

DIEA = N,N-diisopropylethylamine

DMT or DMTr = 4,4'-dimethoxytrityl or bis-(4-methoxyphenyl)phenylmethyl

DMSO = dimethyl sulfoxide

EtOAc or EA = ethyl acetate

ETT = 5-ethylthio-1H-tetrazole

h or hr = hour

HBTU = 3-[bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate

HOBt = hydroxybenzotriazole

imid = imidazole

IPAC = isopropyl acetate

iPrOH = isopropyl alcohol

MOE = methoxyethyl

MS = molecular sieve

MTBE or TBME = methyl tert-butyl ether

NMI = N-methylimidazole

TBS = tert-Butyldimethylsilyl

Py = pyridine

RBF = round bottom flask

RT = retention time

TBAF = tetra-n-butylammonium fluoride

TBuAA = tributylamine acetate

TBDPSCl = tert-butyl(chloro)diphenylsilane

TCA = trichloroacetic acid

TEA = triethylamine

TEAB = tetraethylammonium bromide

TFA = trifluoroacetic acid

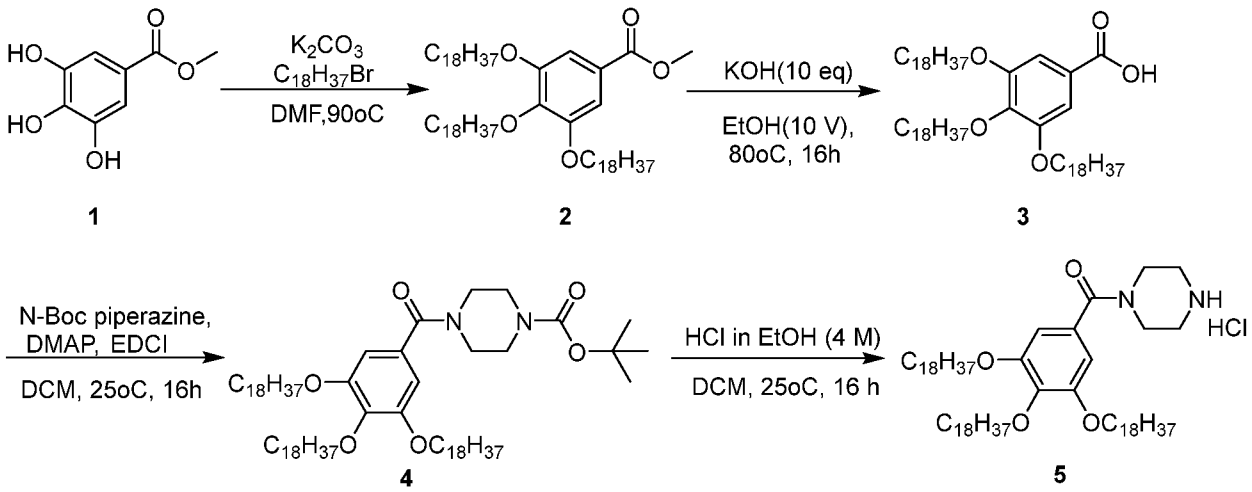
THF = tetrahydrofuran

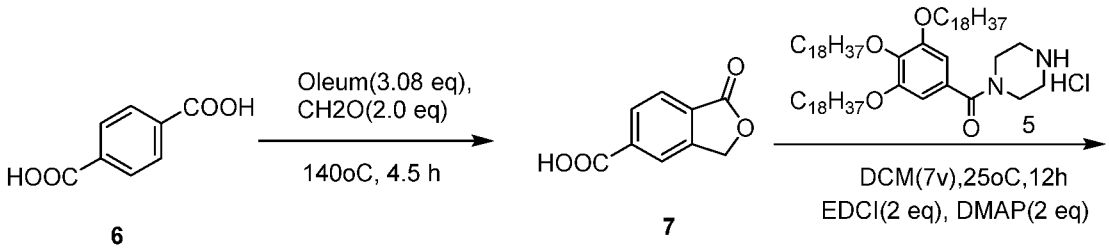
TLC = thin layer chromatography

Tol = toluene

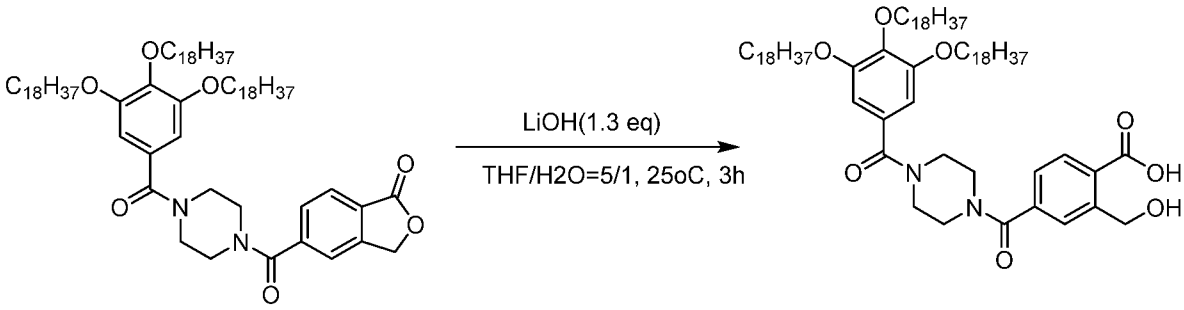
Example 1. Synthesis of Compound M19

[0222] *a. Scheme for Synthesis of Compound M19*

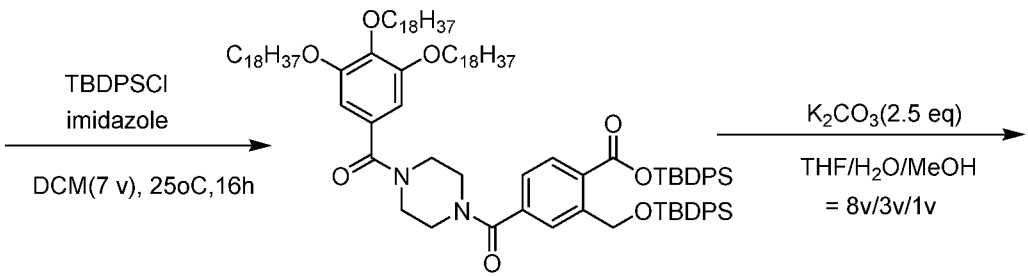

HO
HO
OH
O
O
K2CO3
C18H37Br
DMF,90oC
C18H37O
C18H37O
OC18H37
KOH(10 eq)
EtOH(10 V),
80oC, 16h
OH
1
2
3
N-Boc piperazine,
DMAP, EDCI
DCM, 25oC, 16h
N
HCl in EtOH (4 M)
DCM, 25oC, 16 h
NH
HCl
4
5



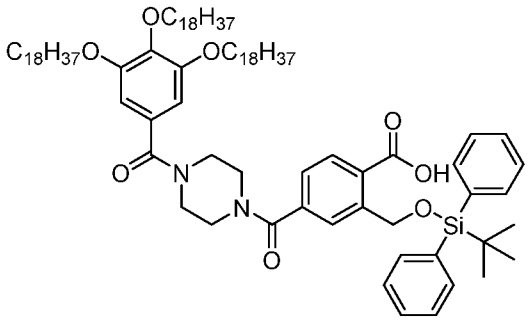
6 7



M-17 M-18



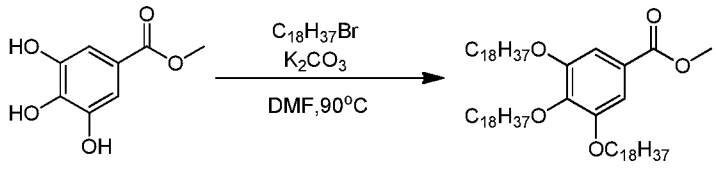
M-19-A



SiLHPG M19

[0223] *b. Procedures for Synthesis of Compound M19*

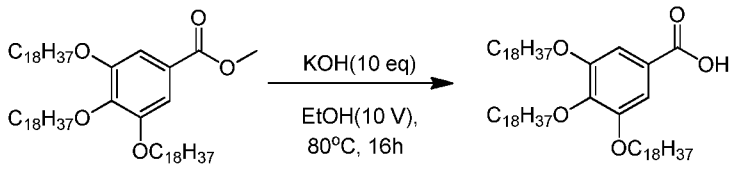
[0224] General procedure for preparation of compound 2



1 2

[0225] To a mixture of compound 1 (1.20 kg, 6.52 mol, 1.00 *eq*) and K_2CO_3 (5.40 kg, 39.1 mol, 6.00 *eq*) in DMF (12 L) was added 1-bromooctadecane (8.69 kg, 26.1 mol, 4.00 *eq*) in one portion at 25 °C under N_2. The mixture was stirred at 90 °C and stirred for 16 h. TLC (dichloromethane/methanol = 5/1, Start material, R_f = 0.52, product, R_f = 0.88) indicated no start material was detected. Added 18 L H_2O, cool down to 25 °C, filtered and washed with 7 L H_2O and 10 L acetone. The solid was recrystallized with 30 L n-heptanes at 55 °C for 1 h. Cool down to 25 °C, filtered and washed the solid with 5 L n-heptanes. Compound **2** (8.60 kg, crude) was obtained as a white solid.

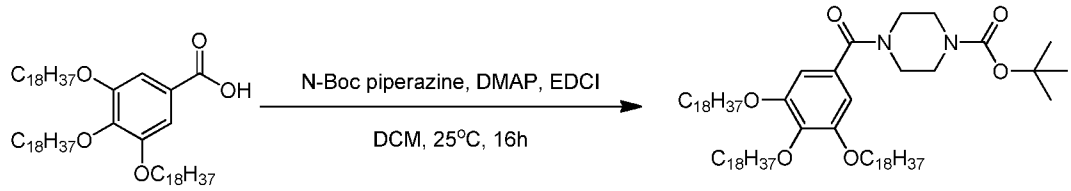
[0226] General procedure for preparation of compound 3



2 3

[0227] To a mixture of compound **2** (3.00 kg, 3.19 mol, 1.00 eq) in EtOH (15 L) was added solution of KOH (268 g, 4.78 mol, 1.50 eq) in H_2O (3 L) in one portion at 25 °C under N_2. The mixture was stirred at 80 °C and stirred for 16 h. TLC (petroleum ether/ethyl acetate = 5/1, start material R_f = 0.33, product R_f = 0.86) indicated no start material was detected. Adjust pH to 2~3 with 2N HCl (6 L), cool down to 25 °C, poured into 75 L H_2O. Filtered and washed the solid with 20 L H_2O, and 10 L acetone. Dried under oven at 50 °C for 24 h. Dissolved in 28 L DCM, triturated at 40 °C for 1 h. Cool down to 25 °C. Filtered and washed with 40 L MeOH. Dried under oven at 50 °C for 48 h. Compound **3** (4.20 kg, 4.53 mol, 71.1% yield) was obtained as a white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 7.33 (s, 2H), 4.06-4.01 (m, 6H), 1.84-1.76 (m, 6H), 1.50-1.26 (m, 6H), 1.26 (m, 86H), 0.90-0.87 (t, J = 6.8 Hz, 9H).

[0228] General procedure for preparation of compound 4

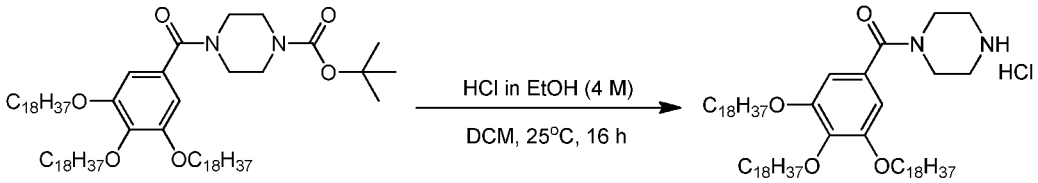


3 4

[0229] To a mixture of compound **3** (4.00 kg, 4.31 mol, 1.00 *eq*), EDCI (1.65 kg, 8.62 mol, 2.00 *eq*) and DMAP (105 g, 862 mmol, 0.200 *eq*) in DCM (28 L) was added tert-butyl piperazine-1-carboxylate (1.04 kg, 5.61 mol, 1.30 *eq*) at 25 °C. Stirred for 16 h at 25 °C under N_2. TLC (dichloromethane / methanol = 20/1, start material R_f = 0.32, product R_f =

0.53) indicated no start material was detected. Poured into 50 L MeOH, filtered and washed the cake with 30 L MeOH. Compound **4** (4.62 kg, 4.22 mol, 97.7% yield) was obtained as a white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 6.57 (s, 2H), 3.97-3.94 (m, 6H), 3.57-3.29 (m, 8H), 1.82-1.71 (m, 6H), 1.47 (m, 16H), 1.25 (m, 16H), 0.89-0.86 (t, J = 6.8 Hz, 9H).

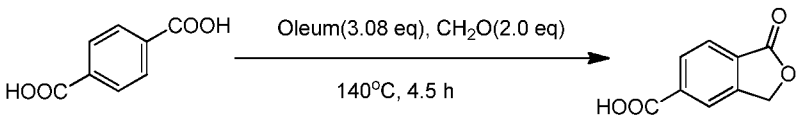
[0230] General procedure for preparation of compound 5



4 5

[0231] To a mixture of compound **4** (1.50 kg, 1.37 mol, 1.00 *eq*) in DCM (10 L) was added 4N HCl in EtOH (4 M, 3.42 L, 10.0 *eq*) in one portion at 25 °C under N_2. The mixture was stirred for 16 h at 25 °C. TLC (DCM/MeOH = 20/1, product R_f = 0.74, start material R_f = 0.18) indicated compound **4** was disappeared. Filtered and washed the solid with 5L EtOH. Compound **5** (3.80 kg, 3.68 mol, 89.6% yield, HCl salt form) was obtained as a white solid.

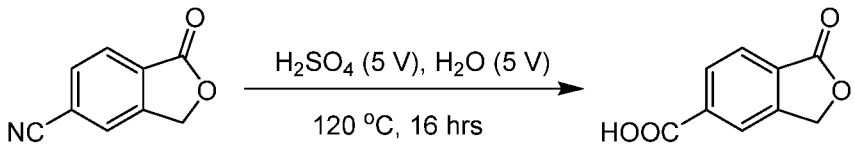
[0232] General procedure for preparation of compound 7



6 7

[0233] To a mixture of compound **6** (500 g, 3.01 mol, 1.00 eq) and CH_2O (181 g, 6.02 mol, 2.00 eq) was added Oleum (825 mL) in one portion at 25 °C under N_2. The mixture was stirred at 140 °C for 15 h. Exhaust gas absorption was charged with 10% NaOH aqueous. HPLC (start material: RT = 2.73 min; product: RT = 2.84 min) showed compound **6** was disappeared. Cooled down to 25 °C, quenched with 3300 mL H_2O. Filtered and washed with H_2O until pH was 3~4. Recrystallized with 3200 mL DMF at 80 °C. Filtered and washed with 2L EtOH. Dried under vacuum. Compound **7** (1.60 kg, crude) was obtained as a gray solid. A mixture of compound 7 (1.60 kg, 8.98 mol, 1.00 *eq*) in DMF (3200 mL) was stirred at 80 °C for 1 h. Cooled down to 25 °C slowly through a period of 16 h. Filtered and washed with 500 mL EtOH. Dried under vacuum. Compound **7** (620 g, 3.48 mol, 38.7% yield) was obtained as a light orange solid.

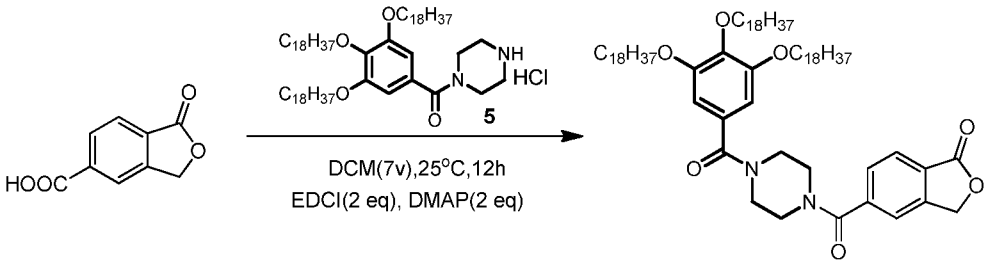
[0234] Alternatively, compound **7** was prepared by the following procedure:



6A 7

[0235] To a solution of 50% H_2SO_4 aq. (15.0 L) was added compound **6A** (1.50 kg, 9.43 mol, 1.00 eq) in one portion at 25 °C under N_2. The mixture stirred at 120 °C for 16 hrs. LCMS (ET49477-3-P1A2, product: RT = 0.597 min) showed the starting material was consumed completely. Cooled down to 25 °C. Pour into H_2O (ice, 15.0 L), filter and wash the solids with H_2O (2.00 L x 5). The filter cake dried under vacuum oven (55 °C for 48 hrs). Compound 7 (2.50 kg, 14.0 mol, 74% yield, 98.8% purity) was obtained as a white solid. ESI$^+$: MS: calcd for $C_9H_6O_4$ $(M+H)^+$ 178.0 found 178.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 8.09-8.07 (d, J = 8.0 Hz, 1H), 7.93-7.91 (d, J = 8.0 Hz, 1H), 5.45 (s, 2H). $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 169.9, 166.5, 147.6, 135.7, 129.8, 128.5, 125.1, 124.0, 70.1.

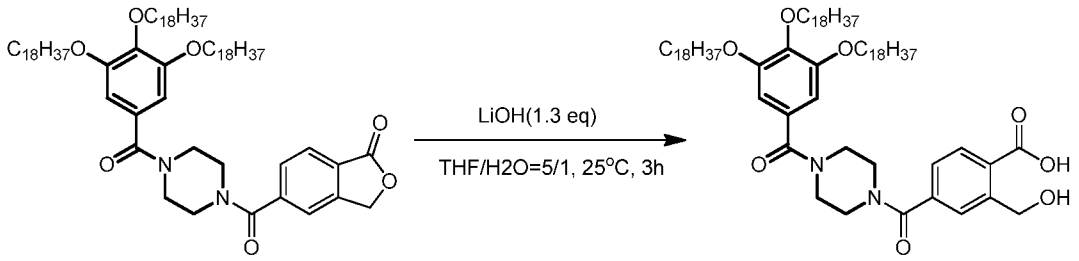
[0236] General procedure for preparation of compound M-17



7 M-17

[0237] To a mixture of compound **5** (3.60 kg, 3.49 mol, 1.00 *eq*, HCl) and compound **7** (683 g, 3.84 mol, 1.10 *eq*) in DCM (24 L) was added DMAP (852 g, 6.98 mol, 2.00 *eq*), EDCI (1.34 kg, 6.98 mol, 2.00 *eq*) in one portion at 25 °C under N_2. The mixture was stirred at 25 °C for 2 h. TLC (DCM/MeOH = 20/1, start material R_f = 0.62, product R_f = 0) indicated the start material was consumed completely. Poured into EtOH (50 L), filtered and washed with EtOH (20 L). Compound **M-17** (3.80 kg, 3.29 mol, 94.3% yield) was obtained as a white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 7.99-7.97 (d, J = 6.8 Hz, 1H), 7.56-7.53 (m, 2H), 6.59 (s, 2H), 5.36 (s, 2H), 3.97-3.94 (t, J = 6.4 Hz, 6H), 3.78-3.44 (m, 8H), 1.82-1.71 (m, 7H), 1.46-1.42 (m, 7H), 1.30-1.26 (m, 93H), 0.89-0.86 (t, J = 6.8 Hz, 9H)

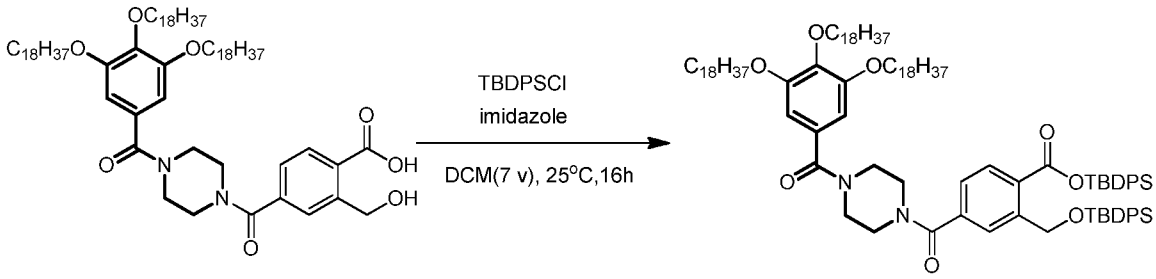
[0238] General procedure for preparation of compound M-18



M-17 M-18

[0239] To a mixture of compound **M-17** in THF (20 L) was added solution of $LiOH.H_2O$ (175 g, 4.16 mol, 1.30 *eq*) in H_2O (4000 mL) in one portion at 25 °C under N_2. The mixture was stirred at 25 °C and stirred for 3 h. TLC (DCM/MeOH = 20/1, start material R_f = 0.46, product R_f = 0.05) indicated the start material was consumed completely. Concentrated and diluted with 40 L H_2O, adjust pH to 4~5 with 1N HCl (10 L). Filtered and washed with 45 L H_2O until the pH was 6~7. Washed with 4 L ACN. Compound **M-18** (3.90 kg, crude) was obtained as a white solid. ^1H NMR: ET29928-65-P1A1 400 MHz $CDCl_3$ 8.05-8.00 (m, 1H), 7.57-7.54 (m, 1H), 7.38-7.36 (m, 1H), 6.59 (s, 2H), 6.6 (s, 2H), 4.79 (s, 2H), 3.97-3.94 (m, 8H), 3.81-3.45 (m, 8H), 1.82-1.77 (m, 4H), 1.45 (m, 7H), 1.29-1.25 (m, 90H), 0.89-0.86 (t, *J* = 7.2 Hz, 9H).

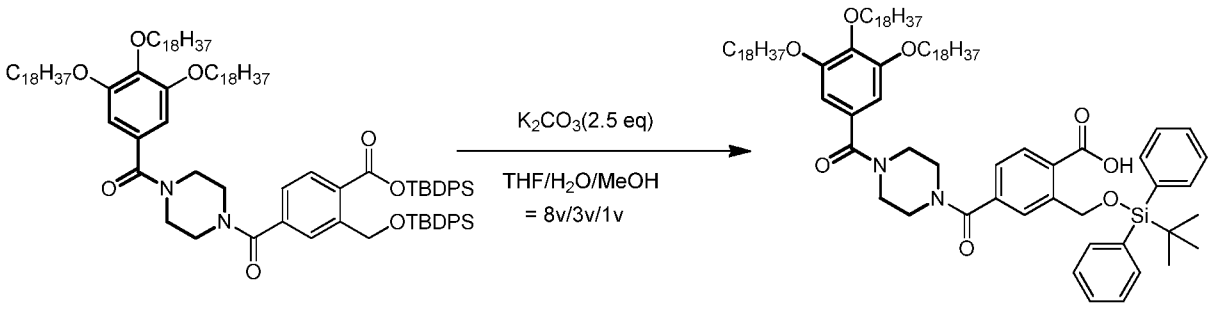
[0240] General procedure for preparation of compound M-19-A



M-18 M-19-A

[0241] To a mixture of compound **M-18** (1.70 kg, 1.45 mol, 1.00 *eq*) in DCM (20 L) was added imidazole (986 g, 14.5 mol, 10.0 *eq*) and TBDPSCl (3.98 kg, 14.5 mol, 3.72 L, 10.0 *eq*) in one portion at 25 °C under N_2. The mixture was stirred at 25 °C and stirred for 2 h. TLC (DCM/MeOH = 10/1, start material R_f = 0.18, product R_f = 0.92) indicated the start material was consumed completely. The reaction worked up with ET29928-70 together. Washed with H_2O (15 L x 2), separated the organic layer and dried over anhydrous Na_2SO_4 and concentrated. Compound **M-19-A** (5.80 kg, crude) was obtained as a white solid.

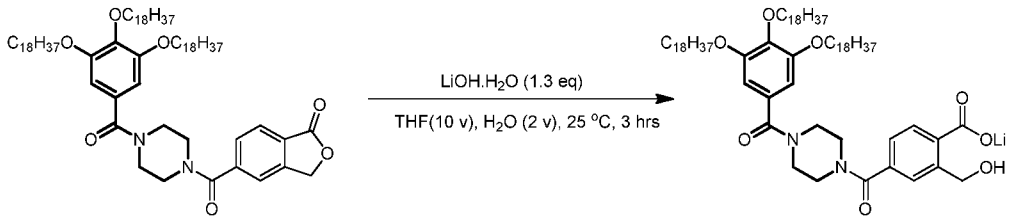
[0242] General procedure for preparation of compound SiLHPG M19



M-19-A SiLHPG M19

[0243] To a mixture of compound **M-19-A** (2000 g, 485 mmol, 1.00 *eq*) in THF (16 L) was added solution of K_2CO_3 (87.1 g, 630 mmol, 1.30 *eq*) in H_2O (6000 mL) and MeOH (2000 mL) one portion at 25 °C under N_2. The mixture was stirred for 16 h at 25 °C. TLC (PE/EA = 2/1, start material R_f = 0.43, product R_f = 0) indicated the start material was consumed completely. Concentrated and diluted with 10 L H_2O, adjust pH to 5 with 1 M $KHSO_4$, extracted with DCM (10 L x 2), dried over anhydrous Na_2SO_4. Concentrated to ~5 L, poured into 10 L MeOH, filtered and washed with MeOH (5 L x 4) to remove the TBDPS byproduct. Dissolved in DCM (5 L) and dropwised into MeCN (10 L)，filtered and washed with MeCN (2 L x 4), dissolved in DCM(12 L), filtered through silica gel pad, and washed with DCM/EtOAc = 1/1 (10 L). Compound **SiLHPG M19** (835 g, 591 mmol, 48.8% yield) was obtained as a white solid. **^{1}H NMR**: 400 MHz $CDCl_3$ δ 8.10-8.08 (d, J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.66-7.64 (m, 4H), 7.43-7.32 (m, 7H), 6.59 (s, 2H), 5.21 (s, 2H), 3.99-3.93 (m, 6H), 3.78-3.44 (m, 8H), 1.82-1.75 (m, 6H), 1.47-1.42 (m, 7H), 1.31-1.27 (m, 93H), 1.12 (s, 10H),0.90-0.87 (t, J = 6.8 Hz, 9H).

[0244] General procedure for preparation of compound M-18A:

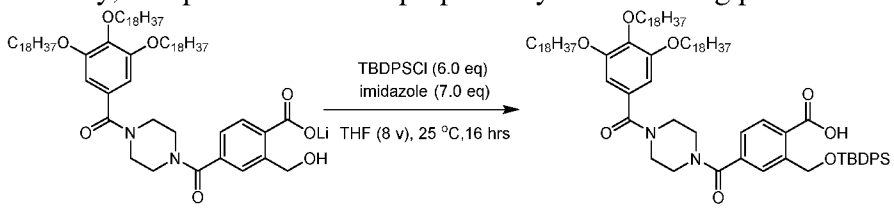


M-17 M-18A

To a mixture of compound M-17 (2.00 kg, 1.73 mol, 1.00 eq) in THF (20.0 L) was added solution of $LiOH.H_2O$ (94.4 g, 2.25 mol, 1.30 eq) in H_2O (4000 mL) in one portion at 25 °C under N_2. The mixture was stirred at 25 °C and stirred for 3 hrs. TLC (dichloromethane / methanol = 20/1, start material R_f = 0.5, product R_f = 0.1) indicated the start material was consumed completely. Poured into ACN (20.0 L) and filtered. Collect solids combined then dry under vacuum oven (50 °C, 10 days). The reactions were carried out with five batches in

parallel. Compound **M-18A** (10.8 kg, 9.15 mol, 106% yield, 87.4% purity) was obtained as a white solid. HRMS: calcd for $C_{74}H_{129}N_2O_8$ $(M\text{-}Li+2H)^+$ 1173.9671, found 1173.9755. **^{1}H NMR**: (Li salt was neutralized to free acid for H-NMR). 400 MHz, $CDCl_3$ δ 8.05-8.00 (m, 1H), 7.57-7.54 (m, 1H), 7.38-7.36 (m, 1H), 6.59 (s, 2H), 6.6 (s, 2H), 4.79 (s, 2H), 3.97-3.94 (m, 8H), 3.81-3.45 (m, 8H), 1.82-1.77 (m, 4H), 1.45 (m, 7H), 1.29-1.25 (m, 90H), 0.89-0.86 (t, J = 7.2 Hz, 9H).

[0245] Alternatively, compound **M19** was prepared by the following procedure:

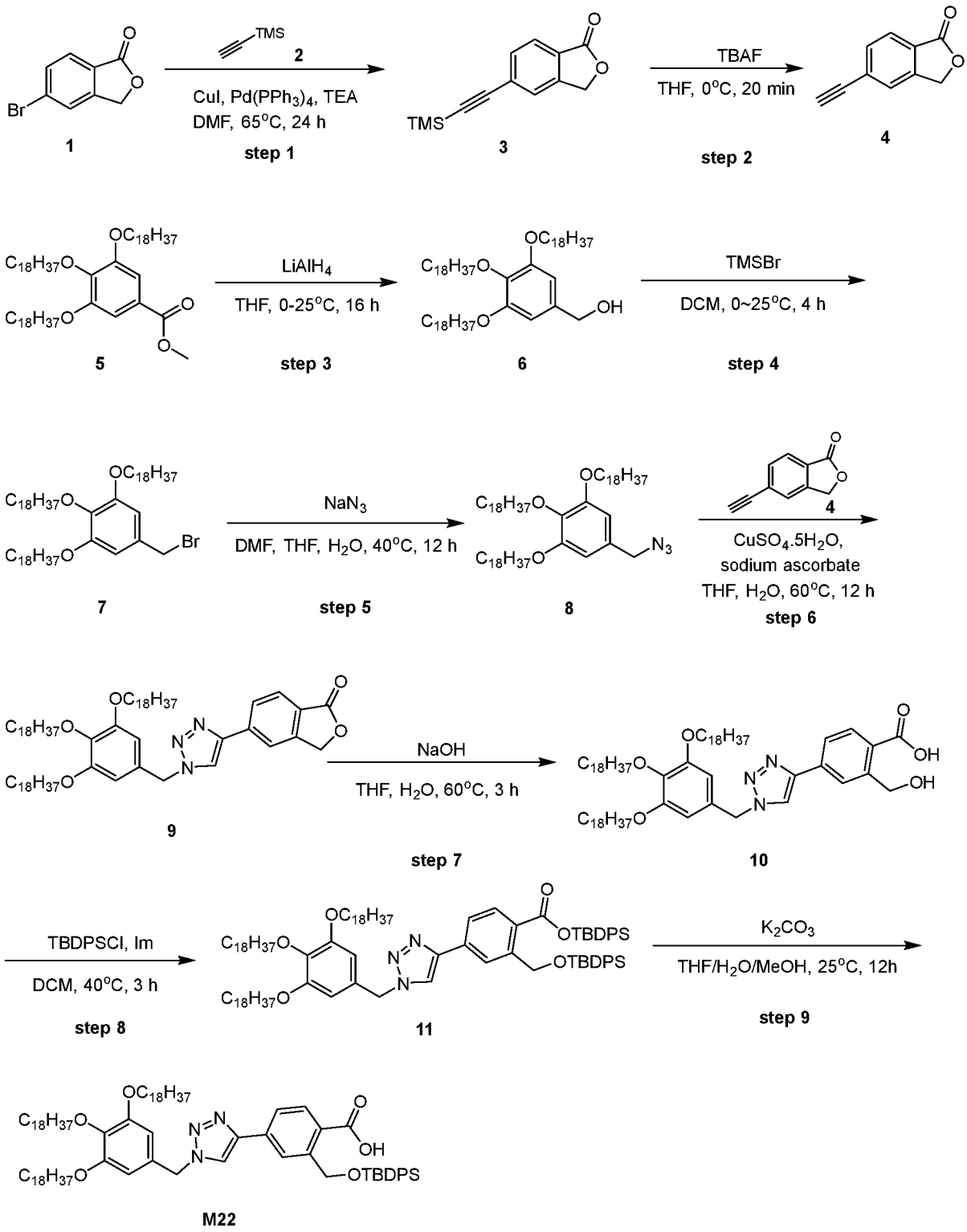


M-18A SiLHPG M19

[0246] To a mixture of compound M-18A (3.00 kg, 2.54 mol, 1.00 eq) in THF (24.0 L) was added imidazole (1.21 kg, 17.80 mol, 7.0 eq) and TBDPSCl (4.19 kg, 15.3 mol, 3.92 L, 6.0 eq) in 5 portions at 25 °C under N_2. The mixture was stirred at 25 °C and stirred for 16 hrs. HPLC (start material t_R= 5.92 min, product t_R = 9.32 min) indicated the start material was ~5% remained. The reaction diluted with DCM (20.0 L) and wash with H_2O (20.0 L x 2). Then the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated ~7 L, poured into 15 L MeOH, filtered and washed with MeOH (5 L x 4) to remove the TBDPS byproduct. Dissolved in DCM (7 L) and dropwised into MeCN (15 L), filtered and washed with MeCN (5L x 4), the filter cake combined and dried under oven (50 °C, 72 hrs) Compound **SiLHPG M19** (13.6 kg, crude, 89% purity, 5% M-17, 3% M-18) was obtained as a white solid. The residue was purified by column chromatography (SiO2, Petroleum ether / Ethyl acetate = 1/10 to 4/1) (10%DCM was added into PE eluent). The reactions were carried out with three batches in parallel. Concentrate and compound SiLHPG M19 (7.20 kg, 5.09 mol, 66.5% yield, 95.08% purity) was obtained as a white solid. HRMS: calcd for $C_{90}H_{147}N_2O_8Si$ $(M+H)^+$ 1412.0848, found 1412.0908. **^{1}H NMR** (400 MHz, CDCl3) δ 8.10-8.08 (d, J = 8.0 Hz, 1H), 7.99 (s, 1H), 7.66-7.64 (m, 4H), 7.43-7.32 (m, 7H), 6.59 (s, 2H), 5.21 (s, 2H), 3.99-3.93 (m, 6H), 3.78-3.44 (m, 8H), 1.82-1.75 (m, 6H), 1.47-1.42 (m, 7H), 1.31-1.27 (m, 90H), 1.12 (s, 10H),0.90-0.87 (t, J = 6.8 Hz, 9H). ^{13}C{1H} NMR (100 MHz, $CDCl_3$) δ 170.8, 169.9, 153.3, 144.7, 139.8, 139.4, 135.4, 134.8, 133.1, 132.1, 129.9, 129.6, 127.9, 127.7, 125.6, 125.1, 105.7, 73.6, 69.3, 64.0, 60.4, 47.5, 42.3, 31.9, 30.3, 29.4-29.7,26.9, 26.1, 22.7, 14.1.

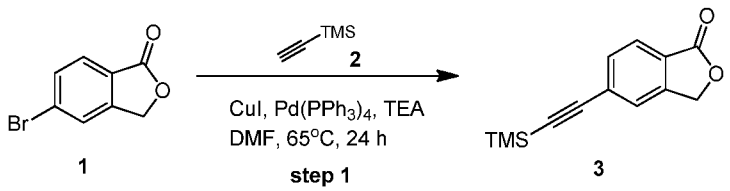
Example 2. Synthesis of Compound M22

[0247] *a. Schemes for Synthesis of Compound M22*



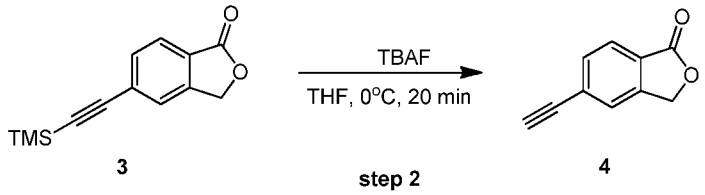
[0248] *b. Procedures for Synthesis of Compound M22*

[0249] General procedure for preparation of compound 3



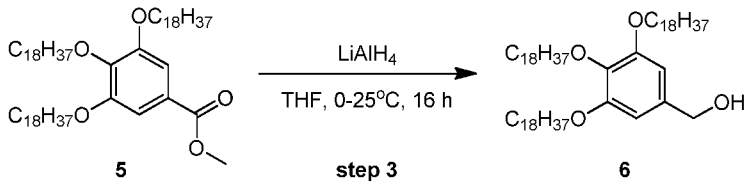
[0250] A mixture of compound 1 (24.00 g, 113 mmol), compound 2 (44.2 g, 451 mmol, 62.4 mL), CuI (6.45 g, 33.8 mmol), Pd(PPh3)4 (6.51 g, 5.64 mmol) and TEA (5.70 g, 56.3 mmol, 7.83 mL) in DMF (144 mL) was degassed and purged with N2 for 3 times, and then the mixture was stirred at 65°C for 24 h under N2 atmosphere. The desired product was detected in TLC (Petroleum ether/Ethyl acetate = 10/1, product: RT = 0.43). The filtrate was diluted with EtOAc (600 mL) and washed with brine (450 mL x 3). The organic layer was dried over anhydrous Na_2SO_4 (45.0 g), filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate = 100/1 to 5/1). Compound 3 (15.0 g, 57.8% yield) was obtained as a brown solid.

[0251] General procedure for preparation of compound 4



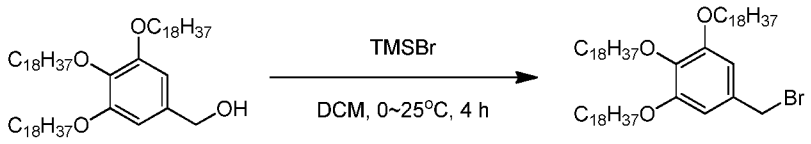
[0252] To a solution of compound 3 (15.0 g, 65.1 mmol) in THF (90.0 mL) was added TBAF (1.00 M, 65.1 mmol, 65.1 mL). The mixture was stirred at 0°C for 20 min. The desired product was detected in LCMS (ET25847-215-P1B, RT = 1.446) and TLC (Petroleum ether/Ethyl acetate = 5/1, product: Rf = 0.43). The reaction mixture was diluted with DCM (300 mL) and washed with brine (300 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 (30.0 g), filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Petroleum ether/Ethyl acetate = 30/1 to 5/1). Compound 4 (3.20 g, 31.1% yield) was obtained as a yellow solid. ^1H NMR: 400 MHz $CDCl_3$ 7.84-7.86 (d, J = 8.0 Hz, 1H), 7.53-7.63 (m, 2H), 5.29 (s, 2H), 0.28 (s, 9H).

[0253] General procedure for preparation of compound 6



[0254] To a solution of compound **5** (48.0 g, 51.0 mmol) in THF (288 mL) was added $LiAlH_4$ (3.18 g, 83.6 mmol) at 0°C. The mixture was stirred at 25°C for 16 h. TLC (DCM/MeOH = 5/1, product: R_f = 0.80) indicated compound 5 was consumed completely and one new spot formed. The reaction mixture was quenched by addition $Na_2SO_4.10H_2O$ (90.0 g) and then filtered. The filtrate was concentrated. The residue was diluted with DCM (900 mL) and washed with water (450 mL x 2), brine (600 mL) and dried over anhydrous Na_2SO_4 (90.0 g), filtered and concentrated under reduced pressure to give a residue. Compound **6** (40.0 g, crude) was obtained as a white solid. ^1H NMR: 400 MHz $CDCl_3$ 6.57 (s, 2H), 4.60-4.61 (d, J = 4.0 Hz, 2H), 3.93-4.00 (m, 6H), 1.71-1.84 (m, 6H), 1.27-1.31 (m, 90H), 0.87-0.91 (t, J = 6.4 Hz, 9H).

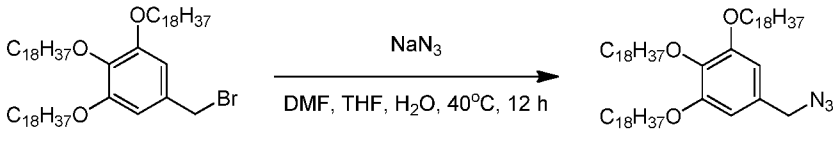
[0255] General procedure for preparation of compound 7



6 step 4 7

[0256] To a solution of compound 6 (40.0 g, 43.8 mmol) in DCM (240 mL) was added TMSBr (8.04 g, 52.5 mmol, 6.82 mL) at 0°C and stirred for 1 h. Then the mixture was stirred at 25°C for 3 h. TLC (DCM/MeOH = 20/1, product: Rf = 0.95) indicated compound 6 was consumed completely and one new spot formed. The reaction mixture was combined and concentrated under reduced pressure to remove solvent. The residue was dissolved in DCM (200 mL) and triturated by ACN (1.00 L). The solid was washed with ACN (200 mL x 3) and filtered. Then it was concentrated. Compound 7 (42.0 g, 98.2% yield) was obtained as a light yellow solid. ^1H NMR: 400 MHz $CDCl_3$ 6.58 (s, 2H), 4.44 (s, 2H), 3.93-3.99 (m, 6H), 1.72-1.84 (m, 6H), 1.27-1.47 (m, 90H), 0.87-0.91 (t, J = 6.4 Hz, 9H).

[0257] General procedure for preparation of compound 8

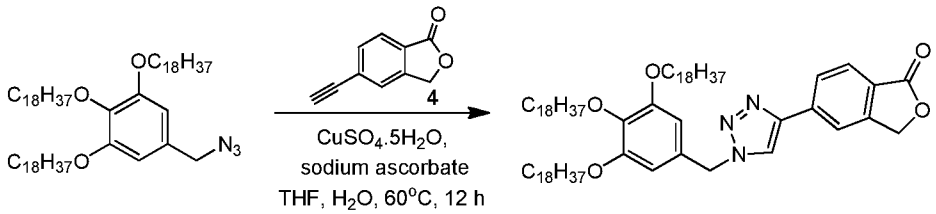


7 step 5 8

[0258] To a solution of compound 7 (42.0 g, 43.0 mmol) in DMF (252 mL) and THF (200 mL) was added NaN3 (4.20 g, 64.5 mmol) in H2O (36.0 mL). The mixture was stirred at 40°C for 12 h. TLC (Petroleum ether/Ethyl acetate = 10/1, product: RT = 0.66) indicated compound 7 was consumed completely and one new spot formed. The reaction mixture was diluted with DCM (300 mL) and washed with brine (450 mL x 3). The combined organic

layers were dried over anhydrous Na_2SO_4 (30.0 g), filtered and concentrated under reduced pressure to give a residue. Compound 8 (40.0 g, crude) was obtained as a white solid. ^1H NMR: 400 MHz $CDCl_3$ 6.49 (s, 2H), 4.25 (s, 2H), 3.94-4.00 (m, 6H), 1.75-1.84 (m, 6H), 1.27-1.49 (m, 90H), 0.87-0.91 (t, J = 6.4 Hz, 9H).

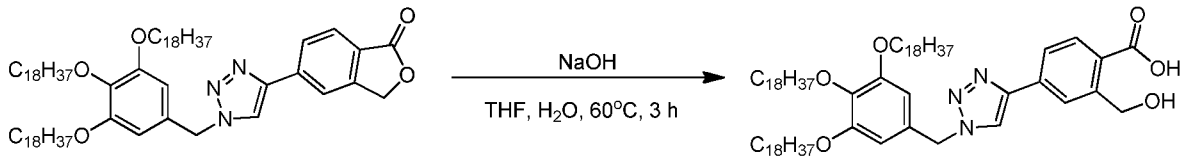
[0259] General procedure for preparation of compound 9



8 step 6 9

[0260] A mixture of compound **9** (2.70 g, 2.88 mmol) , compound 4 (682 mg, 4.32 mmol) , sodium ascorbate (570 mg, 2.88 mmol), $CuSO_4.5H_2O$ (360 mg, 1.44 mmol) in THF (16.2 mL) and H_2O (5.40 mL) was degassed and purged with N_2 for 3 times, and then the mixture was stirred at 70°C for 12 h under N_2 atmosphere. The desired product was detected in TLC (Petroleum ether/Ethyl acetate = 3/1, R_f = 0.22). Then it was filtered and concentrated under reduced pressure to remove solvent. The residue was dissolved in DCM (60.0 mL) and triturated with MeOH (600 mL). Compound **9** (3.00 g, crude) was obtained as a yellow solid.

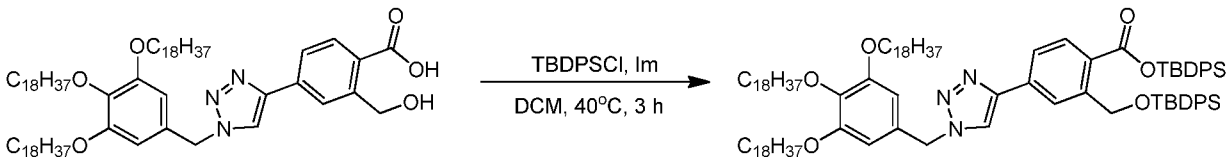
[0261] General procedure for preparation of compound 10



9 step 7 10

[0262] To a solution of compound **9** (3.00 g, 2.74 mmol) in THF (18.0 mL) was added NaOH (438 mg, 11.0 mmol) in H_2O (3.60 mL). The mixture was stirred at 60°C for 3 h. TLC (Petroleum ether/Ethyl acetate = 2/1, product: R_f = 0.04) indicated compound 9 was consumed completely and one new spot formed. This reaction was combined with the reaction (ET258474-259). The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H_2O (300 mL). The solution was adjusted to pH ~ 2 with HCl (1.00 M) and filtered to pH ~ 7 with H_2O and concentrated under reduced pressure to give a residue. Then it was washed by ACN (100 mL). Compound **10** (4.80 g, crude) was obtained as a light yellow solid.

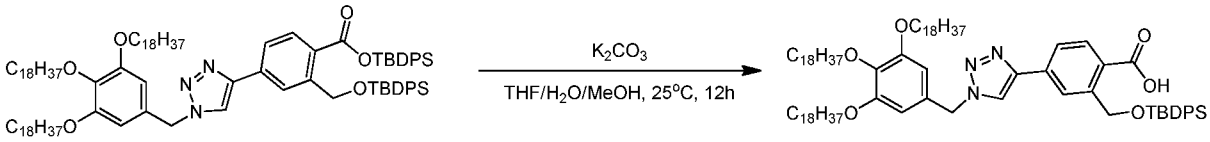
[0263] General procedure for preparation of compound 11



10 step 8 11

[0264] To a solution of compound **10** (4.80 g, 4.32 mmol) in DCM (28.8 mL) was added TBDPSCl (2.96 g, 10.8 mmol, 2.77 mL) and Im (880 mg, 12.9 mmol). The mixture was stirred at 40°C for 3 h. TLC (Petroleum ether/Ethyl acetate = 2/1, product: R_f = 0.94) indicated compound 10 was consumed completely and one new spot formed. The reaction mixture was quenched by addition $NaHCO_3$ (150 mL), and extracted with DCM (300 mL). The combined organic layers were washed with brine (300 mL x 2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was re-dissolve in DCM (90.0 mL) and dropped into MeOH (600 mL) with vigorous stirring. Desired product was precipitated out, filtered and concentrated under reduced pressure to give a residue. Then it was washed by MeOH (300 mL x 3). Compound **11** (6.05 g, crude) was obtained as a white solid.

[0265] General procedure for preparation of compound M22

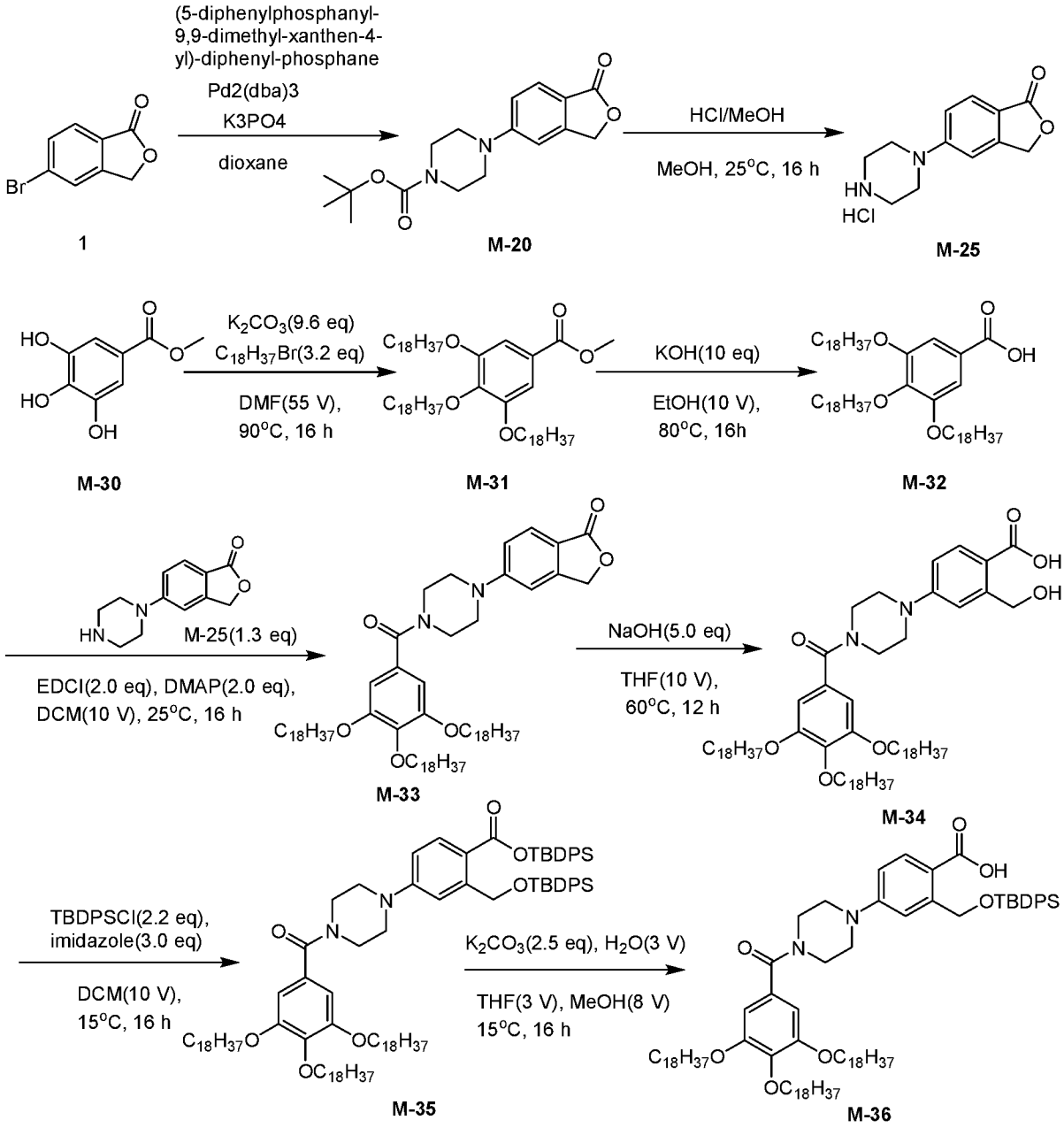


11 step 9 M22

[0266] To a solution of compound **11** (6.00 g, 3.76 mmol) in THF (36.0 mL) and MeOH (6.00 mL) was added K_2CO_3 (1.30 g, 9.42 mmol) in H_2O (12 mL). The mixture was stirred at 25°C for 12 h. TLC (Petroleum ether/Ethyl acetate = 2/1, product: R_f = 0.43) indicated compound 11 was consumed completely and one new spot formed. The reaction was combined and concentrated under reduced pressure. The reaction mixture was quenched by addition brine (200 mL), and adjusted to pH ~ 2 with $KHSO_4$ aqueous solution (200 mL, 1.00 M). Then it was extracted with DCM (300 mL) and washed with brine (100 mL x 2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was re-dissolve in DCM (50.0 mL) and dropped into MeOH (300 mL) with vigorous stirring. Desired product was precipitated out and filtered. Compound **SiLHPG M22** (4.5 g, 88.2% yield) was obtained as a white solid. **^{1}H NMR**: 400 MHz $CDCl_3$ 8.17 (s, 1H), 8.12-8.10 (d, J = 8.0 Hz, 1H), 7.94-7.92 (d, J = 8.0 Hz, 1H), 7.72-7.64 (m, 5H), 7.39-7.30 (m, 6H), 6.52 (s, 2H), 5.47 (s, 2H), 5.18 (s, 2H), 3.97-3.92 (m, 6H), 1.82-1.74 (m, 6H), 1.47-1.42 (m, 6H), 1.31-1.26 (m, 84H), 1.11 (s, 9H), 0.90-0.87 (t, J = 6.8 Hz, 9H).

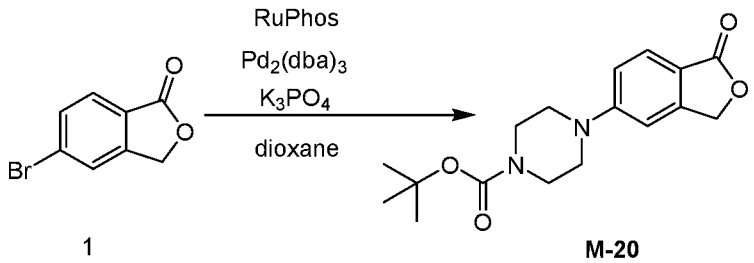
Example 3. Synthesis of Compound M36

[0267] *a. Schemes for Synthesis of Compound M36*



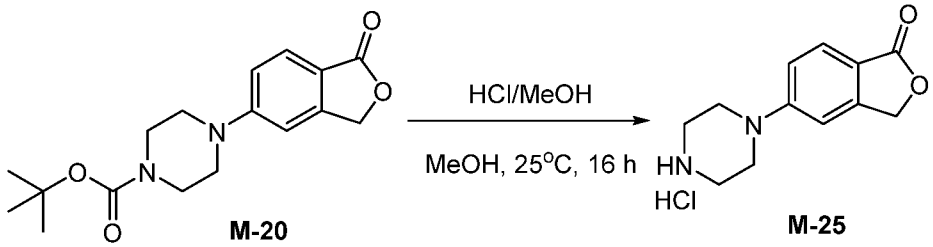
[0268] *b. Procedures for Synthesis of Compound M36*

[0269] General procedure for preparation of compound M20



[0270] To the three-necked round bottom flask charged with N_2 was added compound 1 (70 g, 328 mmol, 1.00 eq) , tert-butyl piperazine-1-carboxylate (61.2 g, 328. mmol, 1 eq), K_3PO_4 (139 g, 657.19 mmol, 2 eq) and Tol. (700 mL). The mixture was purged and degassed with N_2 for 3 times. Then to the solution was added RuPhos (15.3 g, 32.8 mmol, 0.1 eq) and $Pd_2(dba)_3$ (10.71 g, 16.43 mmol, 0.05 eq). The reaction solution was purged and degassed with N2 for 3 times and warmed to 100°C. It was stired at 100°C for 16h. The reaction solution turned to black. TLC (Petroleum ether/Ethyl acetate= 2/1,starting material Rf = 0.70, product Rf = 0.30) indicated the starting material was consumed and the a new point was formed. The reaction was cooled to 20°C. Then it was filtered to remove solid and wash with ethyl acetate twice (1000 mL and 500 mL). The organic layers were combined and concentrated to give a crude solid. The solid was stired with a solution of MTBE:DCM (800 mL,V/V=10/1) for 16 hrs. Then it was filtered to give the solid which was dried under oil pump. M20 was obtained (64 g, 201.03 mmol, 61.18% yield) as off-white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 7.76 (d, *J* = 8.8 Hz 1H), 7.01 (dd, *J* =2.0Hz, *J* =8.8Hz, 1H), 6.80 (s, 1H), 5.21 (s, 2H), 3.59-3.62(m, 4H), 3.35-3.38 (m, 4H), 1.49 (s, 9H).

[0271] General procedure for preparation of compound M25



M-20 M-25

[0272] To a solution of M-20 (30.0 g, 1.0 eq) in DCM (60 mL) was added HCl/MeOH(150 ml, 6.37 eq, 4M). The mixture was stirred at 25 °C for 16 hr. TLC (Dichloromethane : Methanol= 20:1, Rf = 0.0) indicated Reactant M20 was consumed completely. The reaction mixture was concentrated under reduced pressure to remove MeOH and DCM. The crude product was washed with DCM (200 ml). Then it was filtered and concentrated under reduced pressure to give compound M-35 (27 g, crude) as a white solid. ^{1}H NMR: 400 MHz DMSO-d_6 9.43 (s, 1H), 7.66 (d, *J* = 8.4Hz 1H), 7.13-7.18 (m, 2H), 5.28(s, 2H), 3.60-3.63(m, 4H), 3.16-3.19 (m, 4H).

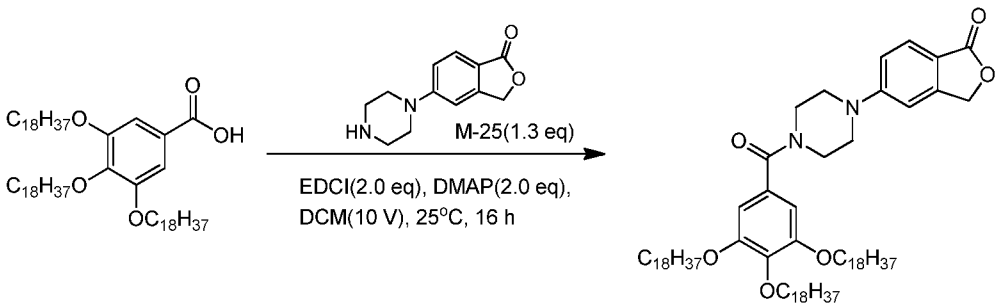
[0273] General procedure for preparation of compound M31

[0274] Compound M31 was prepared based on the same procedure for making compound 2 in Example 1.

[0275] General procedure for preparation of compound M32

[0276] Compound M32 was prepared based on the same procedure for making compound 3 in Example 1.

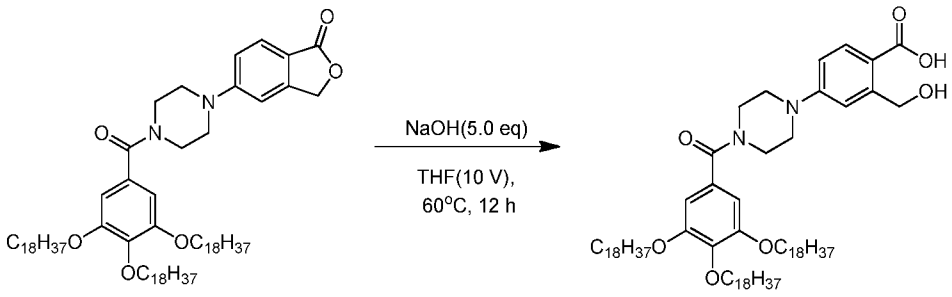
[0277] General procedure for preparation of compound M33



M-32 M-33

[0278] To a solution of compound M-32 (80.0 g, 1.0 eq) in DCM (50 mL) was added DMAP (21.1 g, 2.0 eq), M-25 (28.4 g, 1.3 eq) and EDCI (33.1 g,2.0 eq). The mixture was stirred at 25 °C for 16 hr. TLC (Dichloromethane : Methanol= 20:1, Rf = 0.60) indicated Reactant M-32 was consumed completely. The reaction mixture was quenched by adding NaHCO3(800 mL), and extracted with DCM (800 mL x 3). The combined organic layers were washed with brine (800 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product which was re-dissolve in DCM (160 mL) and dropped into ACN (4800 ml) with vigorous stirring. The solid was collected by filtration and dried under reduced pressure to give compound M-33 (96.0 g, 85.1 mmol, 98.69% yield) as a white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 7.78 (d, J = 8.4Hz 1H), 7.78 (d, J = 8.4Hz 1H), 6.82 (s, 1H), 6.63(s, 2H), 5.22(s, 2H), 3.97(t, J = 6.4Hz 1H), 3.41-3.98 (m, 8H), 1.75-1.84 (m, 6H), 1.26-1.48 (m, 96H), 0.88(t, J = 6.4Hz 9H).

[0279] General procedure for preparation of compound M34

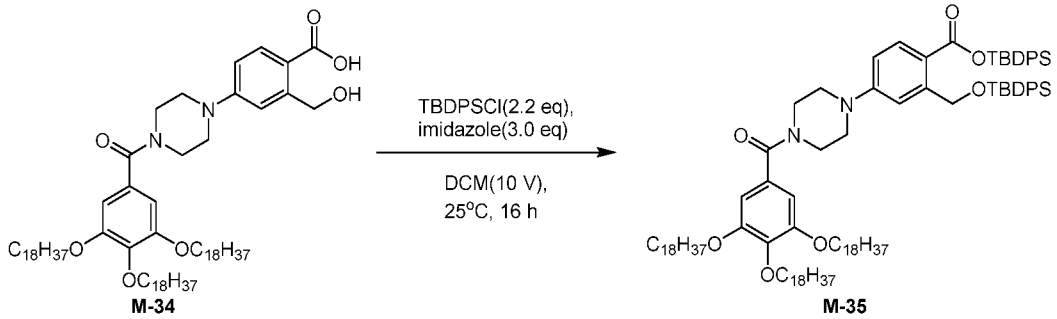


M-33 M-34

To a solution of M-33 (116 g, 1.0 eq) in THF (1160 mL) was added NaOH(20.56 g, 5.0 eq). The mixture was stirred at 60 °C for 12 hr. TLC (Dichloromethane : Methanol= 20:1, Rf = 0.50) indicated Reactant 1 was consumed completely. The reaction mixture was concentrated under reduced pressure to remove THF. The residue was diluted with H_2O 1.5 L. The solution

was adjusted to pH=5~6 with HCl (1M) and filtered and concentrated under reduced pressure to give a residue. The crude product was was co-evaporated with DCM, THF and ACN six times. Compound M-34 (87 g, 75.93 mmol, 73.82% yield) was obtained as a light yellow solid. ^{1}H NMR: 400 MHz $CDCl_3$ 8.01 (d, J = 8.4Hz 1H), 7.22 (s, 1H), 6.85 (s, 1H),6.75 (d, J = 8.8Hz, 1H), 6.68(s, 2H), 4.74(s, 2H), 3.97,\(t, J = 6.4Hz, 6H), 3.41-3.98 (m, 8H), 1.70-1.81 (m, 6H), 1.26-1.48 (m, 96H), 0.83(t, J = 6.4Hz, 9H),

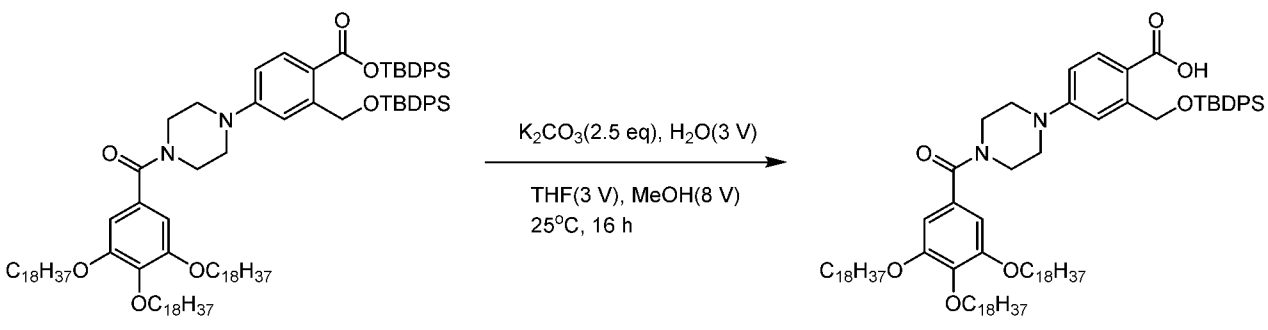
[0280] General procedure for preparation of compound M35



M-34 M-35

[0281] To a solution of compound M-34 (87 g, 1.0 eq) in DCM (870 mL) was added imidazole(14.96 g, 3.0 eq) and TBDPSCl (41.5 mL, 2.2 eq). The mixture was stirred at 25 °C for 16 hr. TLC (Dichloromethane : Methanol= 20:1, Rf = 0.90) indicated Reactant M-34 was consumed completely . The reaction mixture was quenched by addition of $NaHCO_3$ 600 mL, and extracted with DCM (700 mL x 3). The combined organic layers were washed with brine (500 mL x 2), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product which was re-dissolve in DCM (800 mL) and dropped into ACN (2.5 L) with vigorous stirring. The solid was filtered and concentrated under reduced pressure to give compound M-35 (118 g, crude) as a yellow solid. HPLC showed the starting material was consumed completely.

[0282] General procedure for preparation of compound M36



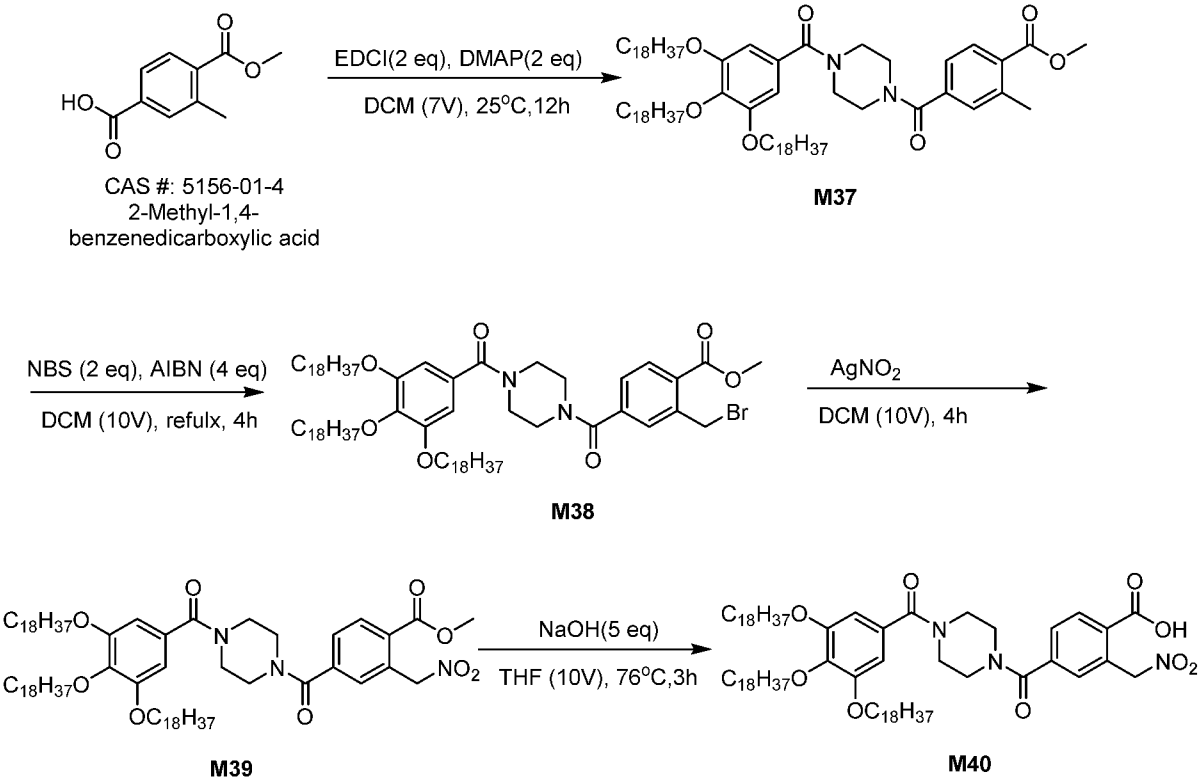
M-35 M-36

To a solution of compound M-35 (115 g, 1.0 eq) in THF (345 mL) and MeOH (920 mL) was added a solution of K_2CO_3 (24.72 g, 2.5 eq) in H_2O (345 mL). The mixture was stirred at

25 °C for 16 hr. TLC (Dichloromethane: Methanol= 20:1, Rf = 0.43) indicated compound M-35 was consumed completely. The reaction mixture was concentrated under reduced pressure to remove one quarter solvent. The residue was diluted with brine 450mL and extracted with DCM (500 mL x 4). The combined organic layers were washed with brine (500 mL x 3), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO2, Dichloromethane /Ethyl acetate=1/0 to 10/1). Compound M-36 (25 g, 93% purity) was obtained as a white solid. ^{1}H NMR: 400 MHz $CDCl_3$ 7.99 (d, J = 8.4Hz, 1H), 7.67-7.69 (m, 4H), 7.33-7.40 (m, 7H), 6.75 (dd, J = 2.0Hz, J = 8.8Hz, 1H), 6.63(s, 2H), 5.18(s, 2H), 3.97-4.00,(m, 6H), 3.38-3.98 (m, 8H), 1.70-1.81 (m, 6H), 1.26-1.48 (m, 90H), 0.90(s, 9H), 0.83(t, J = 6.4Hz, 9H).

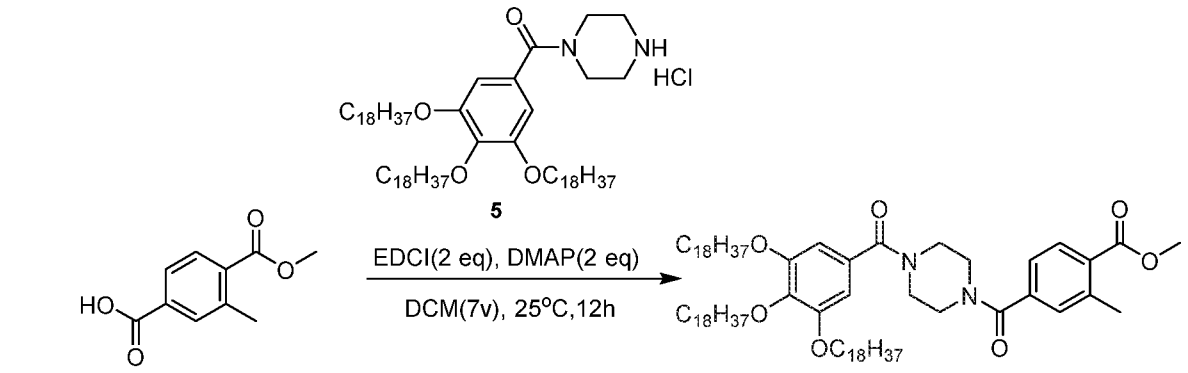
Example 4. Synthesis of Compound M40

[0283] *a. Scheme of M40 Synthesis*



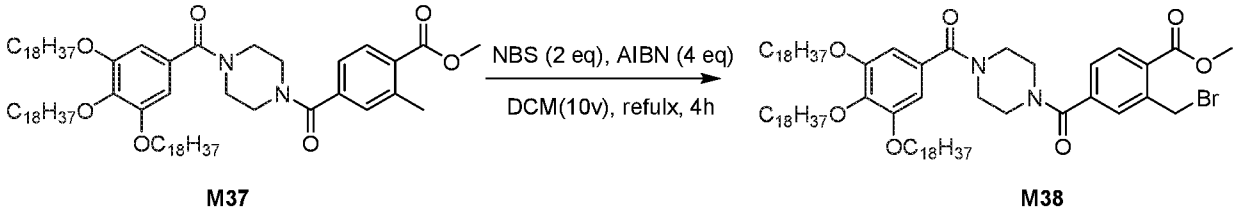
[0284] *b. Procedures for Synthesis of Compound M40*

[0285] General procedure for preparation of compound M37



[0286] To a mixture of compound 2-Methyl-1,4-benzenedicarboxylic acid (1.1 *eq*) and compound 5 (1.10 *eq*) in DCM (7V) was added DMAP (2.00 *eq*), EDCI (2.00 *eq*) in one portion at 25 °C under N_2. The mixture was stirred at 25 °C for 2 h. The TLC indicated that the start material was consumed completely. The reaction mixture was poured into EtOH (50 V), filtered and washed with EtOH (10V). Compound M37 was obtained as a white solid.

[0287] General procedure for preparation of compound M38

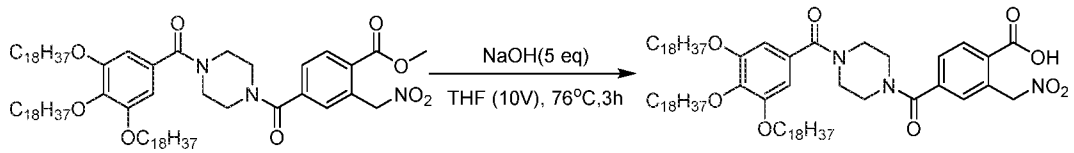


[0288] To a mixture of compound M37 (1.0 *eq*) and AIBN (4.0 eq) in DCM (10 V) was add NBS (2.0 eq) at 25 °C under N_2. The mixture was refluxed for 4 h, The TLC indicated that the start material was consumed completely. After cooling, washed the mixture with saturated aqueous $NaHCO_3$. Dried the mixture with $MgSO_4$ and concentrated the filtrate to residue which was precipitated in ACN to yield a white solid M38. *See JOC,* 2008, 73, 9125-9128.

[0289] General procedure for preparation of compound M39

[0290] To a solution of compound M38 (1.0 *eq*) in DCM (10 V) was add $AgNO_2$ (3.0 eq) at 25 °C under N_2. The mixture was stirred for 2 h. The TLC indicated that the start material was consumed completely. Washed the mixture with saturated aqueous $NaHCO_3$. Dried the mixture with $MgSO_4$ and concentrated the filtrate to residue which was precipitated in ACN to yield a white solid M39. *See Synthesis* 1980, 814-815.

[0291] General procedure for preparation of compound M40

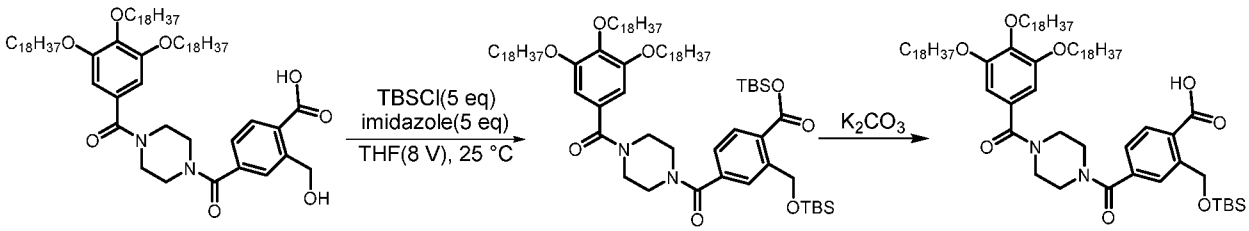


M39 M40

[0292] To a solution of compound M39 (1.0 eq) in THF (10 V) was added the solution of 1.0M NaOH (5.0 eq) slowly in one portion at 25 °C under N_2. The mixture was stirred at 25 °C and stirred for 3 h. The TLC indicated the start material was consumed completely. Concentrated and diluted with H_2O (50 V), adjust pH to 4~5 with 1N HCl. Filtered and washed with H_2O until the pH was 6~7. Washed with ACN. Compound M40 was obtained in 72% yield and 90% purity as a white solid.

Example 5. Synthesis of Compound M50

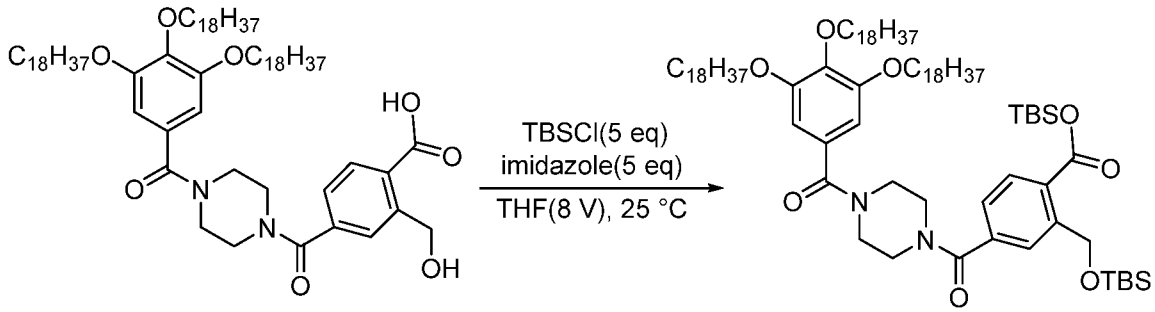
[0293] *a. Scheme for Synthesis of Compound M50*



M-18 M-50-A M-50

[0294] *b. Procedures for Synthesis of Compound M50*

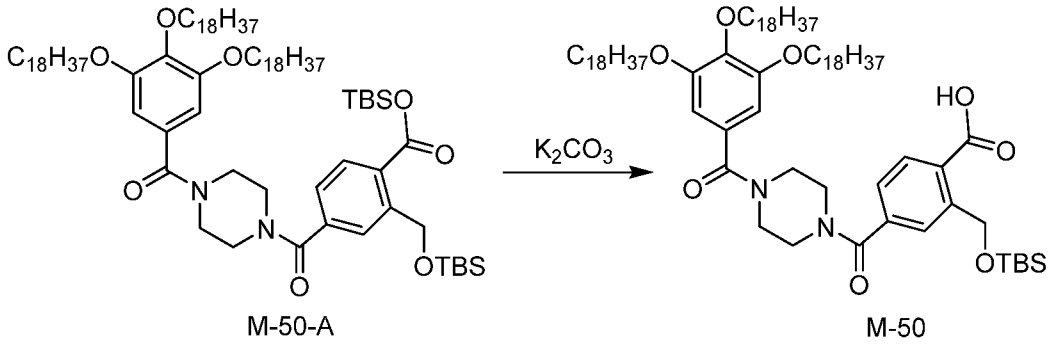
[0295] General procedure for preparation of Compound M-50-A



M18 M-50-A

[0296] To a solution of compound **M-18** (2.0 g, 1.70 mmol, 1.00 eq) in THF (16.0 mL) was added imidazole (579.99 mg, 8.52 mmol, 5.00 eq) and TBSCl (1.28 g, 8.52 mmol, 1.04 mL, 5.00 eq). The mixture was stirred at 25°C for 2 hrs. HPLC showed the starting material was consumed completely. The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Compound **M-50-A** (2.39 g, crude) was obtained as a white solid.

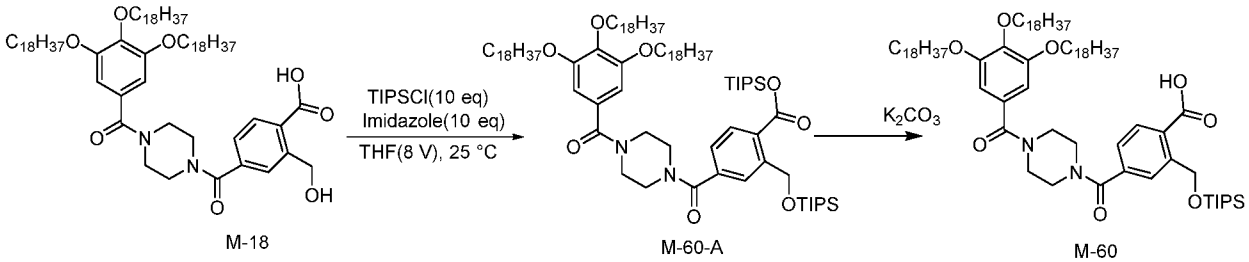
[0297] General procedure for preparation of Compound M-50



[0298] To a solution of compound **M-50-A** (2.39 g, 1.70 mmol, 1.00 eq) in THF (19.0 mL) and MeOH (2.30 mL) was added solution of K_2CO_3 (305.44 mg, 2.21 mmol,1.30 eq) in H_2O (7.0 mL). The mixture was stirred at 25°C for 3 hrs. TLC (DCM/MeOH = 20/1, start material Rf = 0.50, product Rf = 0.30) indicated the start material was consumed completely. Adjust pH to 5 with 1 M $KHSO_4$ (5 mL). The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with ACN (30 V, 70.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **M-50** (1.00 g, 93.8 % purity, 45.67 % yield) was obtained as a white solid.

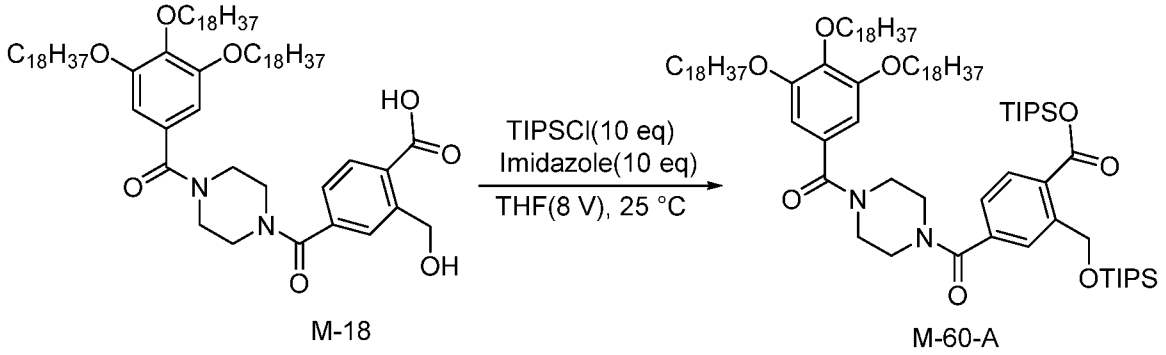
Example 6. Synthesis of Compound M60

[0299] *a. Scheme for Synthesis of Compound M60*



[0300] *b. Procedures for Synthesis of Compound M60*

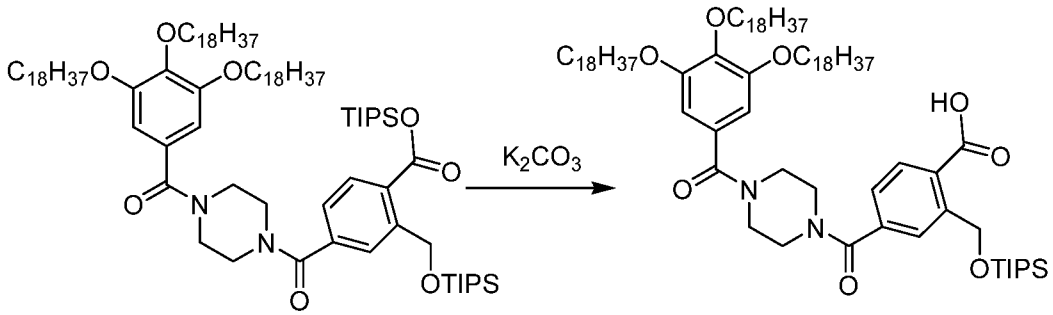
[0301] General procedure for preparation of Compound M-60-A



M-18 M-60-A

[0302] To a solution of compound M-18 (1.0 g, 0.85 mmol, 1.00 eq) in DCM (8.0 mL) was added imidazole (579.99 mg, 8.52 mmol, 10.0 eq) and TIPSCl (1.64 g, 8.52 mmol, 10.00 eq). The mixture was stirred at 25°C for 8 hrs. HPLC showed the starting material was consumed completely. The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Compound M-60-A (1.27 g, crude) was obtained as a white solid.

[0303] General procedure for preparation of Compound M-60



M-60-A M-60

[0304] To a solution of compound **M-60-A** (1.27 g, 0.85 mmol, 1.00 eq) in THF (10.4 mL) and MeOH (1.30 mL) was added solution of K_2CO_3 (153.5 mg, 1.11 mmol,1.30 eq) in H_2O (4.0 mL). The mixture was stirred at 25°C for 3 hrs. Adjust pH to 5 with 1 M $KHSO_4$ (3 mL). The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with ACN (30 V, 70.0 mL) at 25 °C for 30 min. Filtered and concentrated to afford compound **M-60** (0.90 g, 92 % purity, 72.7 % yield) as a white solid. Mass Calcd for $C_{83}H_{149}N_2O_8Si^+$ [M+H$^+$]: 1330.1, found 1330.4.

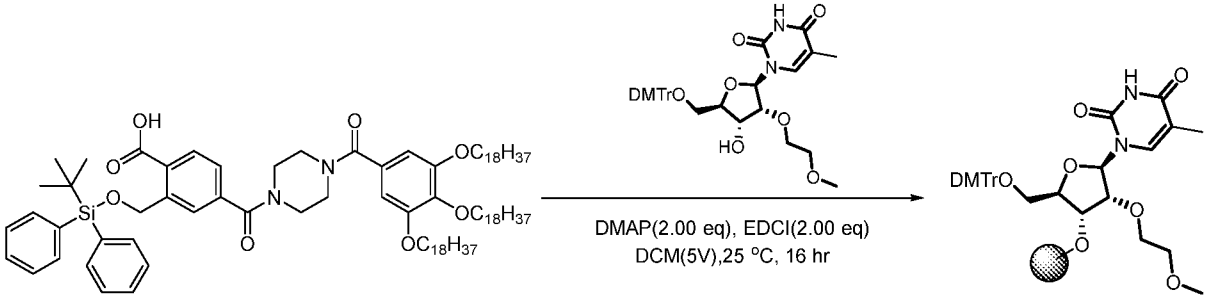
Example 7. Synthesis of Oligonucleotide Fragment A from Reagent M19

[0305] *a. Scheme for Synthesis of Oligonucleotide Fragment A*

[0306] Fragment A was synthesized according to synthetic scheme depicted in FIG 2.

[0307] *b. Procedures for Synthesis of Oligonucleotide Fragment A from M19*

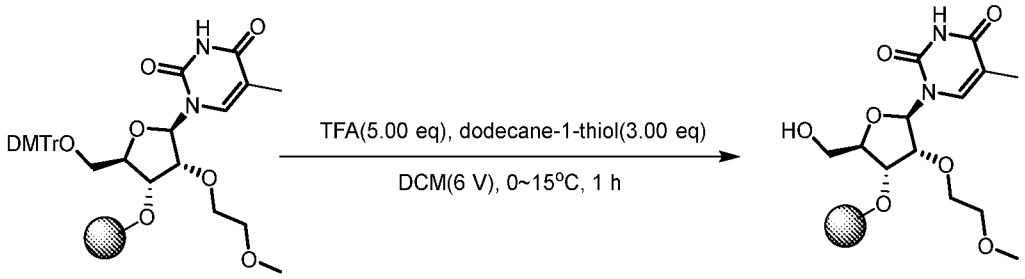
[0308] General procedure for preparation of Compound **M19-Fragment I-U-DMTr**



M19 **M19-Fragment I-U-DMTr**

[0309] To a solution of M19 (20.00 g, 14.16 mmol, 1.00 *eq*) and dU (13.14 g, 21.24 mmol, 1.50 *eq*) in DCM (200 mL) was added DMAP (3.46 g, 28.32 mmol, 2.00 *eq*) and EDCI (5.43 g, 28.32 mmol, 2.00 *eq*). The mixture was stirred at 25 °C for 16 hr. TLC (Dichloromethane: Methanol = 15:1, product, Rf = 0.70) indicated that the reaction was complete and one new spot formed. The reaction was clean according to TLC. The reaction mixture was washed with $NaHCO_3$ (5% aq, 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue to 3 V. The crude was dropped into MeOH (600 ml, 30 V) with vigorous stirring. Desire product was precipitated out. Compound M19-Fragment I-U-DMTr (27.00 g, 13.31 mmol, 94.54% yield) was obtained as a white solid.

[0310] General procedure for preparation of compound M19-Fragment I-U

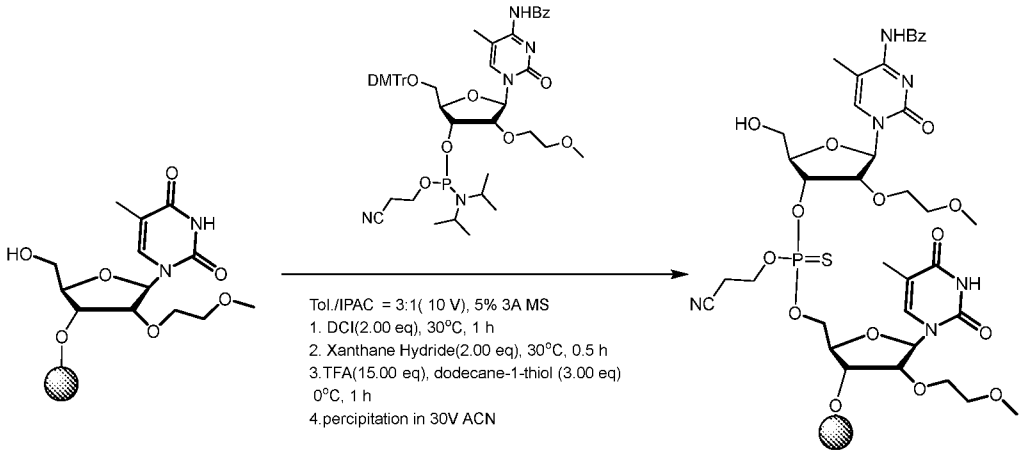


M19-Fragment I-U-DMTr **M19-Fragment I-U**

[0311] To a solution of M19-Fragment I-U-DMTr (27.0 g, 13.31 mmol, 1.00 *eq*) in DCM (170 mL) was added dodecane-1-thiol (8.15 g, 39.93 mmol, 9.64 mL, 3.00 *eq*) and TFA (7.59 g, 66.55 mmol, 4.92 mL, 5.00 *eq*) at 0 °C. The mixture was stirred at 0 °C for 1 hr. TLC (Dichloromethane: Methanol = 15:1, product, Rf = 0.54) indicated Reactant was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was quenched by addition $NaHCO_3$ (5% aq. 100 mL), and then extracted with DCM (100 mL). The combined organic layers were washed with brine (100 mL x 2),

dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude was re-dissolve in DCM (30 mL) and dropped into MeOH/ACN (3:1, 450 ml) with vigorous stirring. Desire product was precipitated outCompound M19-Fragment I-U (15.00 g, 8.77 mmol, 65.51% yield, and 98.5 % purity) was obtained as a white solid.

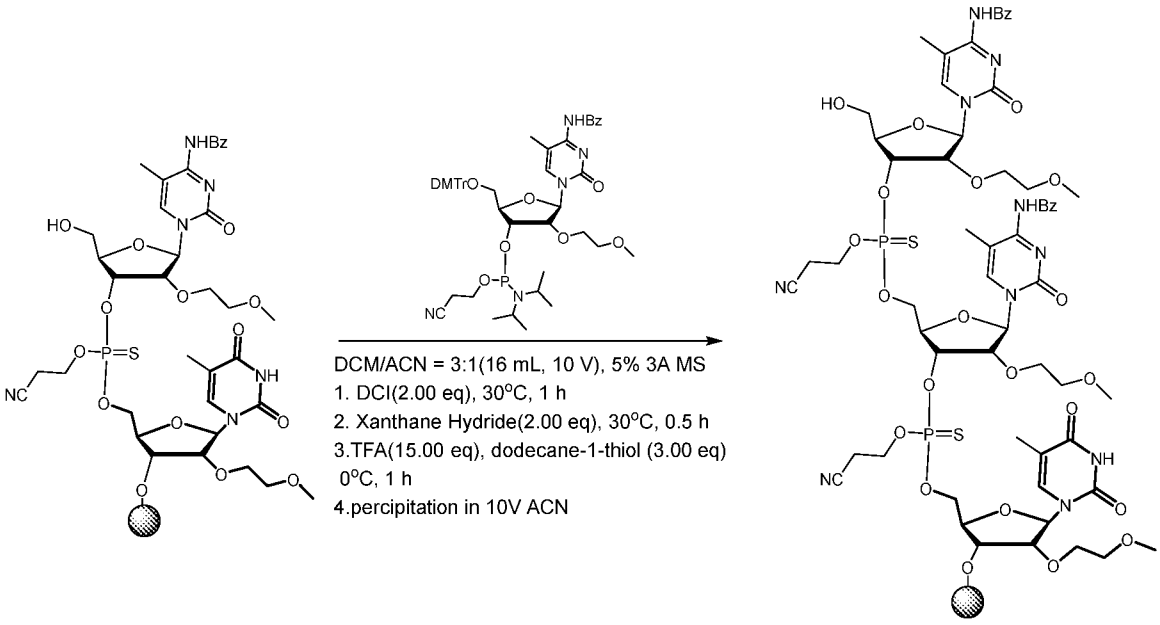
[0312] General procedure for preparation of compound M19-Fragment I-UC



M19-Fragment I-U M19-Fragment I-UC

[0313] To a solution of M19-Fragment I-U (15.00 g, 8.76 mmol, 1.00 *eq*) and dC (12.12 g, 13.14 mmol, 1.50 *eq*) in Tol./IPAC = 3:1 (120 mL) was added 3A MS(7.50 g) and stired for 1 hr. Then to the mixture was added DCI (2.07 g, 17.55 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: R_f = 0.53) indicated the reactant M19-Fragment I-U were consumed completely and one new spot formed. The reaction was clean according to TLC. To the crude was added HX (2.64 g, 17.55 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 0.5 hr, and then to the mixture was added dodecane-1-thiol (5.31 g, 26.31 mmol, 6.30 mL, 3.00 *eq*) and TFA (15.00 g, 134.49 mmol, 9.75 mL, 15.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: R_f = 0.43) indicated the reactant was consumed completely and one new spot formed. The reaction was clean according to TLC. The reaction mixture was diluted with NMI (175.2 mmol, 14 mL, 20.00 *eq*) to pH 7, filtered and concentrated under reduced pressure to give a residue. The filter liquor was dropped into ACN (800 ml), precipitated for 0.5 h, filtered for 3.5 h with 9 cm Buchner funnel to give 14.3 g (98 % yield, 97.7 % purity) as a white solid.

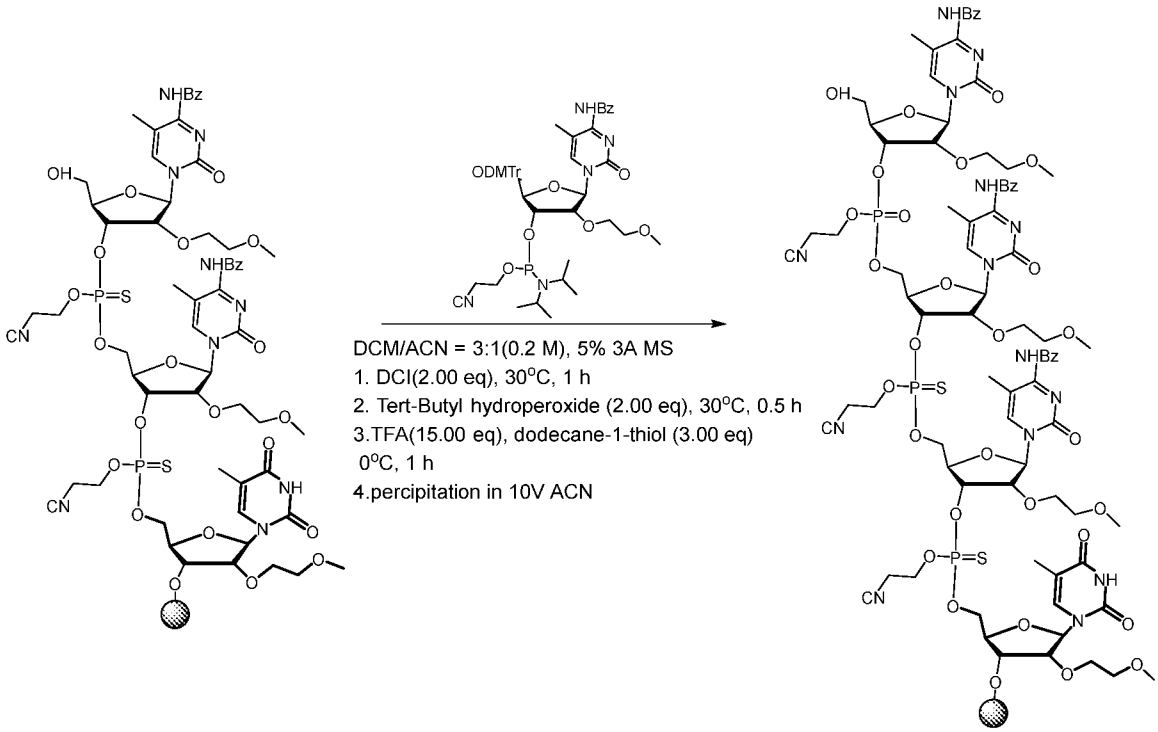
[0314] General procedure for preparation of compound M19-Fragment I-UCC



M19-Fragment I-UC M19-Fragment I-UCC

[0315] To a solution of M19-Fragment I-UC (19.00 g, 8.40 mmol, 1.00 *eq*) and dC (11.55 g, 12.60 mmol, 1.50 *eq*) in Tol./IPAC = 3:1 (150 mL) was added 3A molecular sieve(7.5 g). The mixture was stirred for 1 hr, followed by DCI (1.97 g, 16.80 mmol, 2.00 *eq*). Then it was stirred at 30 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: Rf = 0.53) indicated the compound M19-Fragment I-UC was consumed completely and one new spot was formed. The reaction was clean according to TLC. To the mixture was added HX (2.51 g, 16.80 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 0.5 hr, followed by dodecane-1-thiol (5.04 g, 25.20 mmol, 5.94 mL, 3.00 *eq*) and TFA (14.16 g, 126 mmol, 9.21 mL, 15.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 1 hr. TLC (Dichloromethane: Methanol = 15:1, product: Rf = 0.43) indicated the reaction was complete and one new spot was formed. The reaction was clean according to TLC. To the reaction mixture was added NMI (168 mmol, 14.5 mL, 20.00 *eq*). The mixture was dropped into ACN (800 mL). The solid was precipitated for 0.5 h and filtered for 2.0 h with 15 cm Buchner funnel to afford 15.4 g (94 % yield, 95.0 % purity) as a white solid.

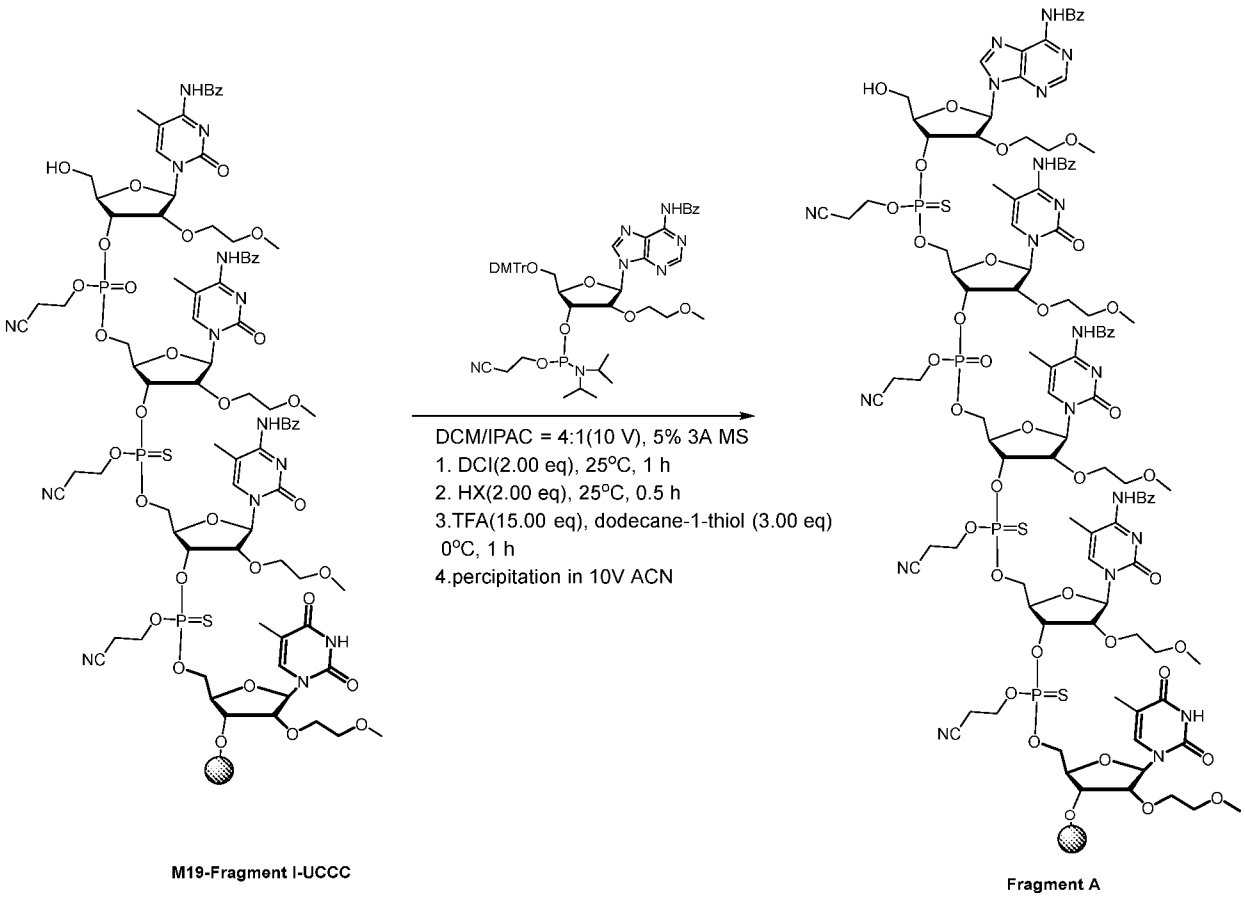
[0316] General procedure for preparation of compound M19-Fragment I-UCCC



M19-Fragment I-UCC M19-Fragment I-UCCC

[0317] To a solution of M19-Fragment I-UCC (5.00 g, 1.78 mmol, 1.00 eq) and dC (2.46 g, 2.67 mmol, 1.50 eq) in Tol./ACN = 3:1 (40 mL) was added 3A MS(2.0 g). The mixture was stirred at 30°C for 1 hr, followed by DCI (420.05 mg, 3.56 mmol, 2.00 eq). The mixture was stirred at 30 °C for 1 hr. TLC (Dichloromethane: Methanol = 15:1, Product: R_f = 0.53) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. To the mixture was added tert-butyl hydroperoxide (320.5 mg, 3.56 mmol, 2.00 eq). The mixture was stirred at 30 °C for 0.5 hr, followed by dodecane-1-thiol (1.08 g, 5.34 mmol, 1.28 mL, 3.00 *eq*) and TFA (3.04 g, 26.68 mmol, 1.98 mL, 15.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: R_f = 0.43) indicated that the reaction was complete and one new spot formed. The reaction mixture was quenched by addition $NaHCO_3$ (2%) 100 mL and Na_2SO_3 (2 eq) at **0** °C, filtered and extracted with DCM (**80** mL x **3**). The combined organic layers were washed with brine (**100** mL x **2**), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was dropped into ACN (200 mL) with vigorous stirring. Desire product was precipitated out. The cake was washed with ACN (30 mL*2), filtered for 1.5 h with 7 cm Buchner funnel to afford 5.00 g (84 % yield, 90.9 % purity) as a white solid.

[0318] General procedure for preparation of Oligonucleotide Fragment A



M19-Fragment I-UCCC

Fragment A

[0319] To a solution of M19-Fragment I-UCCC (4.00 g, 1.20 mmol, 1.00 eq) and dA (1.67 g, 1.79 mmol, 1.50 eq) in Tol./ACN = 3:1 (40 mL) was added 3A MS(2.0 g). The mixture was stirred at 30°C for 1 hr, folloed by DCI (282.36 mg, 2.39 mmol, 2.00 eq). The mixture was stirred at 30 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, Product: R_f = 0.53) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC, and then added glucose (0.5 eq). To the mixture was added HX (358.99 mg, 2.39 mmol, 2.00 eq). The mixture was stirred at 30 °C for 0.5 hr, and then was added dodecane-1-thiol (725.66 mg, 3.59 mmol, 0.86 mL, 3.00 *eq*) and TFA (2.04 g, 17.93 mmol, 1.33 mL, 15.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 1 hr. TLC (Dichloromethane: Methanol = 15:1, product: R_f = 0.43) indicated the reaction was complete and one new spot was formed. The reaction mixture was quenched by addition of $NaHCO_3$ (2%) 80 mL at **0** °C, filtered and extracted with DCM (**50** mL x **3**). The combined organic layers were washed with brine (**80** mL x **2**), dried over anhydrous Na_2SO_4,filtered and concentrated under reduced pressure to give a residue, which was dropped into ACN (200 mL) with vigorous stirring. Desire product was precipitated out. The cake was washed with ACN (30 mL*2), filtered for 1.5 h with 7 cm Buchner funnel, to afford 4.20 g (85 % yield, 86.4 % purity) of fragment A as a white solid.

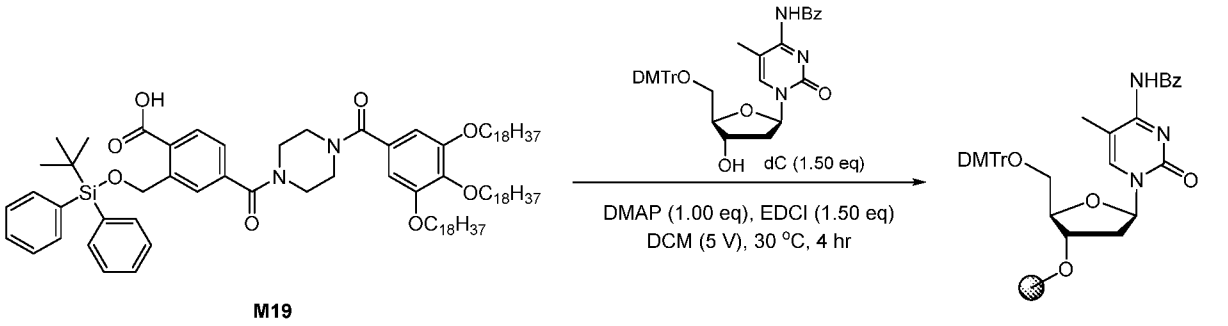
Example 8. Synthesis of Oligonucleotide Fragment B from Reagent M19

[0320] *a. Scheme for Synthesis of Oligonucleotide Fragment B*

[0321] Fragment B was synthesized according to synthetic scheme depicted in FIG. 3.

[0322] *b. Procedures for Synthesis of Oligonucleotide Fragment B from M19*

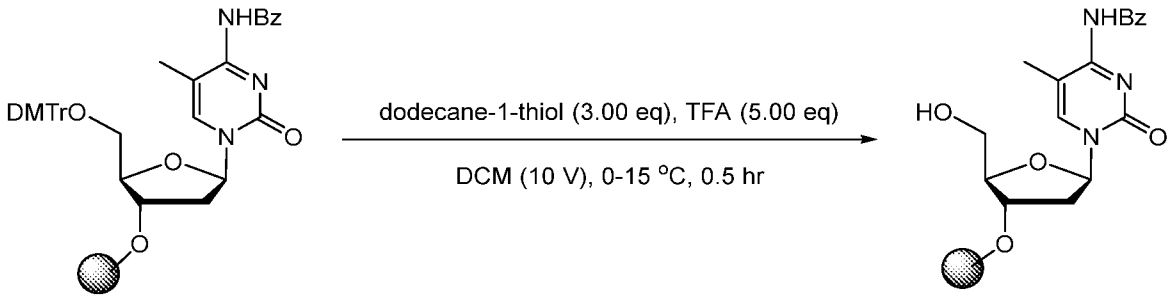
[0323] General procedure for preparation of Compound **M19-Fragment III-C-DMTr**



M19 M19-Fragment III-C-DMTr

[0324] To a solution of **M19** (20.00 g, 14.16 mmol, 1.00 *eq*) and **dC** (13.76 g, 21.24 mmol, 1.50 *eq*) in DCM (200 mL) was added DMAP (1.73 g, 14.16 mmol, 1.00 *eq*) and EDCI (4.08 g, 21.24 mmol, 1.50 *eq*). The mixture was stirred at 30 °C for 12 hr. TLC (Dichloromethane: Methanol = 10:1, product, Rf = 0.70) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The reaction mixture was washed with $NaHCO_3$ (300 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue to 5 V, which was dropped into ACN/MeOH (3:1, 1000 ml, 50 V) with vigorous stirring. Desired product was precipitated out. Compound **M19-Fragment III-C-DMTr** (28.90 g, 14.15 mmol, 99.94% yield) was obtained as a white solid.

[0325] General procedure for preparation of Compound **M19-Fragment III-C**

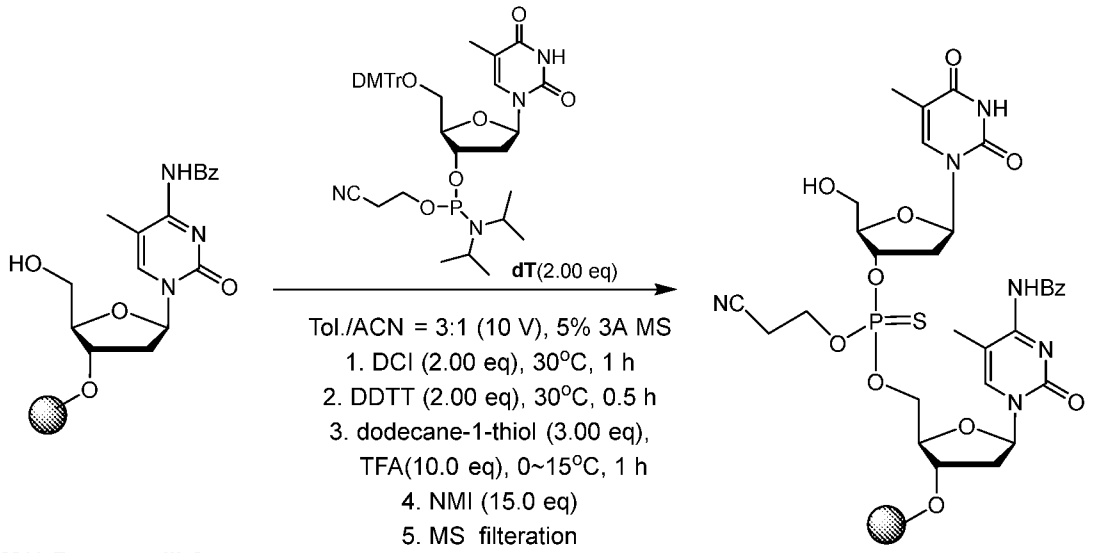


M19-Fragment III-C-DMTr M19-Fragment III-C

[0326] To a solution of **M19-Fragment III-C-DMTr** (28.0 g, 13.72 mmol, 1.00 *eq*) in DCM (250 mL) was added dodecane-1-thiol (8.32 g, 41.12 mmol, 9.84 mL, 3.00 *eq*) and TFA (7.80 g, 68.56 mmol, 5.08 mL, 5.00 *eq*). The mixture was stirred at 0 °C for 1 hr. TLC

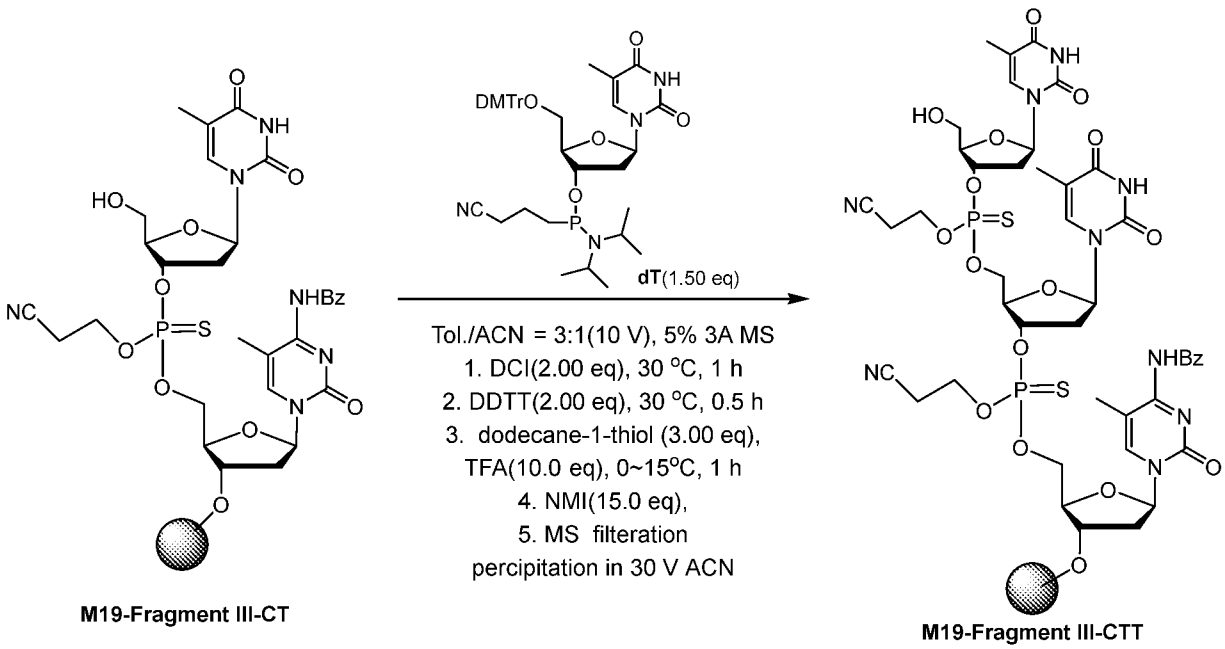
(Dichloromethane: Methanol = 10:1, product, Rf = 0.54) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The reaction mixture was diluted with NMI (9.00 g, 109.72 mmol, 8.76 mL, 8.00 *eq*). The crude was dropped into ACN (900 ml, 30 V) with vigorous stirring. Desired product was precipitated out. Compound **M19-Fragment III-C** (23.00 g, 13.22 mmol, 96.42% yield) was obtained as a white solid.

[0327] General procedure for preparation of Compound **M19-Fragment III-CT**



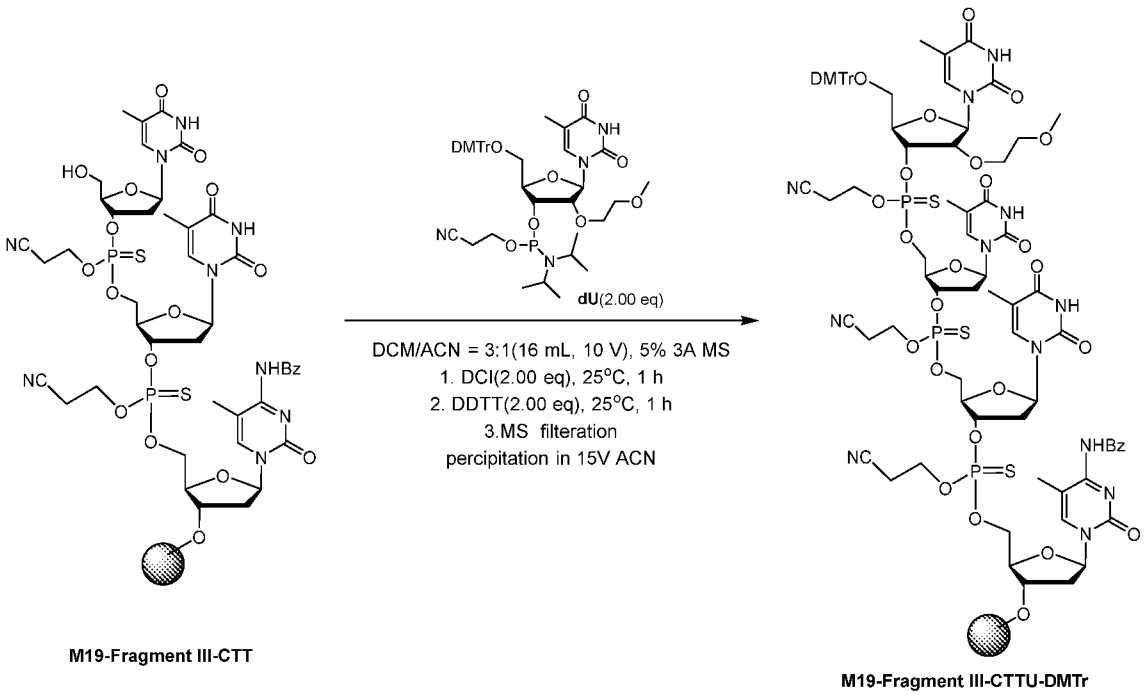
[0328] To a solution of **M19-Fragment III-C** (16.00 g, 9.20 mmol, 1.00 *eq*) and **dT** (10.28 g, 13.80 mmol, 1.50 *eq*) in Tol./ACN = 3:1 (140 mL) was added 3A MS(7.00 g) and stired for 1 hr. The mixture was added DCI (2.17 g, 18.40 mmol, 2.00 *eq*). The mixture was stirred at 25 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: Rf = 0.53) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. To the reaction mixture was added HX (2.77 g, 18.42 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 0.5 hr, followed by dodecane-1-thiol (5.61 g, 27.70 mmol, 6.63 mL, 3.00 *eq*) and TFA (10.53 g, 92.33 mmol, 6.84 mL, 10.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 0.5 hr. TLC (Dichloromethane: Methanol = 20:1, product: Rf = 0.45) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The reaction mixture was diluted with NMI (11.37 g, 138.50 mmol, 11.04 mL, 15.00 *eq*). The crude was dropped into ACN (500 ml, 30 V), precipitated for 0.5 h, filtered for 1.5 h with 15 cm Buchner funnel to give 14.0 g (93.30 % yield, 97.28 % purity) M19-Fragment III-CT as a white solid.

[0329] General procedure for preparation of Compound **M19-Fragment III-CTT**



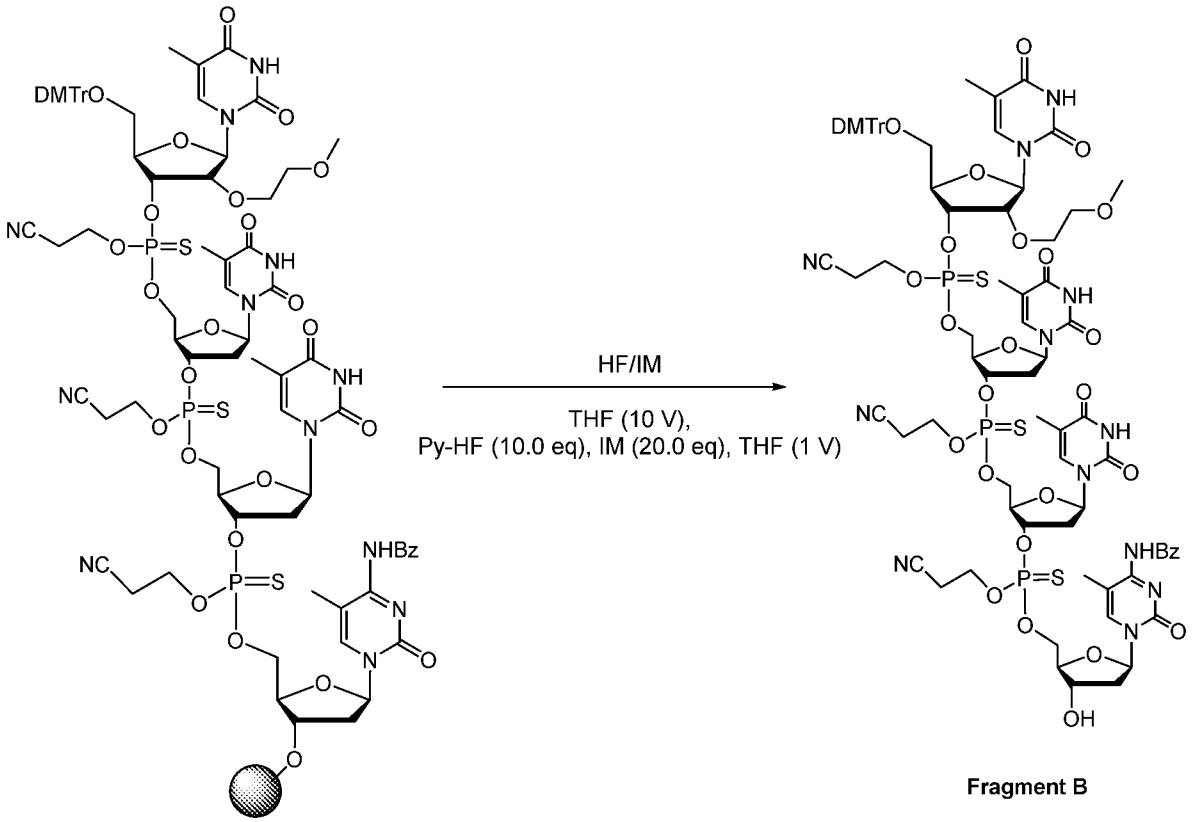
[0330] To a solution of **M19-Fragment III-CT** (15.00 g, 7.10 mmol, 1.00 *eq*) and **dT** (7.93 g, 10.65 mmol, 1.50 *eq*) in Tol./ACN = 3:1 (120 mL) was added 3A molecular sieve(2.00 g). The mixture was stirred for 1 hr, followed by DCI (1.68 g, 14.20 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 1 hr. TLC (Dichloromethane: Methanol = 20:1, product: Rf = 0.42) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The crude was added HX (2.16 g, 14.37 mmol, 2.00 *eq*). The mixture was stirred at 30 °C for 0.5 hr, and then was added dodecane-1-thiol (4.37 g, 21.57 mmol, 5.17 mL, 3.00 *eq*) and TFA (8.20 g, 71.90 mmol, 5.32 mL, 10.00 *eq*) at 0°C. The mixture was stirred at 0 °C for 0.5 hr. TLC (Dichloromethane: Methanol = 10:1, product: Rf = 0.54) indicated the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The reaction mixture was added NMI (8.86 g, 107.85 mmol, 8.60 mL, 15.00 *eq*). The mixture was dropped into ACN (200 mL, 30 V), precipitated for 0.5 h, filtered for 1.0 h with 7 cm Buchner funnel, to afford 5.76 g (96.60 % yield, 93.99 % purity) of M19-Fragment III-CTT as a white solid.

[0331] General procedure for preparation of Compound **M19-Fragment III-CTTU-DMTr**



[0332] To a solution of **M19-Fragment III-CTT** (10.5 g, 4.22 mmol, 1.00 eq) and **dU** (5.19 g, 6.34 mmol, 1.50 eq) in Tol./ACN = 3:1 (80 mL) was added 3A MS (0.5 g). The mixture was stirred at 25°C for 1 hr, followed by DCI (997.52 mg, 8.45 mmol, 2.00 eq). The mixture was stirred at 25 °C for 1 hr. TLC (Dichloromethane: Methanol = 10:1, Product: Rf = 0.50) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The crude was added HX (1.74 g, 8.46 mmol, 2.00 eq). The mixture was stirred at 25 °C for 0.5 hr. The reaction mixture was filtered and concentrated under reduced pressure to give a residue to 5V, which was dropped into ACN (150 mL, 30 V), precipitated for 0.5 h, filtered for 1.0 h with 7 cm Buchner funnel, to afford 4.22 g of M19-Fragment III-CTTU-DMTr (92.5 % yield, 91.7 % purity) as a white solid.

[0333] General procedure for preparation of oligonucleotide fragment B



[0334] To a solution of imidazole (631.13 mg, 9.27 mmol, 20.00 eq) in THF (2.00 mL) was dropwise added pyridine hydrofluoride (132.51 mg, 4.64 mmol, 120.46 uL, 70% purity, 10.00 eq) at 0°C. The mixture was added into the solution of **M19-Fragment III-CTTU-DMTr** (1.50 g, 463.54 umol, 1.00 eq) in THF (15.00 mL). The mixture was stirred at 0-20 °C for 1 hr. TLC (Dichloromethane: Ethyl acetate: Methanol = 20:10:1, Product: Rf = 0.05) indicated that the reaction was complete and one new spot was formed. The reaction was clean according to TLC. The reaction mixture was washed with NaHCO3 (30 mL), DI water to PH = 7, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was dissolved with DCM (3 ml, 2V) and dropped into TBME (50 ml) with vigorous stirring. Desired product was precipitated out. Oligonucleotide II (800 mg, 434.37 umol, 93.71% yield) was obtained as a white solid.

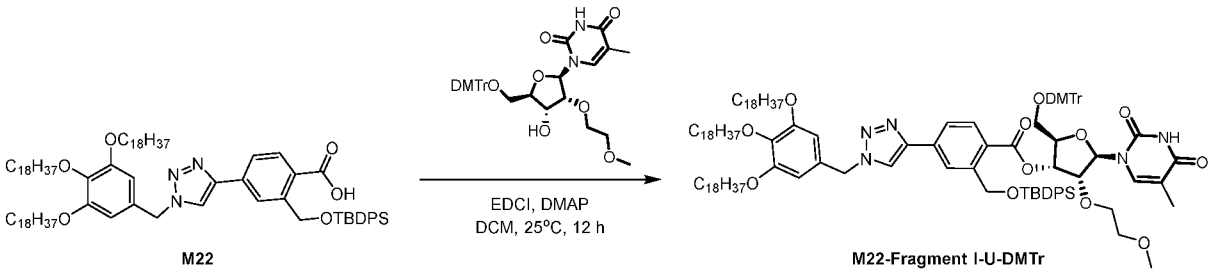
Example 9. Synthesis of Oligonucleotide Fragment C

[0335] *a. Scheme for Synthesis of Oligonucleotide Fragment C from M22*

[0336] Fragment C was synthesized according to synthetic scheme depicted in FIG. 4.

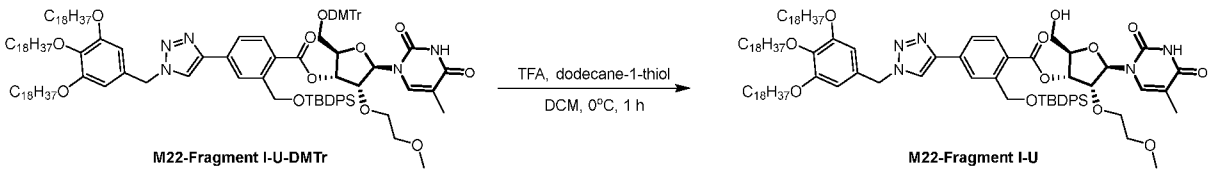
[0337] *b. Procedures for Synthesis of Oligonucleotide Fragment C from M22*

[0338] General procedure for preparation of compound M22-Fragment I-U-DMTr



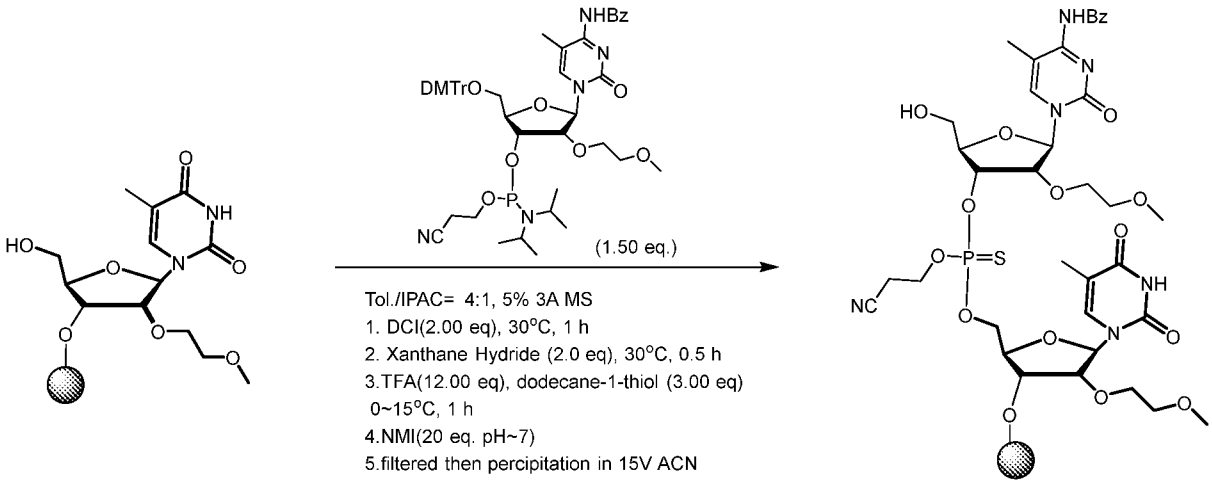
[0339] To a solution of M22 (3.10 g, 2.30 mmol) and compound U-DMTr (2.84 g, 4.58 mmol) in DCM (18.0 mL) was added DMAP (560 mg, 4.58 mmol) and EDCI (879 mg, 4.58 mmol). The mixture was stirred at 25°C for 12 h. TLC (Petroleum ether/Ethyl acetate = 1/1, product: $R_f = 0.34$) indicated M22 was consumed, and one major new spot was detected. The reaction mixture was concentrated under reduced pressure. The residue was diluted with DCM (100 mL) and washed with saturated $NaHCO_3$ solution (40.0 mL x 3). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was re-dissolve in DCM (60.0 mL) and dropped into MeOH/ACN (3/1, 300 mL) with vigorous stirring. Desired product was precipitated out, filtered and concentrated under reduced pressure to give a residue. M22-Fragment I-U-DMTr (5.20 g, crude) was obtained as a white solid.

[0340] General procedure for preparation of compound M22-Fragment I-U



[0341] To a solution of M22-Fragment I-U-DMTr (5.10 g, 2.61 mmol) in DCM (30.0 mL) was added dodecane-1-thiol (1.58 g, 7.83 mmol, 1.88 mL) and TFA (2.38 g, 20.9 mmol, 1.55 mL). The mixture was stirred at 0°C for 1 h. TLC (Petroleum ether/Ethyl acetate = 1/1, product: $R_f = 0.28$) indicated M22-Fragment I-U-DMTr was consumed completely and two new spots formed. The reaction mixture was quenched by addition Py (26.0 eq) at 0°C, and then diluted with DCM (150 mL) and washed with saturated $NaHCO_3$ aqueous solution (150 mL x 4). The combined organic layers were washed with brine (150 mL x 3), dried over anhydrous Na_2SO_4 Na_2SO_4 (15.0 g), filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, DCM/MeOH = 150/1 to 100/1). M22-Fragment I-U (2.90 g, 64.6% yield, 96.0% purity) was obtained as a white solid.

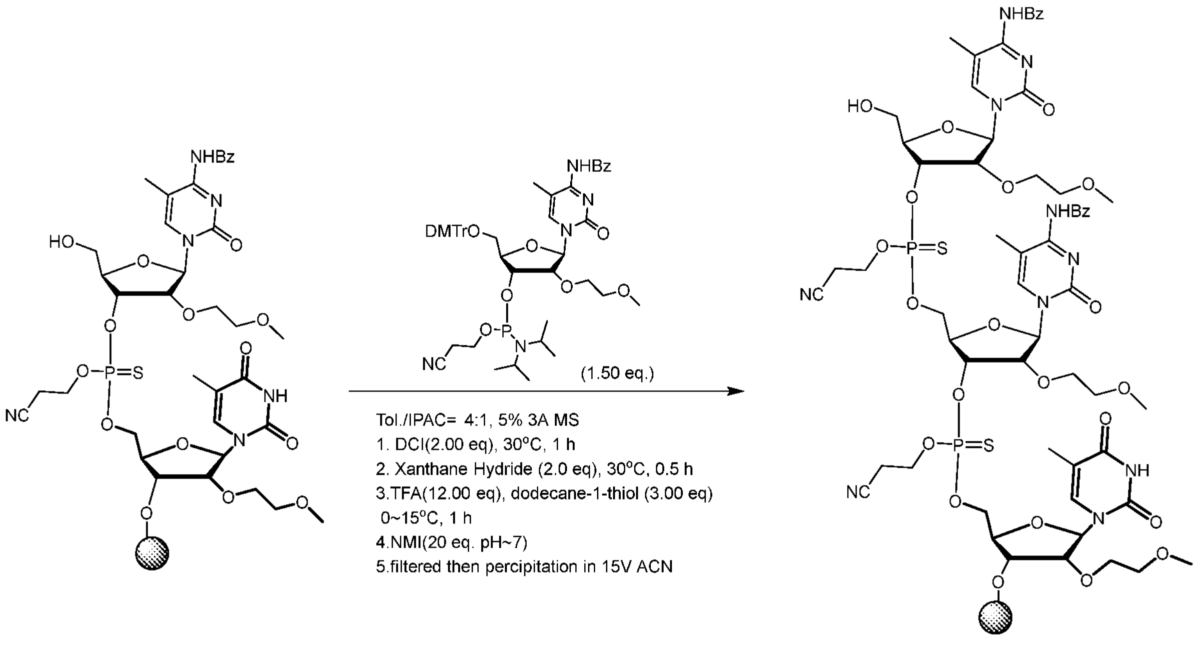
[0342] General procedure for preparation of compound M22-Fragment I-UC



M22-Fragment I-U M22-Fragment I-UC

[0343] To a solution of M22-Fragment I-U (2.00 g, 1.21 mmol) and C-DMTr (1.67 g, 1.82 mmol) in Tol. (9.60 mL) and IPAC (2.40 mL) was added DCI (286 mg, 2.42 mmol) and Molecular sieve 3A (2.00 g). The mixture was stirred at 30°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, product: R_f = 0.43) indicated M22-Fragment I-U was consumed completely and many new spots formed. Then Xanthane Hydride (365 mg, 2.42 mmol) was added into the reaction mixture. Then mixture was stirred at 30°C for 0.5 h. Then dodecane-1-thiol (727 mg, 3.60 mmol, 860 uL) and TFA (2.04 g, 18.0 mmol, 1.33 mL). The mixture was stirred at 0°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, product: R_f = 0.34) indicated M22-Fragment I-UC-DMTr was consumed completely and many new spots formed. The reaction mixture was quenched by addition NMI (20.0 eq) and then filtered. Then it was triturated by ACN (300 mL) and filtered. M22-Fragment I-UC (2.64 g, 95.2% yield, 95.1% purity) was obtained as a white solid.

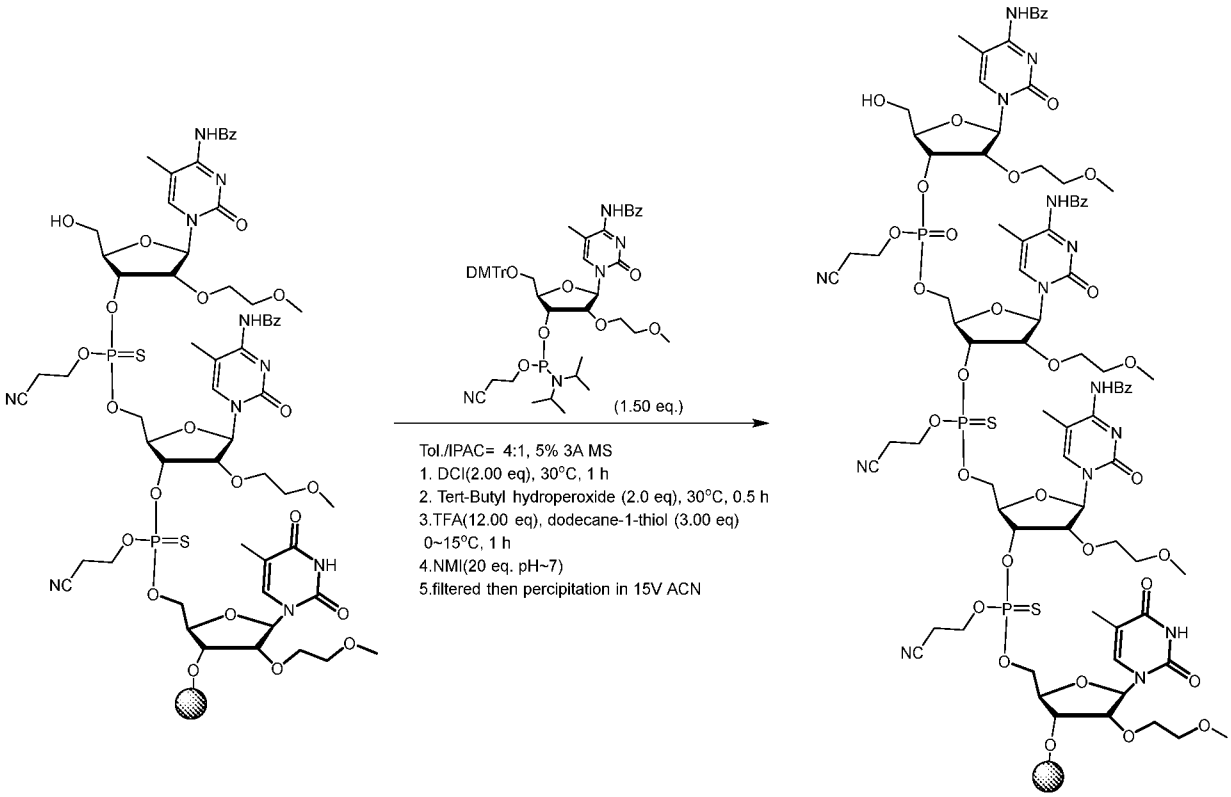
[0344] General procedure for preparation of compound M22-Fragment I-UCC



M22-Fragment I-UC M22-Fragment I-UCC

[0345] To a solution of M22-Fragment I-UC (2.60 g, 1.18 mmol) and C-DMTr (1.64 g, 1.77 mmol) in Tol. (12.6 mL) and IPAC (3.00 mL) was added DCI (279 mg, 2.36 mmol) and Molecular sieve 3A (2.00 g). The mixture was stirred at 30°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, product: Rf = 0.48) indicated M22-Fragment I-UC was consumed completely and many new spots formed. Then Xanthane Hydride (358 mg, 2.38 mmol) was added into the reaction mixture. The mixture was stirred at 30°C for 0.5 h. Then dodecane-1-thiol (716 mg, 3.54 mmol, 846 uL) and TFA (2.02 g, 17.7 mmol, 1.31 mL). The mixture was stirred at 0°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, product: Rf = 0.41) indicated M22-Fragment I-UCC-DMTr was consumed completely and two new spots formed. The reaction mixture was quenched by addition NMI (20.0 eq) and then filtered. Then it was triturated by ACN (300 mL) and filtered. M22-Fragment I-UCC (3.23 g, crude) was obtained as a white solid.

[0346] General procedure for preparation of compound M22-Fragment I-UCCC

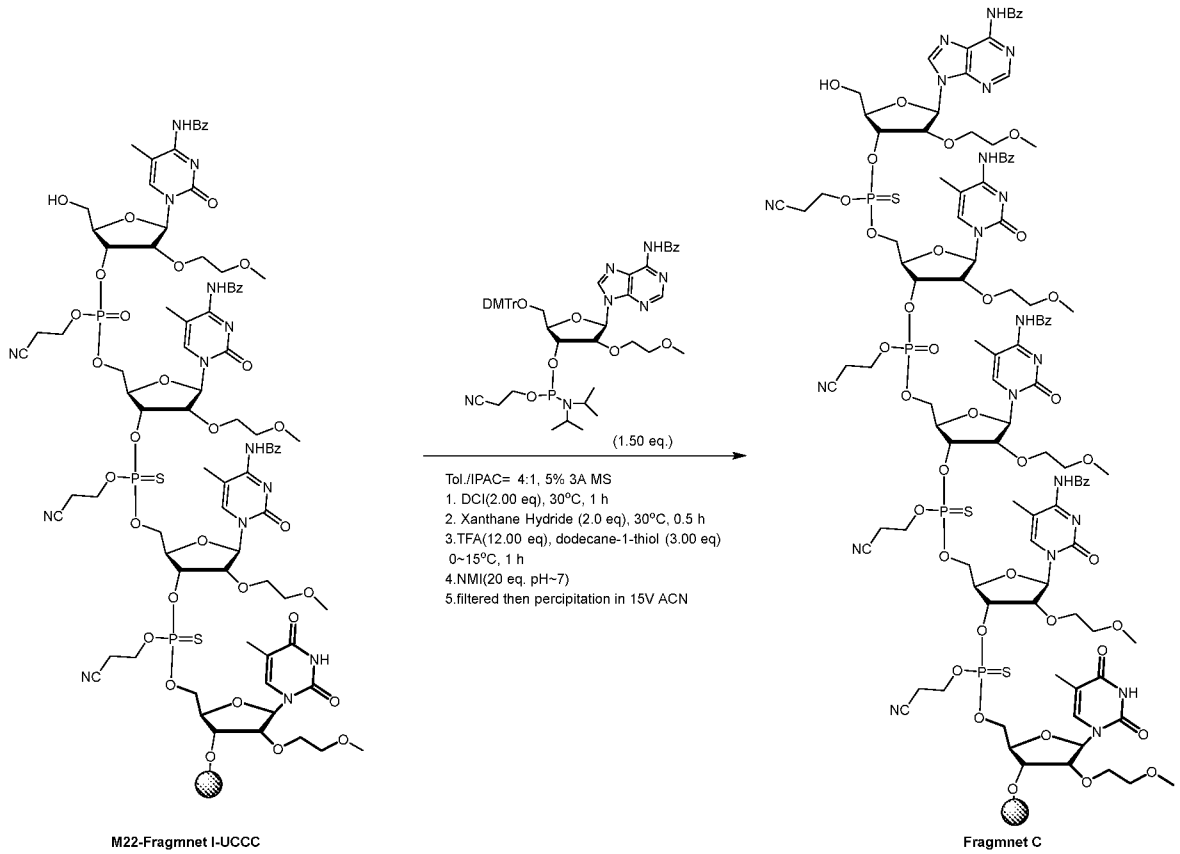


M22-Fragment I-UCC

M22-Fragment I-UCCC

[0347] To a solution of M22-Fragment I-UCC (3.20 g, 1.16 mmol) and C-DMTr (1.61 g, 1.74 mmol) in Tol. (15.4 mL) and ACN (3.80 mL) was added DCI (275 mg, 2.32 mmol) and Molecular sieve 3A (1.50 g). The mixture was stirred at 30°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, two times, R_f = 0.59) indicated M22-Fragment I-UCC was consumed completely and many new spots formed. Then Tert-Butyl hydroperoxide (5.50 M, 1.18 mmol, 106.85 uL) was added into the reaction mixture. The mixture was stirred at 30°C for 0.5 h. Then dodecane-1-thiol (711 mg, 3.52 mmol, 840 uL) and TFA (2.00 g, 17.6 mmol, 1.30 mL). The mixture was stirred at 0°C for 1 h. TLC (Dichloromethane/Methanol = 20/1, three times, product: R_f = 0.54) indicated M22-Fragment I-UCCC-DMTr was consumed completely and two new spots formed. The reaction mixture was quenched by addition NMI (20.0 eq) and then filtered. Then it was triturated by ACN (300 mL) and filtered. The white solid was concentrated under reduced pressure to give a residue. M22-Fragment I-UCCC (3.60 g, crude) was obtained as a white solid.

[0348] General procedure for preparation of oligonucleotide fragment C



[0349] To a solution of M22-Fragment I-UCCC (3.40 g, 1.03 mmol) and A-DMTr (1.45 g, 1.55 mmol) in Tol. (16.0 mL) and ACN (4.00 mL) was added DCI (244 mg, 2.06 mmol) and Molecular sieve 3A (1.00 g). The mixture was stirred at 30°C for 1 h. TLC (Dichloromethane/Methanol = 15/1, three times, $R_f = 0.47$) indicated M22-Fragment I-UCCC was consumed completely and many new spots formed. Then Xanthane Hydride (307 mg, 2.04 mmol) was added into the reaction mixture. The mixture was stirred at 30°C for 0.5 h. Then dodecane-1-thiol (615 mg, 3.04 mmol, 727 uL) and TFA (1.73 g, 15.2 mmol, 1.12 mL) was added into the reaction mixture, and the mixture was stirred at 0°C for 1 h. TLC (Dichloromethane/Methanol = 15/1, three times, product: $R_f = 0.45$) indicated M22-Fragment I-UCCCA-DMTr was consumed completely and two new spots formed. The reaction mixture was quenched by addition NMI (20.0 eq) and then filtered. Then it was triturated by ACN (600 mL) and filtered. Oligonucleotide fragment C (3.90 g, 90.3% yield and 84.2% purity) was obtained as a white solid.

Example 10. Synthesis of Oligonucleotide Fragment D

[0350] *a. Scheme for Synthesis of Oligonucleotide Fragment D*

[0351] Fragment D was synthesized according to synthetic scheme depicted in FIG. 5.

[0352] *b. Procedures for Synthesis of Oligonucleotide Fragment D*

[0353] Procedures for synthesizing oligonucleotide fragment D were similar to those described for the synthesis of oligonucleotide fragment A.

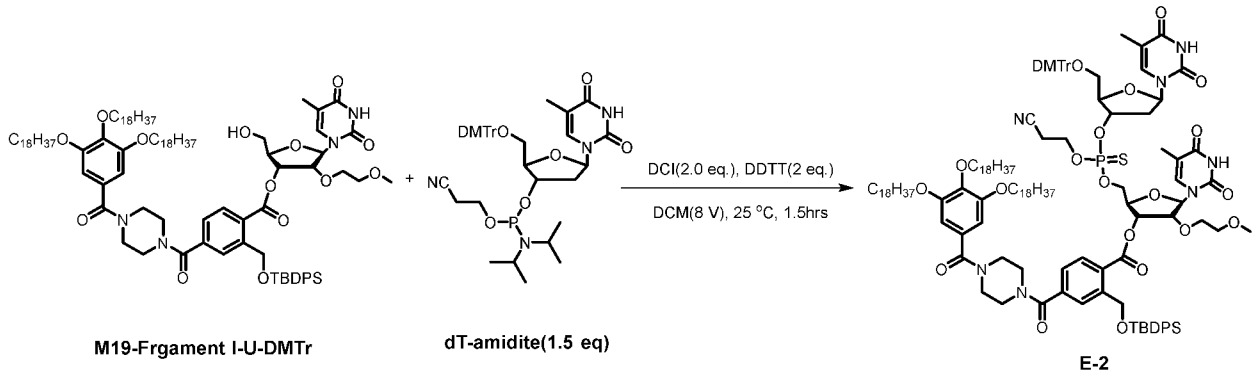
Example 11. **Synthesis of Oligonucleotide Fragment E**

[0354] *a. Scheme for Synthesis of Oligonucleotide Fragment E*

[0355] Fragment E was synthesized according to synthetic scheme depicted in FIG. 6.

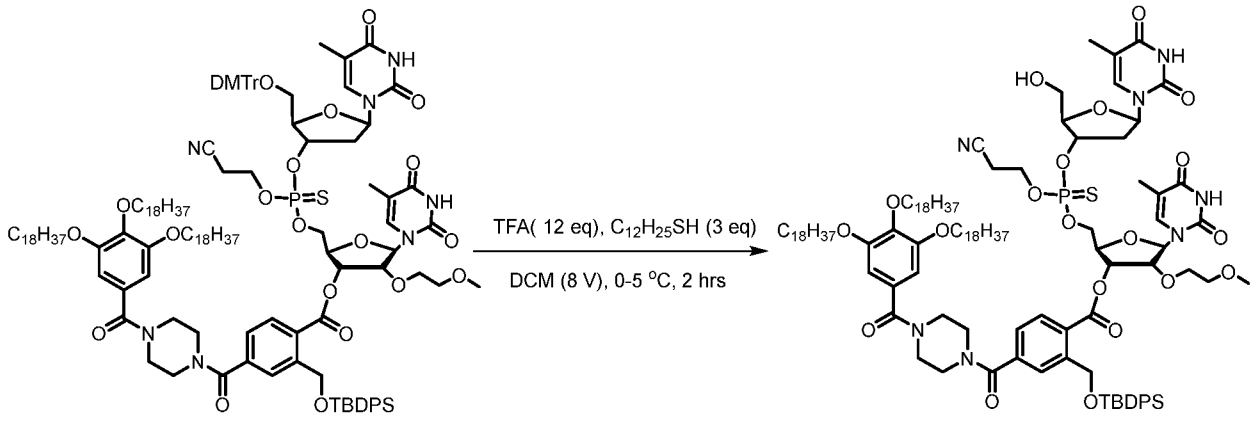
[0356] *b. Procedures for Synthesis of Oligonucleotide Fragment E*

[0357] General procedure for preparation of Compound E-2



[0358] Compound M19-Fragment I-U-DMTr (2.00 g, 1.17 mmol, 1.00 eq) with dry DCM (12.0ml) and CH_3CN (4.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound M19-Fragment I-U-DMTr (2.00 g, 1.17 mmol, 1.00 eq) in DCM (16 mL) was added 3A MS (1.60 g) in one portion at 25°C under Ar and stir for 0.5 hour. dT-amidite (1.31 g, 1.75 mmol, 1.5 *eq*) and DCI (276 mg, 2.34 mmol, 2.00 eq) were added, and the mixture was stirred at 25°C for 1 hour. HPLC showed the starting material was consumed completely. DDTT (480 mg, 2.34 mmol, 2.00 *eq*) was added into reaction solution. The mixture was stirred at 25°C for 0.5 hr. HPLC showed the starting material was consumed completely. The crude product was triturated with ACN (160 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **E-2** (2.3 g, 964 umol, 83.0% yield) was obtained as a white solid. Mass calcd for $C_{137}H_{198}N_7O_{22}PSSiNa^+$ [M+Na^+]: 2408.4, found: 2408.4.

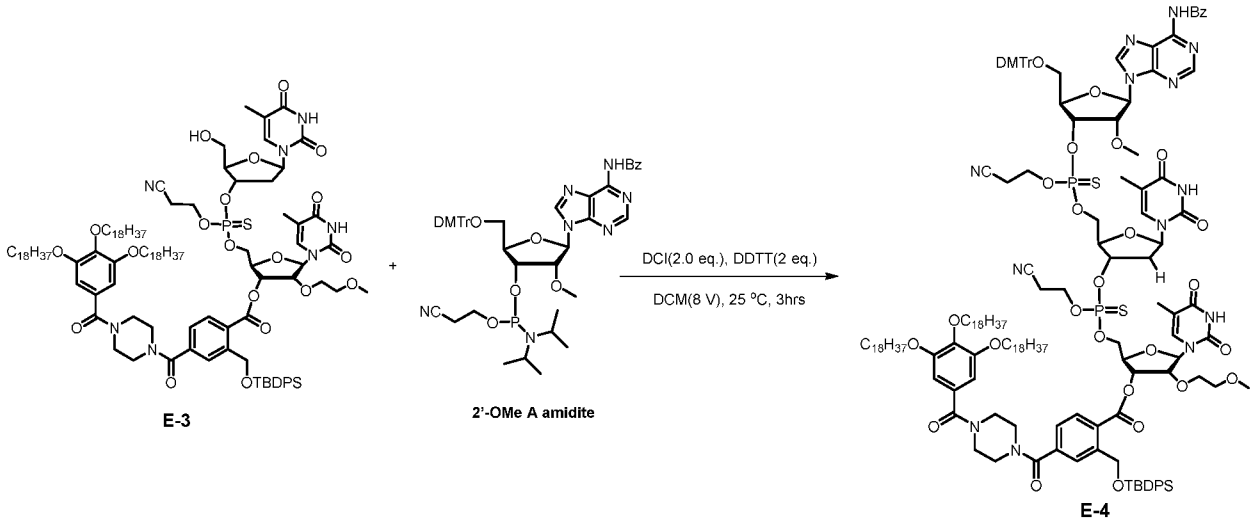
[0359] General procedure for preparation of Compound E-3



E-2 E-3

[0360] To a solution of compound **E-2** (2.30 g, 964 umol, 1.00 *eq*) in DCM (20.0 mL) was added $C_{12}H_{25}SH$ (585 mg, 2.89 mmol, 693 uL, 3.00 eq) in one portion at 25°C under N_2. TFA (1.32 g, 11.57 mmol, 856.38 uL, 12 *eq*) was added into solution, and the mixture was stirred at 0-5 °C and stirred for 2 hours. LCMS showed the starting material was consumed completely. NMI (1.19 g, 14.5 mmol, 1.15 mL, 15.0 *eq*) was added into the reaction and stir at 0-5 °C for 0.5 hr. The crude product was triturated with ACN (200 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **E-3** (2.00 g, 960 umol, 99 % yield) was obtained as a white solid. Mass calcd for $C_{116}H_{181}N_7O_{20}PSSi^+$ [M+H$^+$]: 2083.3, found: 2083.3.

[0361] General procedure for preparation of Compound E-4

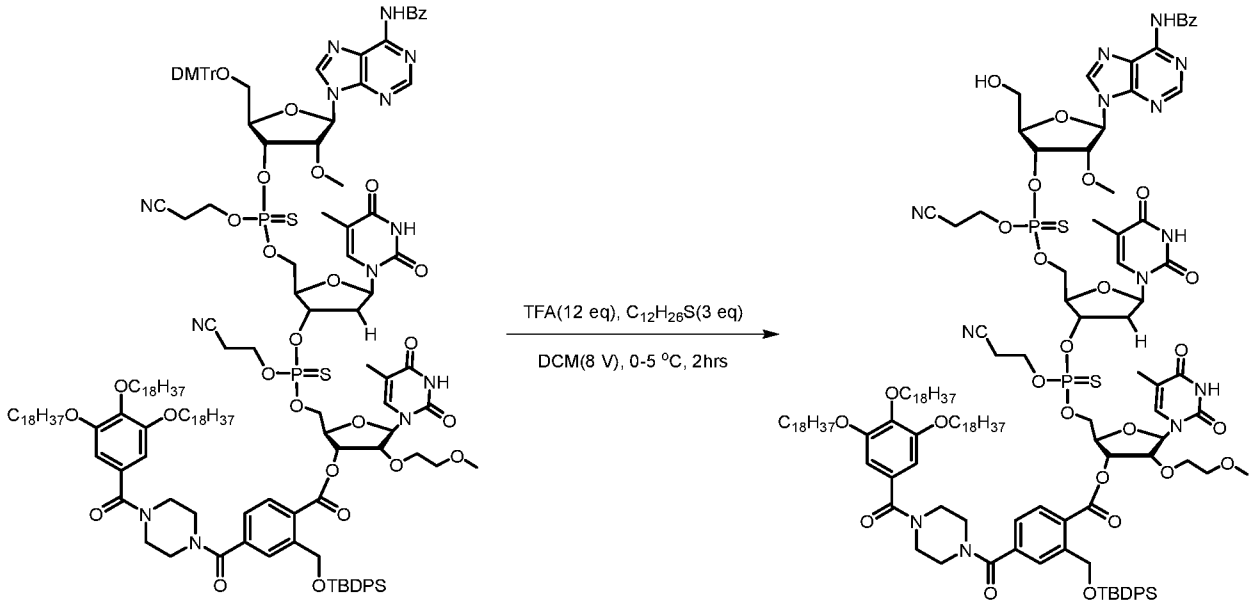


E-3 2'-OMe A amidite E-4

[0362] Compound E-3 (2.00 g, 959 umol, 1.00 eq) with dry DCM (12.0ml) and ACN (4.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound E-3 (2.00 g, 959 umol, 1.00 eq) in DCM (16 mL) was added 3A MS (1.60 g) in one portion at 25°C under Ar and stir for 0.5hr. 2'-OMe A amidite (1.28 g, 1.44 mmol, 1.50

eq) and DCI (227mg, 1.92 mmol, 2.00 eq) were added, and the mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (395 mg, 1.92 mmol, 2.00 eq) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was consumed completely. The crude product was triturated with ACN (160 mL) at 25 oC for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound E-4 (2.11 g, 727 umol, 75.6% yield) was obtained as a white solid. Mass calcd for C158H219N13O28P2S2SiNa+ [M+Na+]: 2923.5, found: 2924.5.

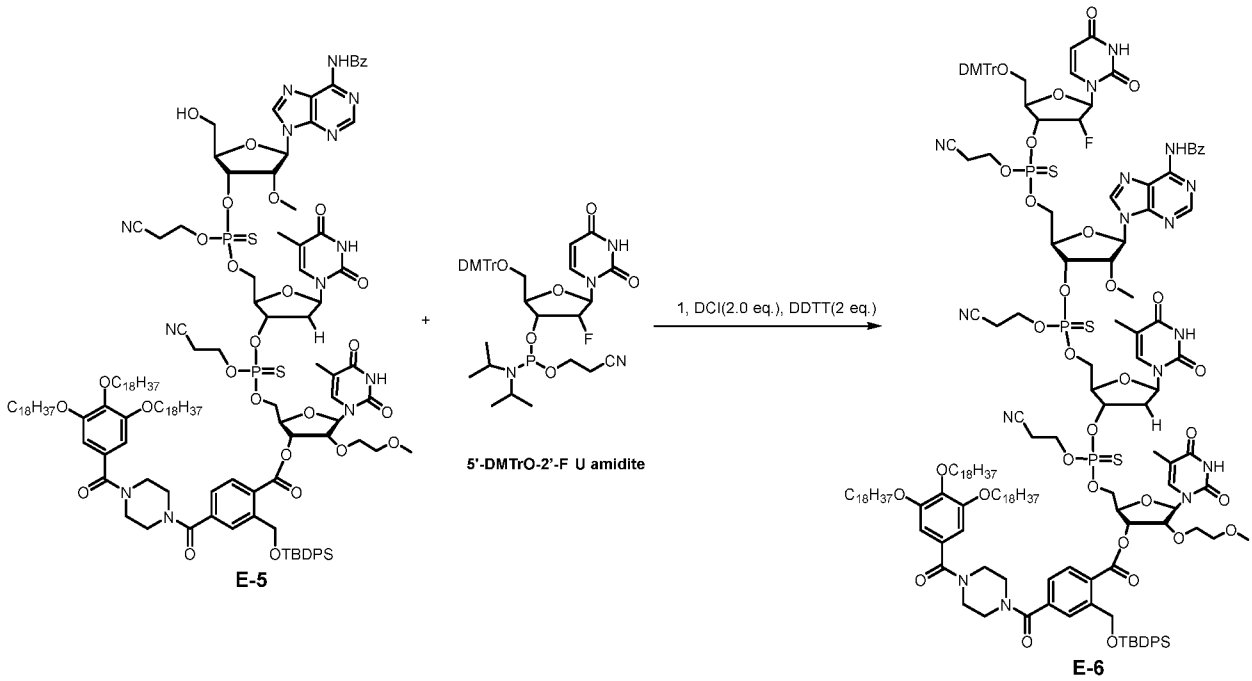
[0363] General procedure for preparation of Compound E-5



E-4 **E-5**

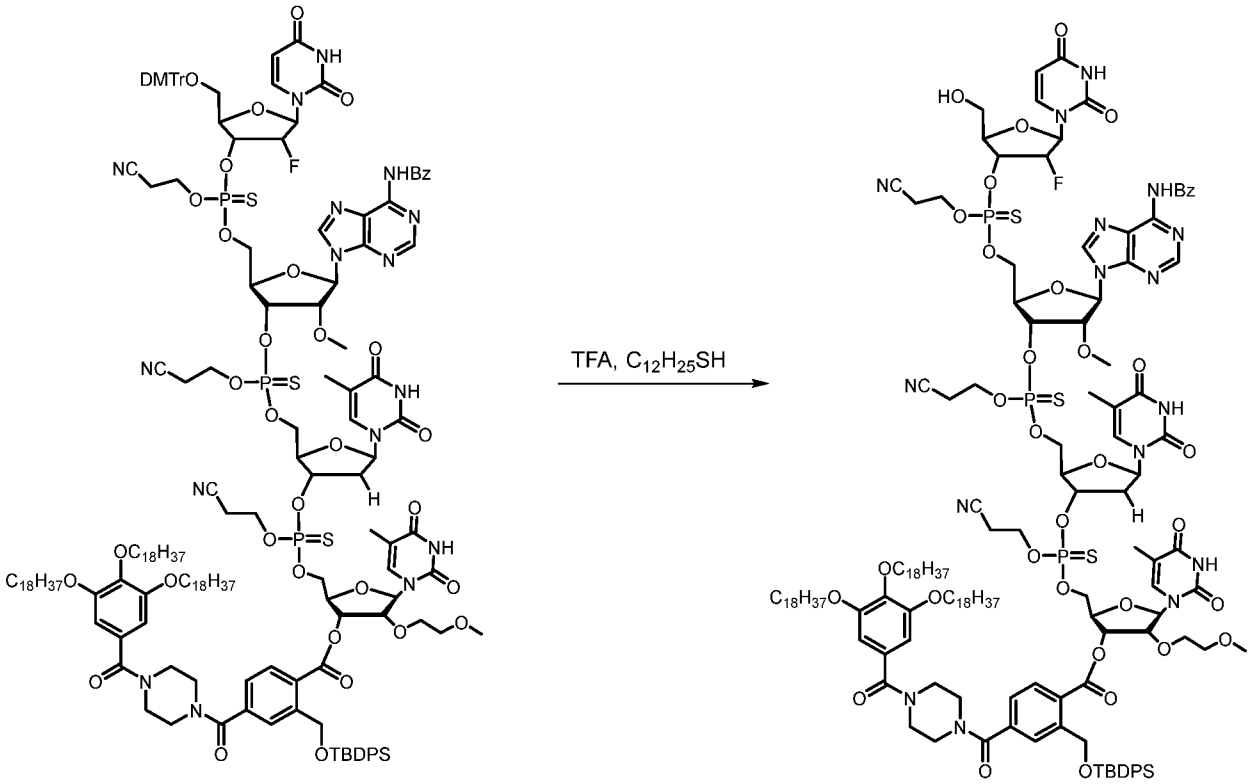
[0364] Compound **E-4** (2.1 g, 723 umol, 1.00 eq) with dry DCM (12.0ml) and ACN (4.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound **E-4** (2.1 g, 723 umol, 1.00 eq) in DCM (20.0 mL) was added $C_{12}H_{25}SH$ (439 mg, 2.17 mmol, 520 uL, 3.00 eq) in one portion at 25°C under N_2. TFA (990 mg, 8.68 mmol, 643 uL, 12.0 eq) was added into solution, and the mixture was stirred at 0-5 °C and stirred for 2 hrs. LCMS showed the starting material was consumed completely. NMI (891 mg, 10.8 mmol, 865 uL, 15 eq) was added into the reaction and stir at 0-5 °C for 0.5 hr. The crude product was triturated with ACN (200 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **E-5** (1.7 g, 653.77 umol, 90.37% yield) was obtained as a white solid. Mass calcd for $C_{137}H_{202}N_{13}O_{26}P_2S_2Si^+$ [M+H]: 2599.3, found: 2599.4.

[0365] General procedure for preparation of Compound E-6



[0366] Compound **E-5** (1.00 g, 385 umol, 1.00 eq) with dry DCM (6.00 ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound **E-5** (1.00 g, 385 umol, 1.00 eq) in DCM (8.00 mL) was added 3A MS (1.60 g) in one portion at 25°C under Ar and stir for 0.5hr. 5'-DMTrO-2'-F U amidite (440 mg, 577 umol, 1.50 eq) and DCI (90.8 mg, 769 umol, 2.00 eq) were added into the mixture, and the mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (151 mg, 736 umol, 2.00 eq) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was consumed completely. The crude product was triturated with ACN (80 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **E-6** (0.9 g, 273 umol, 74.3% yield) was obtained as a white solid. Mass calcd for $C_{170}H_{233}FN_{16}O_{34}P_3S_3Si^+$ [M+H]: 3278.5118, found: 3278.6255.

[0367] General procedure for preparation of Fragment E



E-6 **Fragment E**

[0368] Compound **E-6** (800 mg, 244 umol, 1.00 eq) with dry DCM (6.00ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of Compound **E-6** (800 mg, 244 umol, 1.00 eq) in DCM (8.00 mL) was added $C_{12}H_{25}SH$ (148 mg, 732 umol, 176 uL, 3.00 eq) in one portion at 25°C under N_2. TFA (334 mg, 2.93 mmol, 217 uL, 12.0 eq) was added into solution, and the mixture was stirred at 0-5 °C and stirred for 2 hrs. HRMS showed the starting material was consumed completely. NMI (300 mg, 3.66 mmol, 292 uL 15.0 eq) was added into the reaction and stir at 0-5 °C for 0.5 hr. The crude product was triturated with ACN (80.0 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. **Fragment E** (600 mg, 201 umol, 82.6% yield) was obtained as a white solid, HRMS calcd for $C_{149}H_{214}FN_{16}O_{32}P_3S_3Si^+$ [M+H]: 2976.3811, found: 2976.3875.

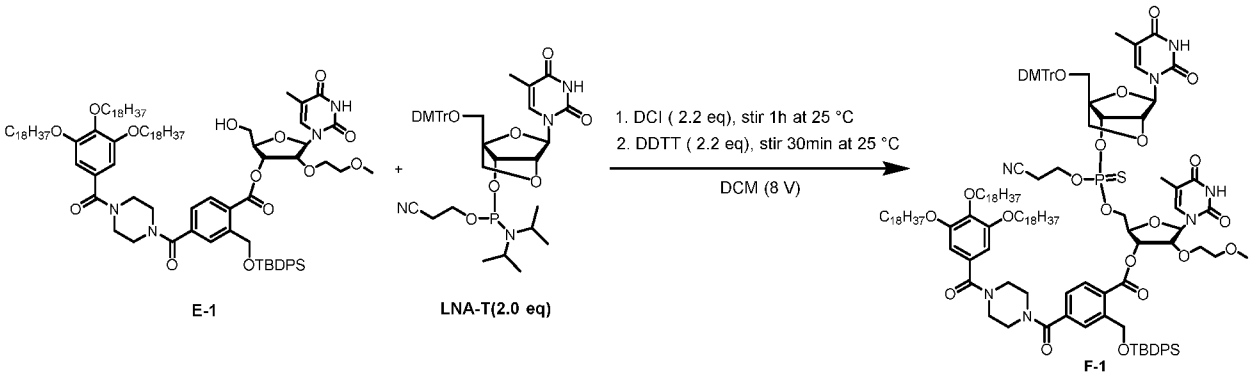
Example 12. **Synthesis of Oligonucleotide Fragment F**

[0369] *a. Scheme for Synthesis of Oligonucleotide Fragment F*

[0370] Fragment F was synthesized according to synthetic scheme depicted in FIG. 7.

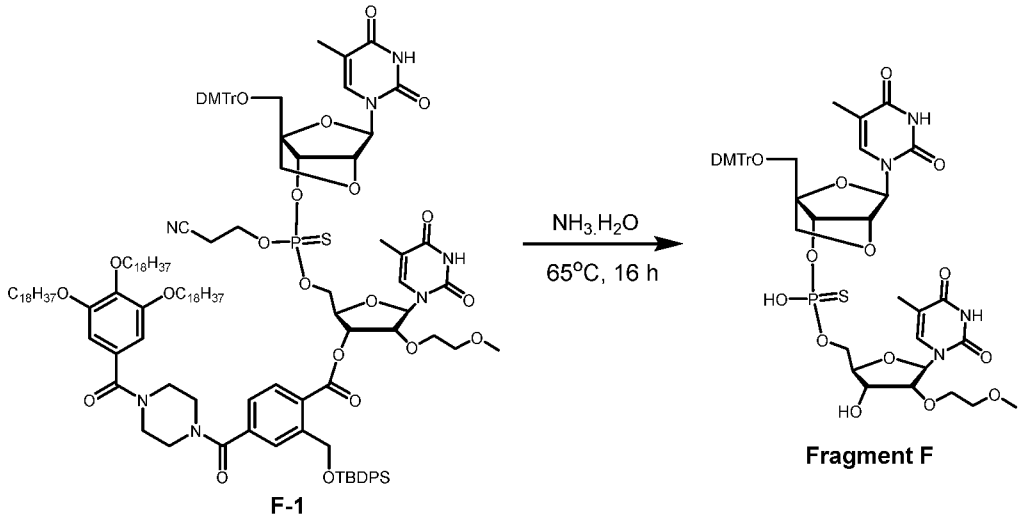
[0371] *b. Procedures for Synthesis of Oligonucleotide Fragment F*

[0372] General procedure for preparation of Compound F-1



[0373] Compound **E-1** (0.5 g, 292.31 umol, 1.00 eq) with dry DCM (4.0ml) and CH_3CN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound **E-1** (0.5 g, 292.31 umol, 1.00 eq) in DCM (5.00 mL) was added 3A MS (0.50 g) in one portion at 25°C under Ar and stirred for 0.5hr. LNA-T amidite (451.81 mg, 584.62 umol, 2.00 eq) and DCI (75.95 mg, 643.08 umol, 2.20 eq) were added, and the mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (480 mg, 2.34 mmol, 2.00 *eq*) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was consumed completely. The crude product was triturated with ACN (50 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **F-1** (2.3 g, 964 umol, 83.0% yield) was obtained as a white solid.

[0374] General procedure for preparation of Fragment F

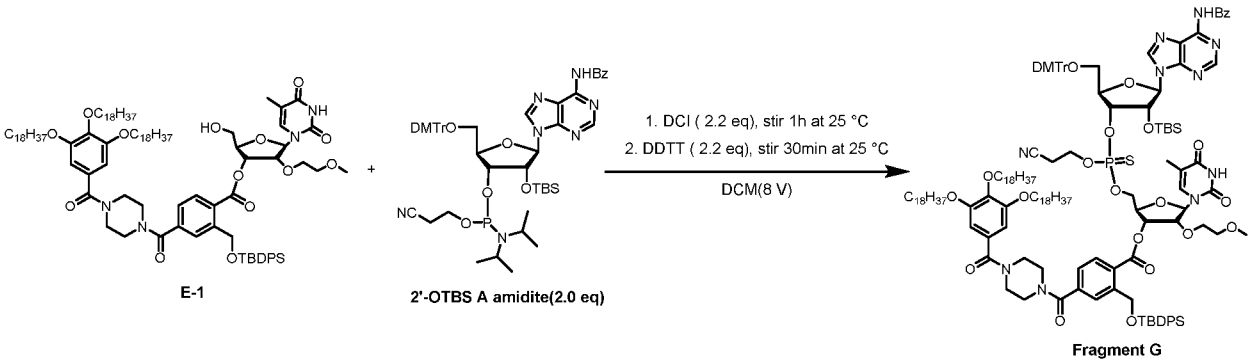


[0375] A solution of compound **F-1** (50 mg, 19.23 umol, 1.00 eq) saturated with $NH_3 \cdot H_2O$ (2.00 mL) was stirred at 70 °C for 16 hours in a 4 mL of sealed tube. Without any purification and the reaction mixture was filtered, the filtrate was provided for LCMS. Fragment F is confirmed by LCMS: HRMS calcd for $C_{45}H_{50}N_4O_{16}PS^-$ [M-H^+]: 965.2686, found: 965.2846.

Example 13. **Synthesis of Oligonucleotide Fragment G**

[0376] *a. Scheme for Synthesis of Oligonucleotide Fragment G*

[0377] Fragment G was synthesized according to synthetic scheme depicted below.



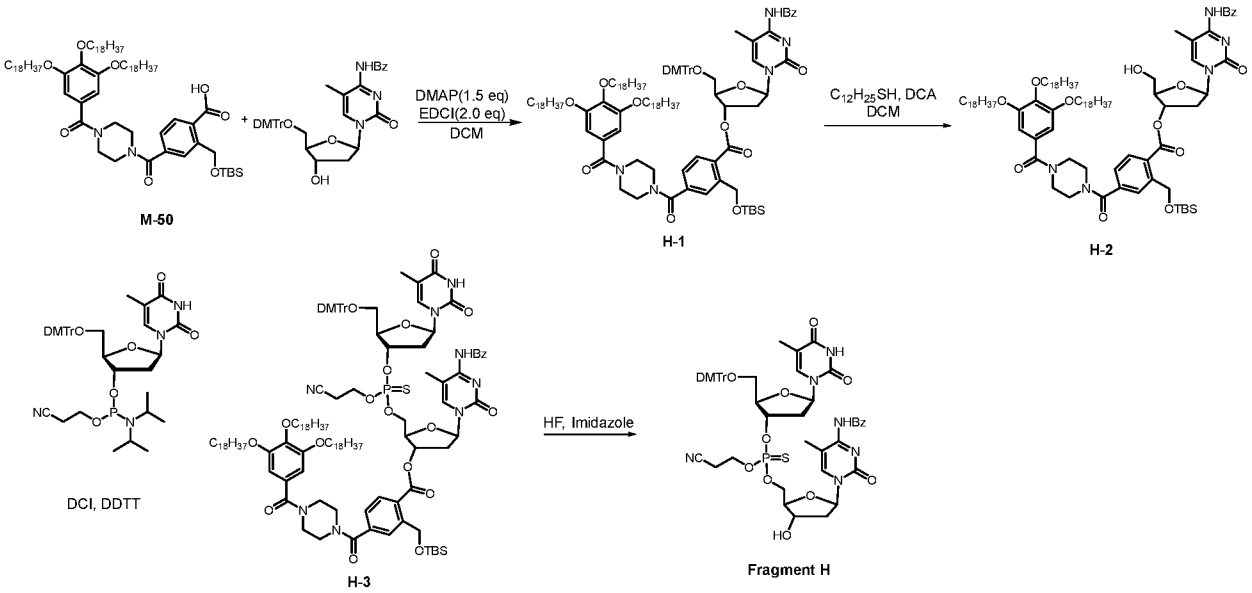
[0378] *b. Procedures for Synthesis of Oligonucleotide Fragment G*

[0379] Compound E-1 (500 mg, 292 umol, 1.00 eq) with dry DCM (4.0ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound E-1 (500 mg, 292 umol, 1.00 eq) in DCM (4.00 mL) was added 3A MS (500 mg) in one portion at 25°C under Ar and stir for 0.5 hr. 2'-OTBS A amidite (578 mg, 585 umol, 2.00 eq) and DCI (75.9 mg, 643 umol, 2.20 eq) were added into above mixture, and the mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (480 mg, 2.34 mmol, 2.00 eq) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was consumed completely. The crude product was triturated with ACN (40 mL) at 25 oC for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Fragment G (700 mg, 266 umol, 91.1% yield) was obtained as a white solid. Mass calcd for C150H216N10O22PSSi2+ [M+H+]: 2628.5043, found: 2628.5959.

Example 14. **Synthesis of Oligonucleotide Fragment H**

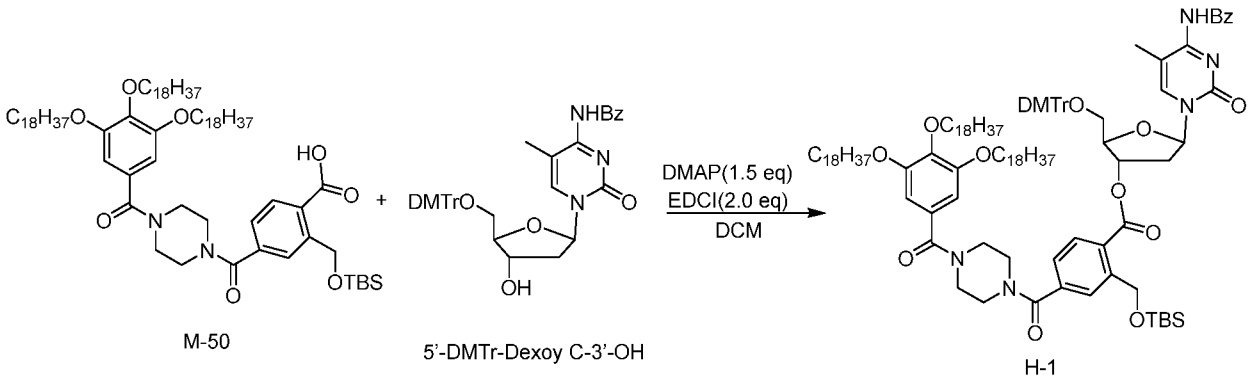
[0380] *a. Scheme for Synthesis of Oligonucleotide Fragment H*

[0381] Fragment H was synthesized according to the synthetic scheme depicted below:



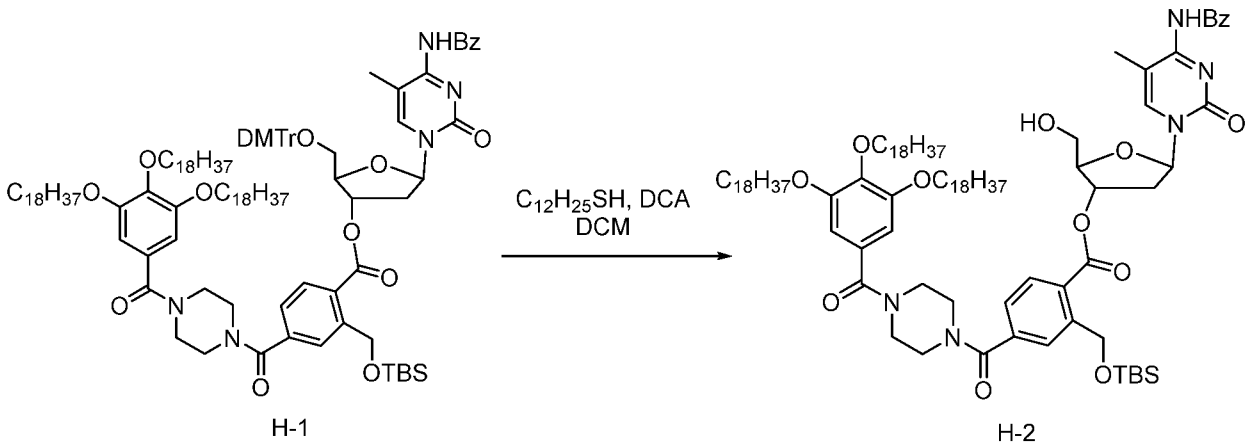
[0382] *b. Procedures for Synthesis of Oligonucleotide Fragment H*

[0383] General procedure for preparation of Compound H-1



[0384] To a solution of compound **M-50** (0.8 g, 621.08 umol, 1.00 eq) and 5'-DMTr-Dexoy C-3'-OH (804.57 mg, 1.24 mmol, 2.00 eq) in DCM (6.0 mL) was added DMAP (113.82 mg, 931.62 umol,1.50 eq). The mixture was stirred at 25°C for 0.5 h and EDCI (238.12 mg, 1.24 mmol, 2.00 eq) was added. The mixture was stirred at 25°C for 3 hrs. HPLC showed the starting material was consumed completely. The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with EtOH (30 V, 30.0 mL) at 25 °C for 30 min. The crude product was triturated with ACN (30 V, 30.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **H-1** (0.8 g, 0.41 mmol, 92.0 % purity, 67.17 % yield) was obtained as a white solid. Mass calcd for $C_{118}H_{179}N_5O_{14}Si_2$+ $[M+2H^+]/2$: 959.1, found: 960.

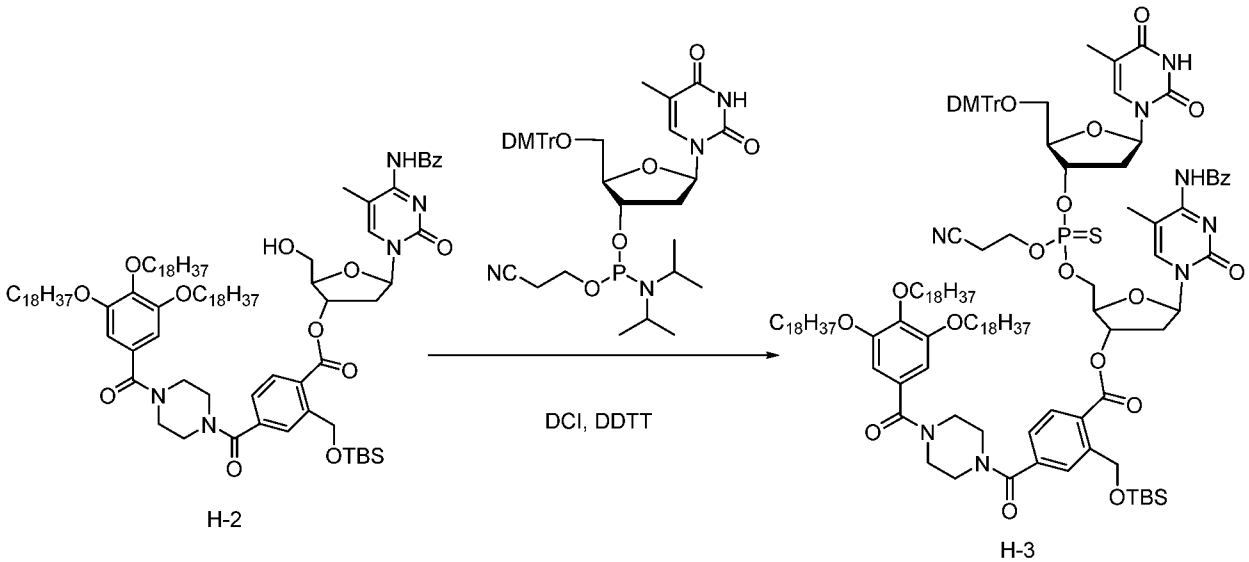
[0385] General procedure for preparation of Compound H-2



H-1 H-2

[0386] To a solution of compound **H-1** (2.0 g, 1.04 mmol, 1.00 eq) in DCM (16.0 mL) was added $C_{12}H_{25}SH$ (274.40 mg, 1.36 mmol, 324.74 uL, 1.30 eq) and DCA (1.08 g, 8.34 mmol, 685.20 uL, 8.00 eq) at 0-5°C. The mixture was stirred at 0-5°C for 2.5 hrs and NMI (856.20 mg, 10.43 mmol, 831.26 uL,10.00 eq) was added. The mixture was stirred at 0-5°C for 0.5 hrs. The residue was diluted with $NaHCO_4/H_2O$ (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na2SO4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with ACN (30 V, 60.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **H-2** (1.4 g, 713.26 umol, 82.3 % purity, 68.4 % yield) was obtained as a white solid. Mass calcd for $C_{97}H_{160}N_5O_{12}Si^+$ [M+H$^+$]: 1615.1, found: 1615.6.

[0387] General procedure for preparation of Compound H-3

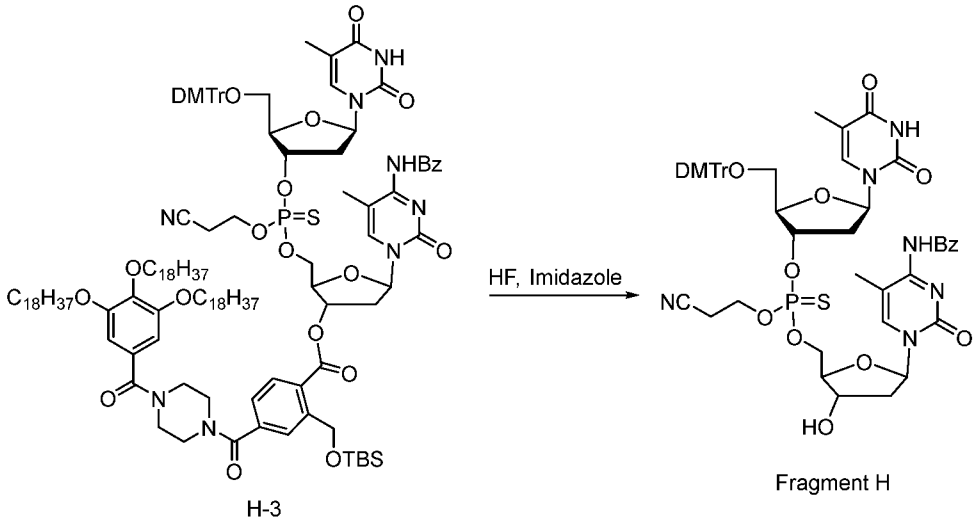


H-2 H-3

[0388] Compound **H-2** (900 mg, 557 umol, 1.00 eq) with dry DCM (8.0 ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound **H-2** (900 mg, 557 umol, 1.00 eq) in DCM (8.00 mL) was added 3A MS (800

mg) in one portion at 25°C under Ar and stir for 0.5 hr. 5'-DMTr-Dexoy T- 3'-phosphoramidite (830 mg, 1.11 mmol, 2.00 eq) and DCI (197.4 mg, 1.67 mmol, 3.0 eq) were added into above mixture. The mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (343 mg, 1.67 mmol, 3.00 *eq*) was added into reaction solution. The mixture was stirred at 25°C for 0.5 hr. LCMS showed the starting material was consumed completely. The crude product was triturated with ACN (40 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **H-3** (600 mg, 145.85 mmol, 47.3% yield) was obtained as a white solid. Mass calcd for $C_{110}H_{176}N_8O_{18}PSSi^+$ [M-DMTr+H$^+$] 1988.2; found 1989.0.

[0389] General procedure for preparation of Fragment H



[0390] To a solution of compound **H-3** (0.2 g, 87.29 umol, 1.00 eq) in THF (1.6 mL) was added solution of imidazole (118.86 mg, 1.75 mmol, 20.0 eq) and pyridine/hydrofluoride (24.94 mg, 872.95 umol, 70% purity, 10.0 eq) in THF (0.6 mL). The mixture was stirred at 0-5°C for 20 hrs (Compound **H-3** was converted to Fragment H with > 95% conversion). The structure of Fragment H was confirmed by LCMS. Mass calcd for $C_{51}H_{54}N_6O_{13}PS^+$ [M+H$^+$]: 1021.3, found: 1021.4

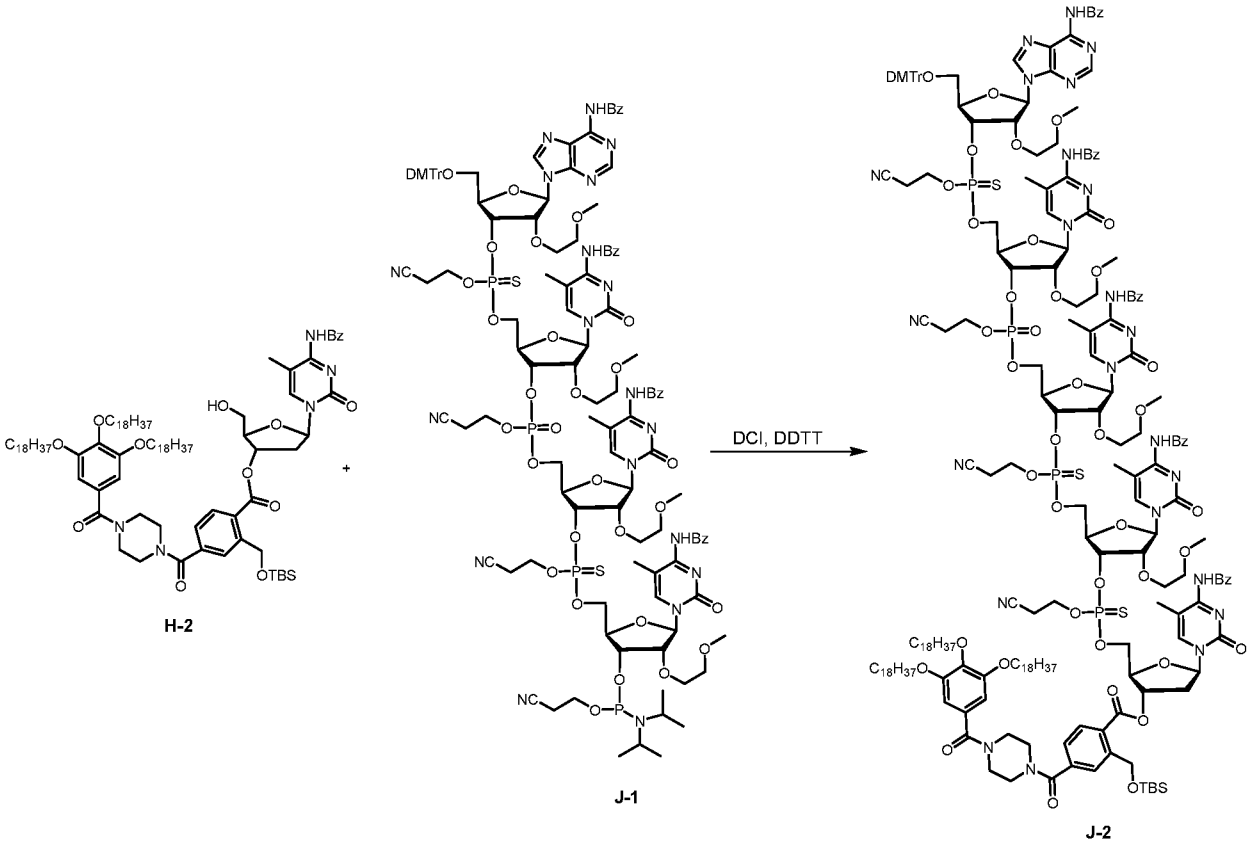
Example 15. **Synthesis of Oligonucleotide Fragment J**

[0391] *a. Scheme for Synthesis of Oligonucleotide Fragment J*

[0392] Fragment J was synthesized according to synthetic scheme depicted in FIG. 8.

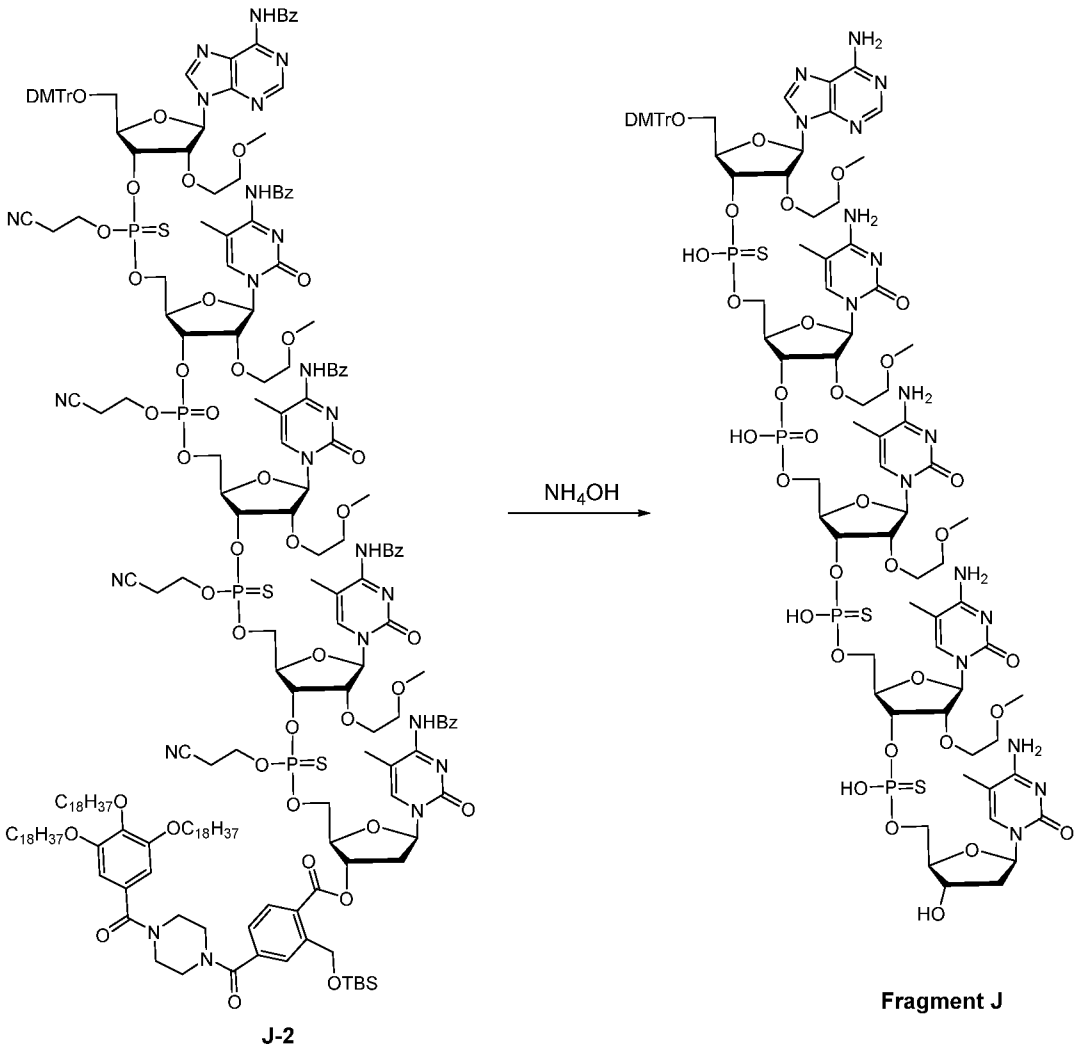
[0393] *b. Procedures for Synthesis of Oligonucleotide Fragment J*

[0394] General procedure for preparation of Compound J-2



[0395] Compound **H-2** (500 mg, 0.309 umol, 1.00 eq) with dry DCM (8.0 ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water three times. To a solution of compound **H-2** (500 mg, 0.309 mmol, 1.00 eq) in DCM (5.00 mL) was added 3A MS (500 mg) in one portion at 25°C under Ar and stir for 0.5 hr. 5'-DMTr-MOE-ACCC- 3'-phosphoramidite (Compound J-1 (synthetic procedures are described in WO2020/227618, paragraphs [0332]-[0333], which is incorporated herein by reference), 1590 mg, 0.62 mmol, 2.00 eq) and DCI (110 mg, 0.927 mmol, 3.0 eq) were added into above mixture, and the mixture was stirred at 25°C for 1hr. DDTT (254 mg, 1.23 mmol, 4.00 *eq*) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. The crude product was triturated with ACN (50 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **J-2** (770 mg, 61% yield) was obtained as a white solid. Mass Calcd for $C_{210}H_{285}N_{23}O_{46}P_4S_3Si^+$ $[M+2H^+]/2$: 2057.4, found 2058.0.

[0396] General procedure for preparation of Fragment J



J-2 Fragment J

[0397] A solution of compound **J-2** (50 mg) saturated with $NH_3 \cdot H_2O$ (2.00 mL) was stirred at 65°C for 8 hours in a 4 mL of sealed tube. Without any purification and the reaction mixture was filtered, the filtrate was provided for LCMS. **Fragment J**: Mass Calcd for $C_{83}H_{109}N_{17}O_{34}P_4S_3$ [M-2H]/2: 1053.8; found 1054.4.

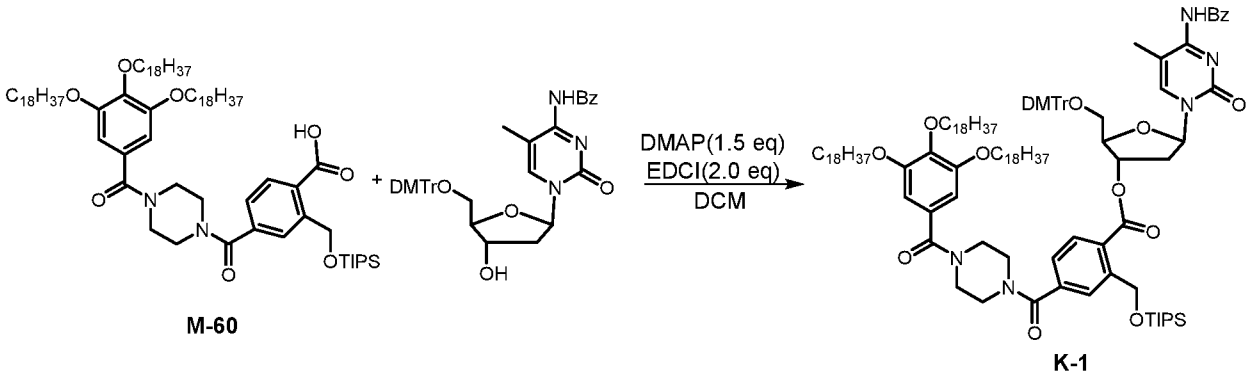
Example 16. **Synthesis of Oligonucleotide Fragment K**

[0398] *a. Scheme for Synthesis of Oligonucleotide Fragment K*

[0399] Fragment K was synthesized according to the synthetic scheme depicted in Fig. 9.

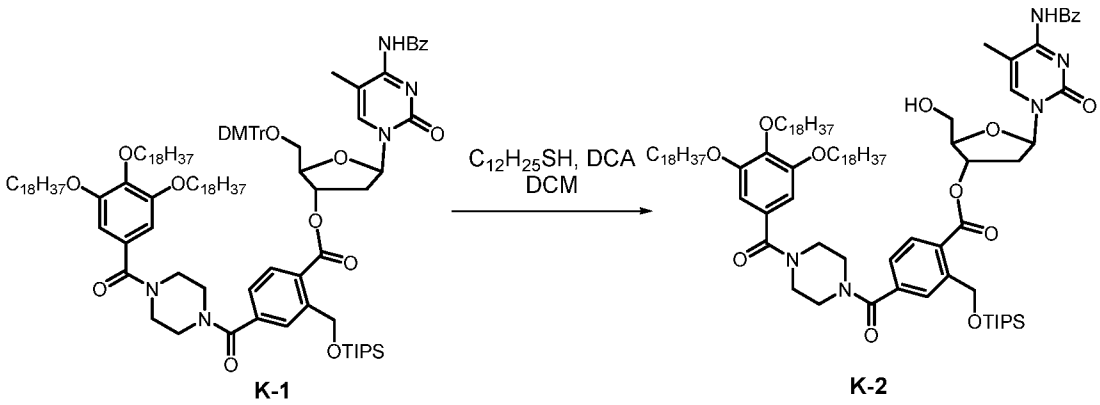
[0400] *b. Procedures for Synthesis of Oligonucleotide Fragment K*

[0401] General procedure for preparation of Compound K-1



[0402] To a solution of compound **M-60** (0.9 g, 676 umol, 1.00 eq) and 5'-DMTr-Dexoy C-3'-OH (876.5 mg, 1.35 mmol, 2.00 eq) in DCM (10.0 mL) was added DMAP (124 mg, 1.01 mmol,1.50 eq). The mixture was stirred at 25°C for 0.5 h and EDCI (260 mg, 1.35 mmol, 2.00 eq) was added. The mixture was stirred at 25°C for 3 hrs. HPLC showed the starting material was consumed completely. The residue was diluted with H_2O (50 mL) and extracted with DCM (2 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with EtOH (30 V, 30.0 mL) at 25 °C for 30 min. The crude product was triturated with ACN (30 V, 30.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **K-1** (1.2 g, 0.56 mmol, 82.4 % yield) was obtained as a white solid. Mass calcd for $C_{121}H_{184}N_5O_{14}Si^+$ $[M+2H^+]/2$: 980.7, found: 981.1.

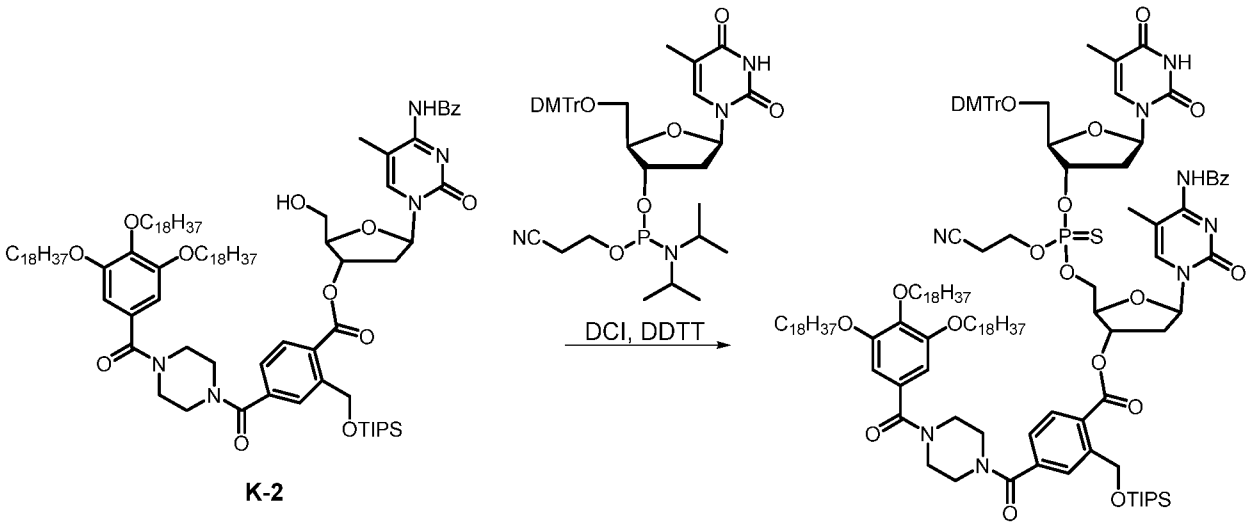
[0403] General procedure for preparation of Compound K-2



[0404] To a solution of compound **K-1** (2.0 g, 1.04 mmol, 1.00 eq) in DCM (16.0 mL) was added $C_{12}H_{25}SH$ (274.40 mg, 1.36 mmol, 324.74 uL, 1.30 eq) and DCA (1.08 g, 8.34 mmol, 685.20 uL, 8.00 eq) at 0-5°C. The mixture was stirred at 0-5°C for 2.5 hrs and NMI (856.20 mg, 10.43 mmol, 831.26 uL,10.00 eq) was added. The mixture was stirred at 0-5°C for 0.5 hrs. The residue was diluted with $NaHCO_4/H_2O$ (50 mL) and extracted with DCM (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with

ACN (30 V, 60.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **K-2** (1.4 g, 713.26 umol, 68.4 % yield) was obtained as a white solid. Mass Calcd for $C_{100}H_{166}N_5O_{12}Si^+$ [M+H$^+$]: 1657.2, found: 1657.9.

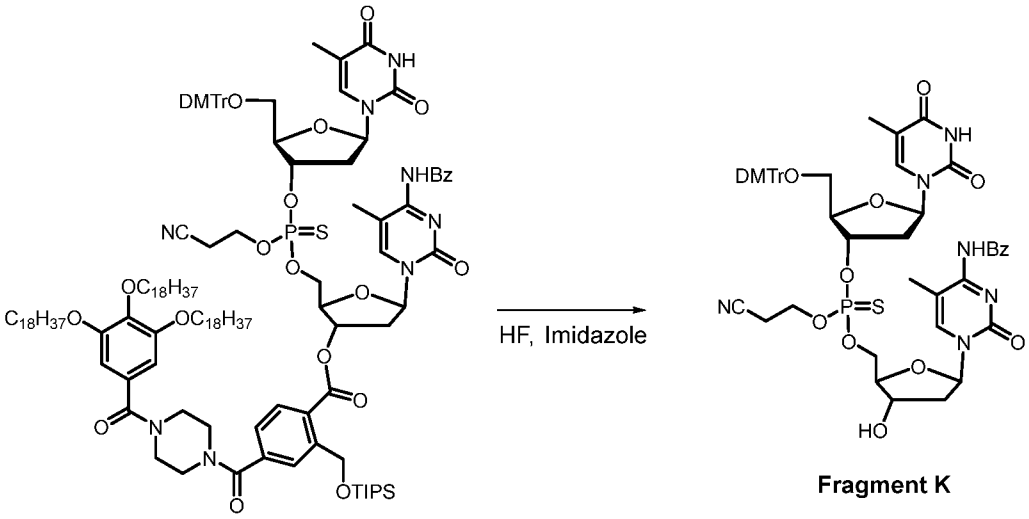
[0405] General procedure for preparation of Compound K-3



K-2 **K-3**

[0406] Compound **K-2** (1.0 g, 603 umol, 1.00 eq) with dry DCM (8.0 ml) and ACN (2.00 mL) were concentrated under reduced pressure to remove water two times. To a solution of compound **K-2** (1.0 g, 603 umol, 1.00 eq) in DCM (8.00 mL) was added 3A MS (800 mg) in one portion at 25°C under Ar and stir for 0.5 hr. 5'-DMTr-Dexoy T- 3'-phosphoramidite (898 mg, 1.21 mmol, 2.00 eq) and DCI (213.7 mg, 1.81 mmol, 3.0 eq) were added into above mixture. The mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. DDTT (372 mg, 1.81 mmol, 3.00 *eq*) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. The crude product was triturated with ACN (40 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **K-3** (1.20 g, 77.3% yield) was obtained as a white solid. Mass calcd for $C_{113}H_{182}N_8O_{18}PSSi^+$ [M-DMTr+H$^+$]: 2031.3, found 2031.0.

[0407] General procedure for preparation of Fragment K



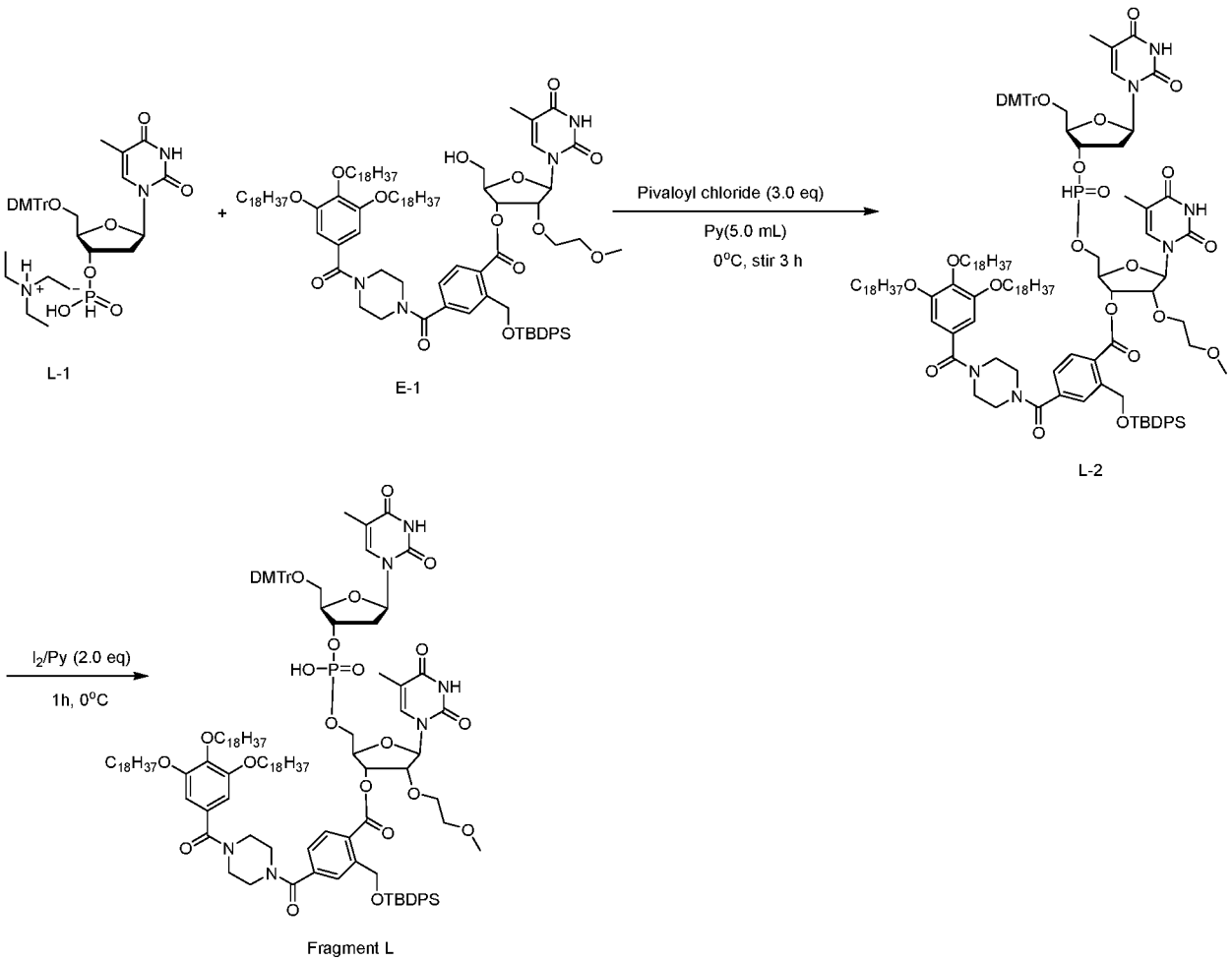
K-3 Fragment K

[0408] To a solution of compound **K-3** (0.2 g, 85.7 umol, 1.00 eq) in THF (1.6 mL) was added solution of imidazole (116.7 mg, 1.71 mmol, 20.0 eq) and pyridine/hydrofluoride (24.5 mg, 857.2 umol, 70% purity, 10.0 eq) in THF (0.6 mL). The mixture was stirred at 25°C for 20 hrs (Compound **K-3** was converted to Fragment K with > 95% conversion). The structure of Fragment K was confirmed by LCMS. Mass calcd for $C_{51}H_{53}N_6NaO_{13}PS^+$ [M+Na^+]: 1043.3, found: 1043.9.

Example 17. H Phosphate Chemistry for Preparing Fragment L

[0409] *a. Scheme for Synthesis of Fragment L*

[0410] Fragment L was synthesized according to synthetic scheme depicted below:



[0411] *b. Procedures for Synthesis of Oligonucleotide Fragment L*

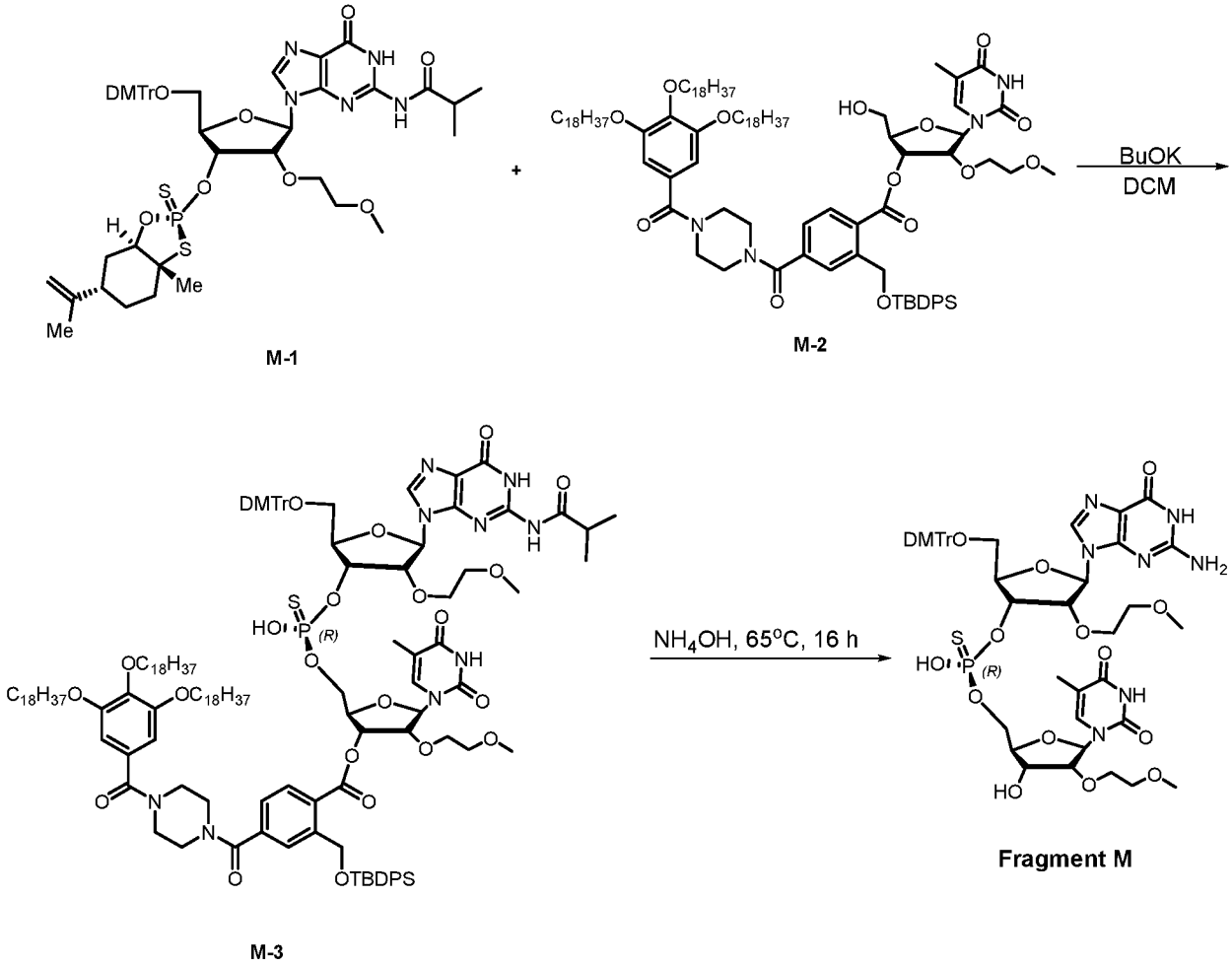
[0412] General procedure for preparation of Fragment L

[0413] A mixture of **compound L-1** (0.5 g, 0.70 mmol, 3.00 eq), pivaloyl chloride (85 mg, 0.70 mmol, 3.0 eq) in pyridine (5 mL) was stirred at 0 °C for 1 h, **compound E-1** (402 mg, 235.1 mmol, 1.0 eq) was added, and the reaction mixture was stirred 0 °C for 2.0 h. A solution of iodine (120 mg, 0.47 mmol, 2.00 eq) and pyridine (121 mg, 1.53 mmol, 6.5 eq) in THF/H_2O (3 mL, 5:1, v/v) was added to the reaction mixture dropwise at 0-5 °C and stirred at 0-5 °C for 1 h. A 4 wt% aqueous solution of $Na_2S_2O_3$ (116 mg, 0.47 mmol, 2.00 eq) was added dropwise at 0-5 °C and stirred at 15-25 °C for 10 min. EtOAc (50 mL) was added and stirred vigorously for 30 min. The top organic layer was separated, washed with 5 wt% $NaHCO_3$ solution (2 x 30 mL), brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was triturated with ACN (40 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Fragment **L** (400 mg) was obtained as a white solid. Mass calcd for $C_{134}H_{195}N_6O_{23}PSi$ [M]/2: 1157.6, found: 1157.6.

Example 18. **Synthesis of Chiral Oligonucleotide Fragment M**

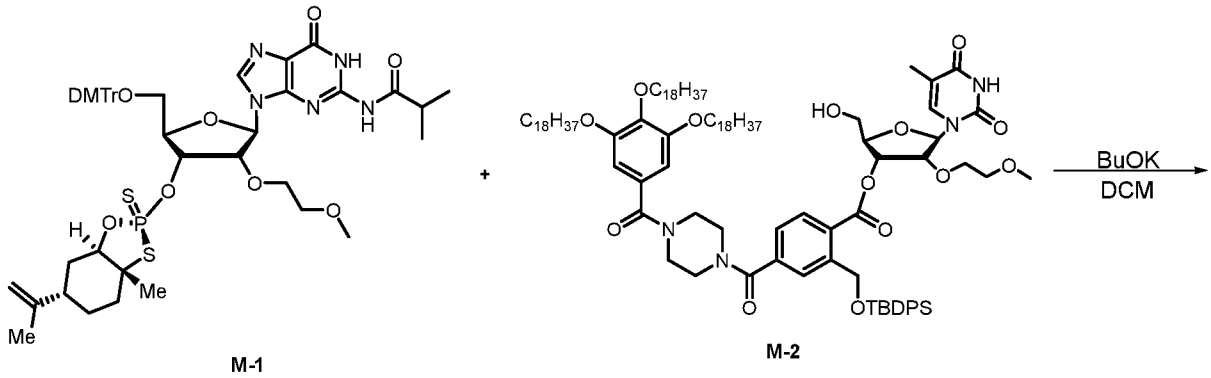
[0414] *a. Scheme for Synthesis of Chiral Oligonucleotide Fragment M*

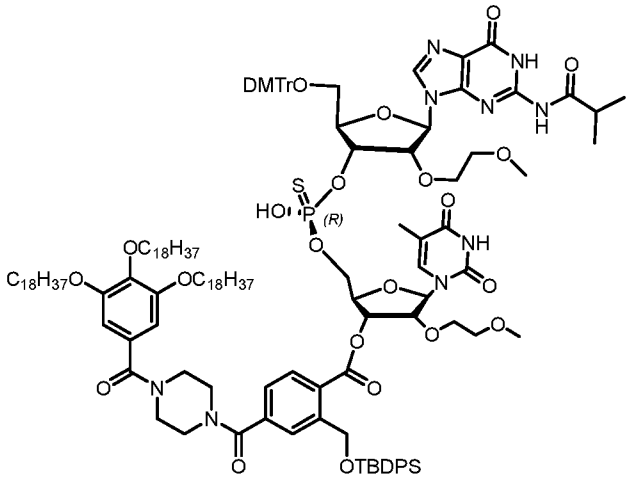
[0415] Chiral oligonucleotide Fragment M was synthesized according to the synthetic scheme depicted below:



[0416] *b. Procedures for Synthesis of Oligonucleotide Fragment M*

[0417] General procedure for preparation of Compound M-3

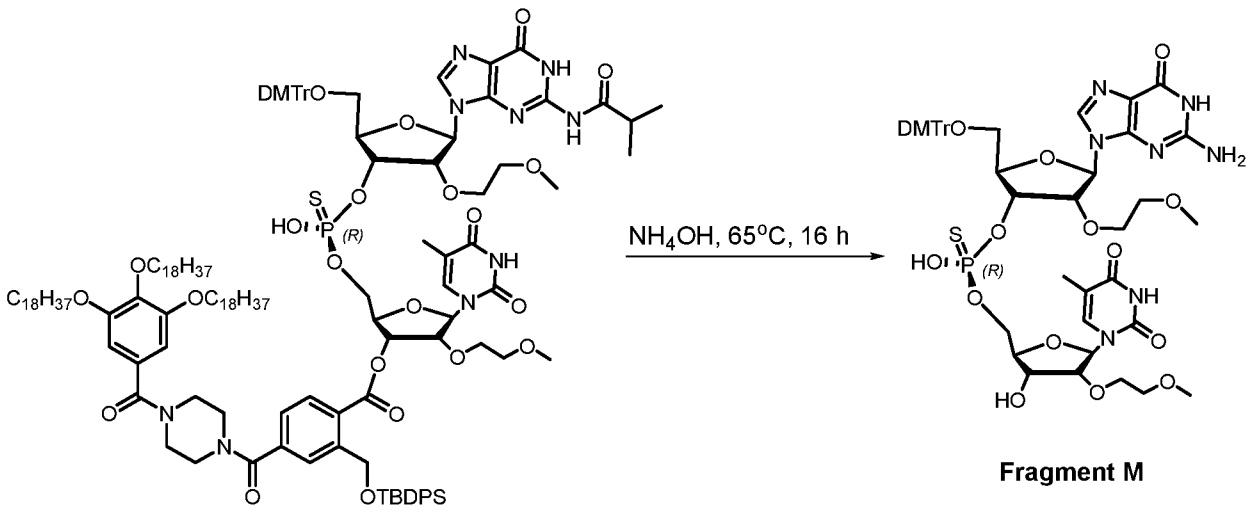




M-3

[0418] Compound M-1 (134.7 mg, 140.3 umol, 1.00 eq) with dry DCM (5.0 ml) and ACN (10.00 mL) were concentrated under reduced pressure to remove water two times. To a solution of compound **M-1** (134.7 mg, 140.3 umol, 1.00 eq) and compound **M-2** (160 mg, 93.5 ummol, 1.0 eq) in DCM (2.00 mL) was added 3A MS (200 mg) in one portion at 25°C under Ar and stir for 0.5 hr. BuOK (1.0 M, 281 uL, 3.00 eq) was added into above mixture. The mixture was stirred at 25°C for 1hr. LCMS showed the starting material was consumed completely. The mixture was filtered and washed with DCM (0.5 mL). The crude product was triturated with ACN (20 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Chiral Fragment M (175 mg, 69.9 umol, 74.7% yield) was obtained as a white solid. Mass calcd for $C_{120}H_{189}N_9O_{22}PSSi^+$ [M-DMTr+H^+]: 2200.3, found: 2200.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 58.6 ppm.

[0419] General procedure for preparation of Fragment M



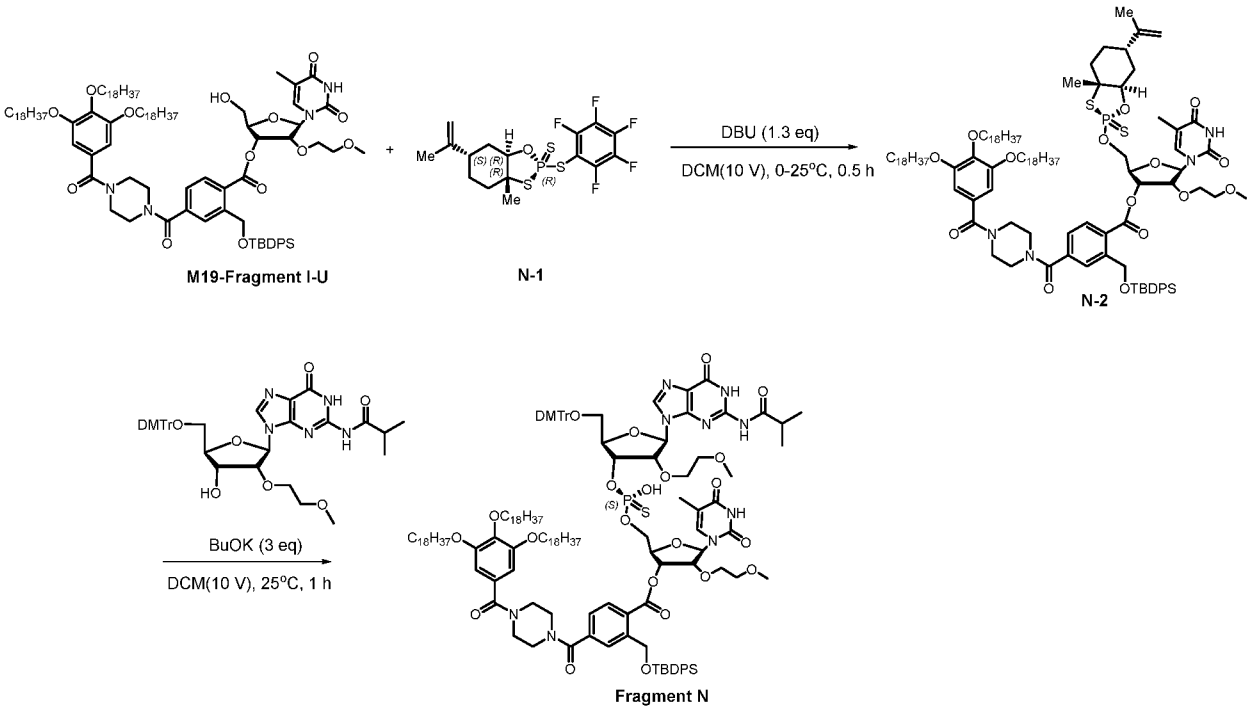
M-3

[0420] A solution of compound M-**3** (30 mg) saturated with $NH_3 \cdot H_2O$ (2.00 mL) was stirred at 65°C for 16 hours in a 4 mL of sealed tube. Without any purification and the reaction mixture was filtered, the filtrate was provided for LCMS. Fragment M was confirmed by LCMS. HRMS Calcd for $C_{47}H_{56}N_7O_{16}P^-$ [M-H^-]: 1036.3169, found: 1036.3502.

Example 19. **Synthesis of Chiral Oligonucleotide Fragment N**

[0421] *a. Scheme for Synthesis of Chiral Oligonucleotide Fragment N*

[0422] Chiral oligonucleotide Fragment N was synthesized according to the synthetic scheme depicted below:



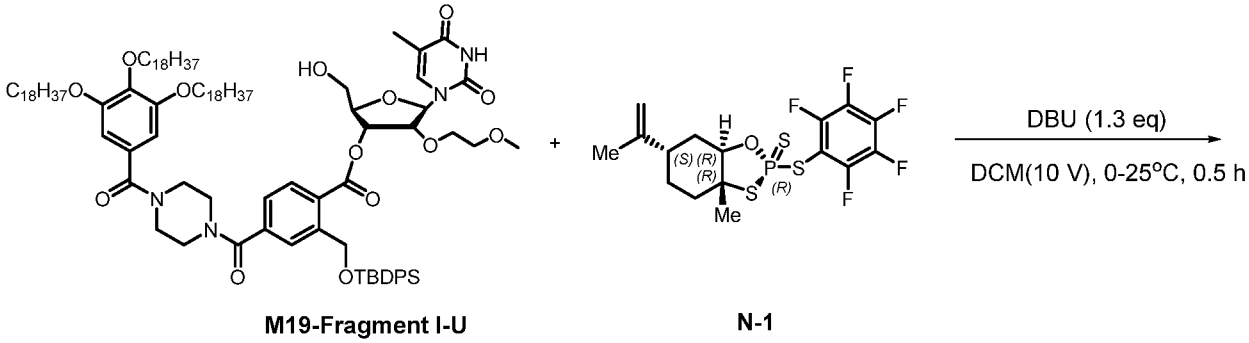
M19-Fragment I-U

N-1

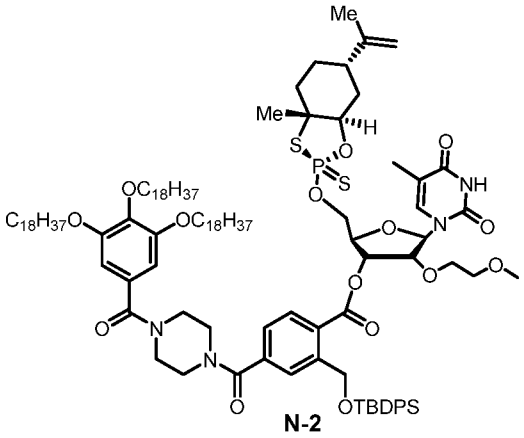
N-2

Fragment N

[0423] General procedure for preparation of Compound N-2



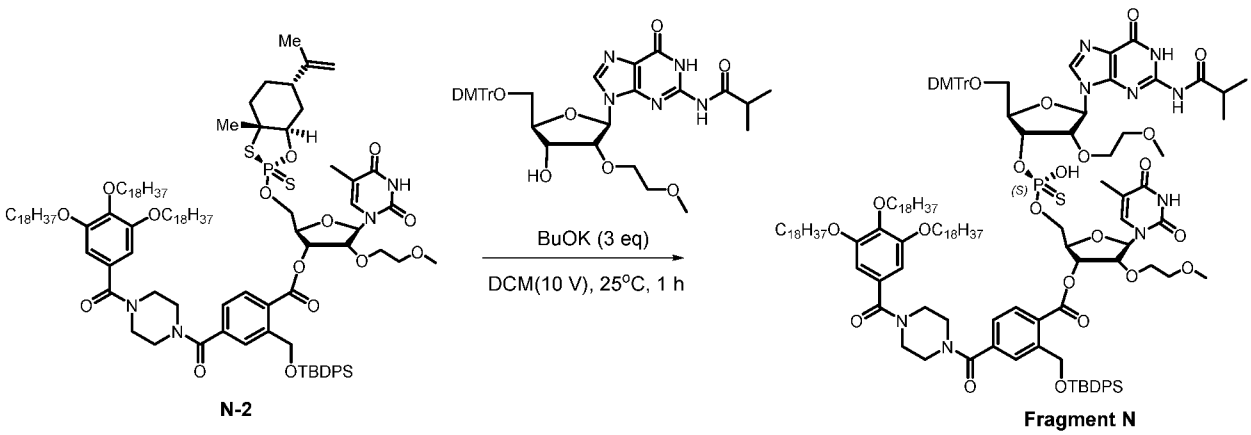
M19-Fragment I-U N-1



N-2

[0424] Compound **M19-Fragment I-U** (200 mg, 117 umol, 1.00 eq) with dry DCM (1.0 ml) and DCM (3.0 mL) were concentrated under reduced pressure to remove water two times. To a solution of compound **M19-Fragment I-U** (200 mg, 117 umol, 1.00 eq) and compound **N-1** (68 mg, 152 ummol, 1.30 eq) in DCM (2.00 mL) was added 3A MS (200 mg) in one portion at 25°C under Ar and stir for 0.5 hr. DBU (23 mg, 152 umol, 1.30 eq) was added into above mixture, and the mixture was stirred at 25°C for 1hr. The mixture was filtered and washed with DCM (1.0 mL). The crude product was triturated with ACN (30 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **N-2** (215 mg, 84.6 umol, 84.6% yield) was obtained as a white solid.

[0425] General procedure for preparation of Fragment N



N-2 Fragment N

[0426] Compound **N-2** (109.4 mg, 153umol, 1.50 eq) with dry CH_3CN (1.0 ml) and DCM (3.0 mL) were concentrated under reduced pressure to remove water two times. To a solution of compound **5** (109.4 mg, 153 umol, 1.50 eq) and 5'-DMTr-MOE G- 3'-OH (200 mg, 102.2 umol, 1.0 eq) in DCM (2.00 mL) was added 3A MS (200 mg) in one portion at 25°C under Ar and stir for 0.5 hr. BuOK (1.0 M, 307 uL, 3.0 eq) was added into above mixture, and the mixture was stirred at 25°C for 1hr. The mixture was filtered and washed with DCM (1.0 mL). The crude product was triturated with ACN (25 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Chiral Fragment N (175 mg, 54.1 umol, 68.1% yield) was obtained as a white solid. Mass calcd for $C_{120}H_{189}N_9O_{22}PSSi^+$ [M-DMTr+H^+]: 2200.3, found: 2200.1. ^{31}P NMR (162 MHz, $CDCl_3$) δ 58.6 ppm.

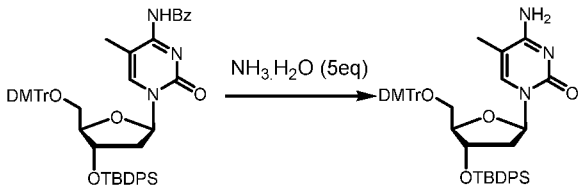
Example 20. **Synthesis of Oligonucleotide Fragment O**

[0427] *a. Scheme for Synthesis of Oligonucleotide Fragment O*

[0428] Oligonucleotide Fragment N was synthesized according to the synthetic scheme depicted in Fig. 10.

[0429] *b. Procedures for Synthesis of Oligonucleotide Fragment O*

[0430] General procedure for preparation of Compound O-1



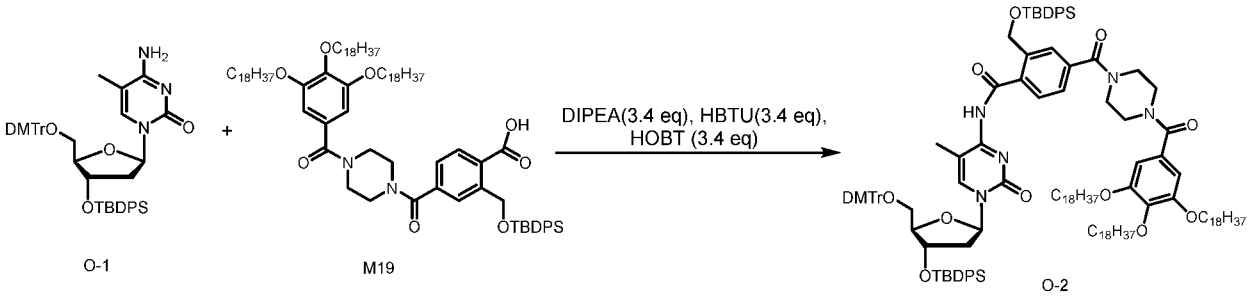
O-1

A solution of 5'-DMTrO-C-OTBDPS-3' (5.0 g, 5.2 mmol, 1.00 equiv), NH3•H2O (25%, 3.6 g,

26.0 mmol, 5.00 equiv) and THF (40 mL) was stirred at 20±5 °C for 1.0 h (HLPC indicated 42.8% conversion of 5'-DMTrO-C-OTBDPS-3'). The mixture was concentrated to give a residue of crude product which was purified by silica gel chromatography (0-10% THF in DCM as eluent). The compound O-1 was obtained as a pale-yellow solid (1.6 g, 36.0% yield). 1H NMR (300 MHz, DMSO-d6) δ 8.02 (s, 1H), 7.90 (d, J = 9.0 Hz, 1 H), 7.64 (d, J = 9.0 Hz, 2 H), 7.58-7.09 (m, 22H), 6.93-6.72 (m, 5H), 5.96 (s, 1H), 4.33 (t, J = 4.5 Hz, 1H), 4.12 (s, 1H), 3.63 (t, J = 3.0 Hz, 1 H), 3.39 (s, 2H), 3.34 (t, J = 3.0 Hz, 2 H), 3.28-3.19 (m, 2H), 3.18 (s, 3H), 3.05-2.93 (m, 1H), 1.39 (s, 3H), 0.94 (s, 9H). 13C{1H} NMR (75 MHz, DMSO-d6) δ 168.4, 165.8, 158.6, 155.3, 114.9, 135.9, 135.7, 135.4, 133.1, 130.2, 128.6, 128.2, 128.1,

127.9, 113.7, 102.0, 87.8, 86.5, 82.5, 81.7, 71.7, 71.4, 69.2, 63.1, 58.6, 55.5, 27.1, 19.3, 13.3. HRMS calcd for C50H58N3O8Si+ [M+H]+: 856.3988, found: 856.4009

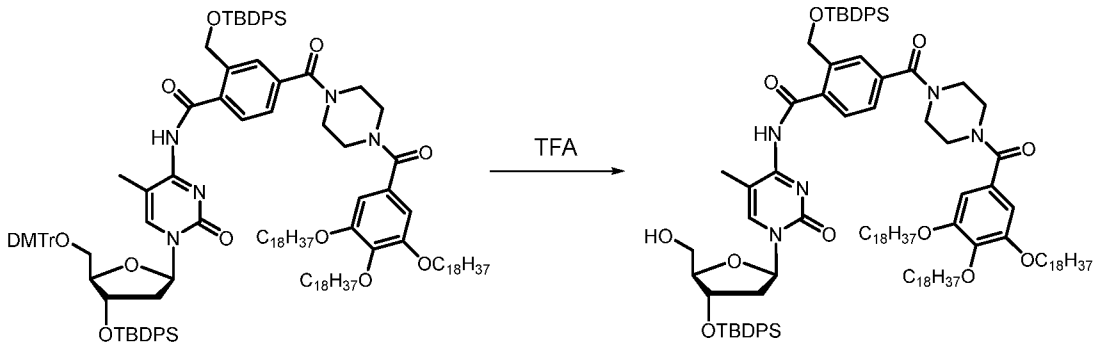
[0431] General procedure for preparation of Compound O-2



O-1 M19 O-2

[0432] To a solution of compound **O-1** (498 mg, 0.64 mmol, 1.50 eq) and compound **M19** (600 mg, 0.42 mmol, 1.0 eq) in DCM (5.00 mL) was added DIPEA (186 mg, 3.4 eq), HBTU (549 mg, 3.4 eq), and HOBT (192 mg, 3.4 eq) at 25°C under and stirred for 4 hours. The crude product was triturated with ACN (40 mL) at 25 °C for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound **O-2** (700 mg, 0.32 mmol, 76.7% yield) was obtained as a white solid. Mass calcd for $C_{137}H_{196}N_5O_{13}PSSi_2^+$ [M+H$^+$]: 2175.4363, found: 2175.4373.

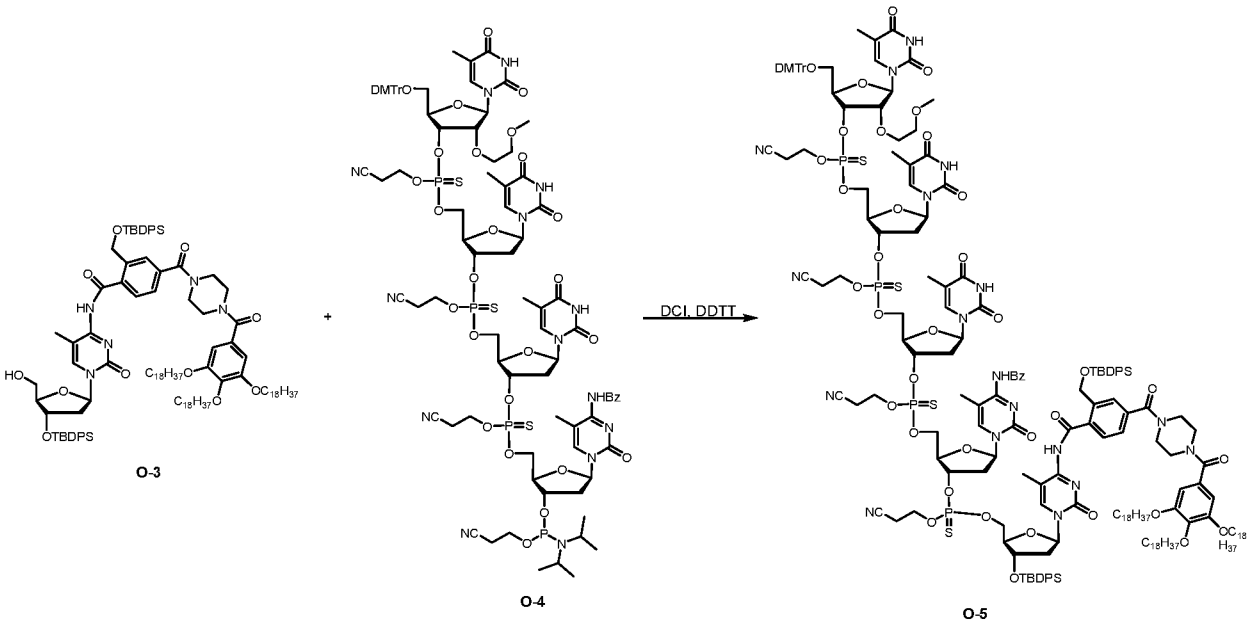
[0433] General procedure for preparation of Compound O-3



O-2 **O-3**

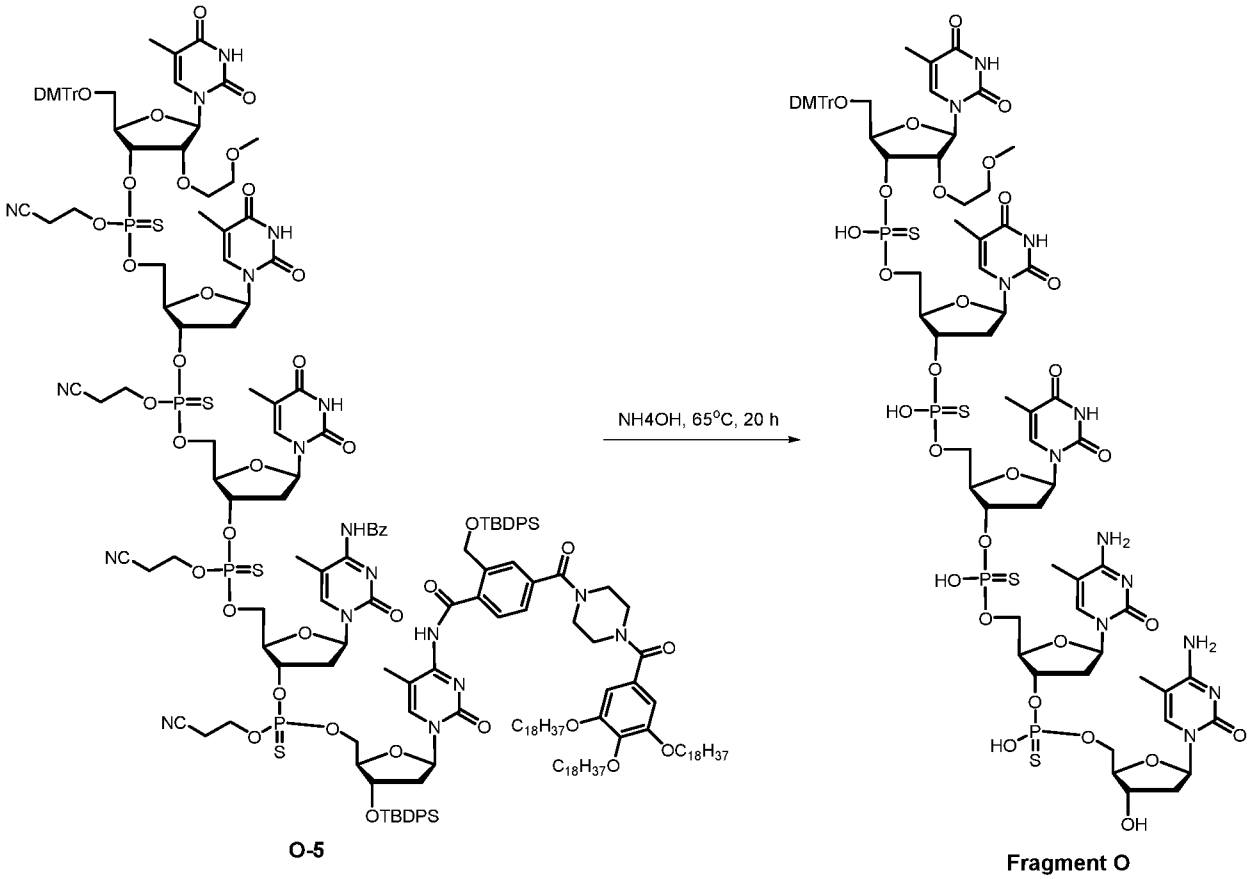
[0434] To a solution of compound **O-2** (650 mg, 0.298 mmol, 1.00 eq) in DCM (6.0 mL) was added $C_{12}H_{25}SH$ (180 mg, 3.0 eq) and DCA (339 mg, 10.0 eq) at 0-5°C. The mixture was stirred at 0-5°C for 2.5 hours and NMI (293.5 mg,12.00 eq) was added. The mixture was stirred at 0-5°C for 0.5 hrs. The residue was diluted with $NaHCO_4/H_2O$ (50 mL) and extracted with DCM (2 x 30 mL). The crude product was triturated with ACN (40.0 mL) at 25 °C for 30 min. Filtered and concentrated. Compound **O-3** (500 mg, 89 % yield) was obtained as a white solid. Mass Calcd for $C_{116}H_{178}N_5O_{11}Si_2^+$ [M+H$^+$]: 1874.3, found: 1874.1.

[0435] General procedure for preparation of Compound O-5



[0436] Compound **O-3** (0.25 g, 133 umol, 1.00 eq) with dry DCM (4.0 ml) and ACN (4.00 mL) were concentrated under reduced pressure to remove water two times. To a solution of compound **O-3** (0.25 g, 133 umol, 1.00 eq) in DCM (4.00 mL) was added 3A MS (200 mg) in one portion at 25°C under Ar and stir for 0.5 hr. Compound O-**5** (816 mg, 0.4 mmol, 3.00 eq) and DCI (50 mg, 426 umol, 3.2 eq) were added into above mixture. The mixture was stirred at 25°C for 1hr. DDTT (60 mg, 292 umol, 2.20 *eq*) was added into reaction solution, and the mixture was stirred at 25°C for 0.5 hr. The crude product was triturated with ACN (40 mL) at 25 ℃ for 1 hr. The mixture was filtered and the cake was concentrated in vacuum. Compound O-5 (0.25 g, 50% yield) was obtained as a white solid.

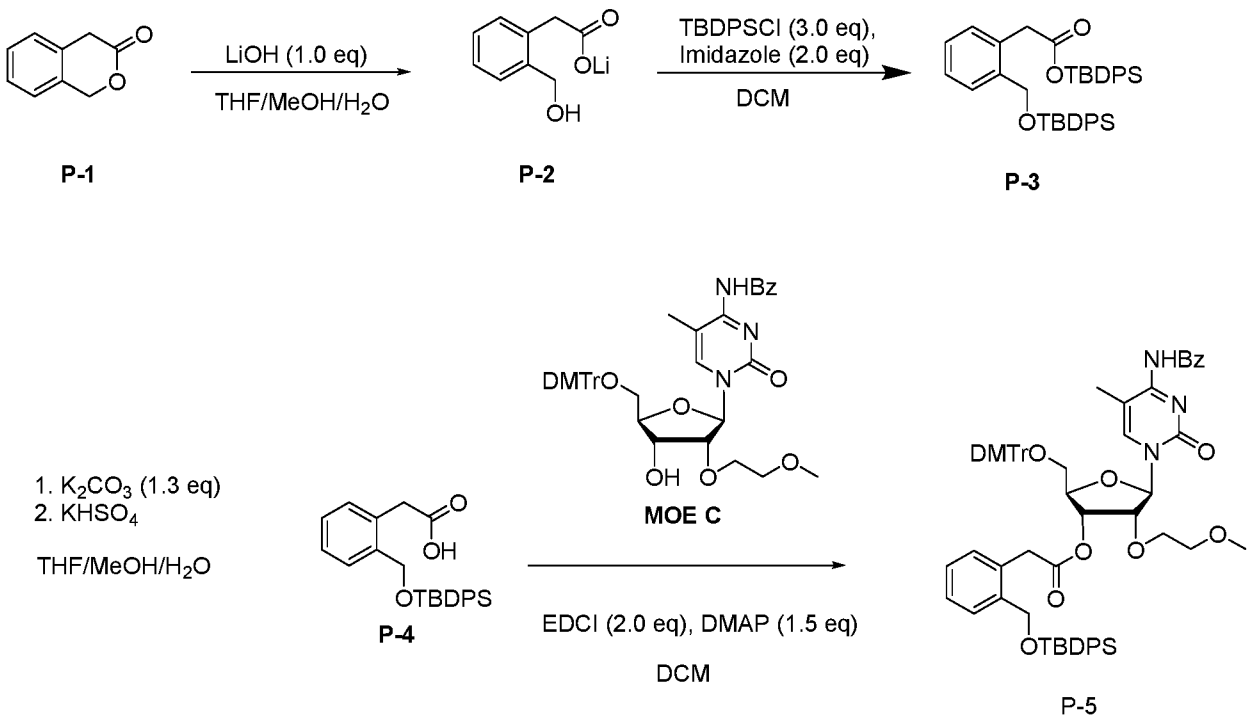
[0437] General procedure for preparation of Fragment O

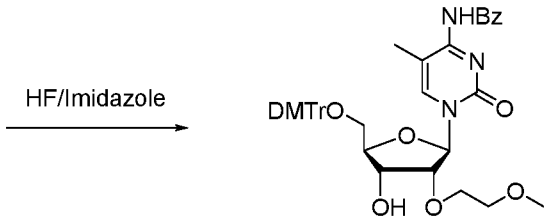


[0438] A solution of compound **O-5** (20 mg) saturated with $NH_3 \cdot H_2O$ (2.00 mL) was stirred at 65°C for 20 hours in a 4 mL of sealed tube. Without any purification and the reaction mixture was filtered, the filtrate was provided for LCMS. Fragment O was confirmed by LCMS. HRMS Calcd for $C_{74}H_{91}N_{12}O_{31}P_4S_4^-$ [M-H^-]: 1895.3752, found: 1895.3716.

Example 21. **Synthesis of monomer Fragment P**

[0439] *a. Scheme for Synthesis of monomer Fragment P*

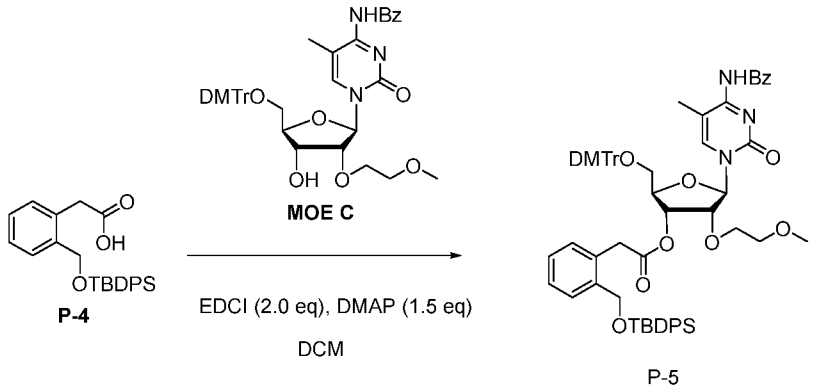




Fragment P

[0440] *b. Procedures for Synthesis of Fragment P*

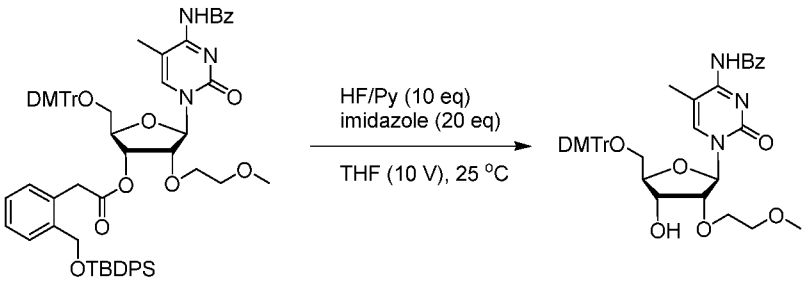
[0441] General procedure for preparation of Compound P-5



[0442] To a solution of compound **P-4** ((synthetic procedures are described in European Journal of Organic Chemistry (2003), (12), 2327-2335, which is incorporated herein by reference), 1.0 g, 2.47 mmol, 1.0 eq) and 5'-DMTr-MOE C-3'-OH (1.78 g, 2.47 mmol, 1.0

eq) in DCM (20.0 mL) was added EDCl (947 mg, 2.0 eq) and DMAP (453 mg, 1.5 eq) at 25°C under and stirred for 24 hours. The mixture was concentrated in vacuum and the residue was purified by silica gel column chromatography (Heptane/EtOAc 5:1 to 3:1). Compound **P-5** was obtained as a white solid (1.7 g, 1.48 mmol, 60% yield). Mass calcd for $C_{66}H_{69}N_3O_{11}Si^+$ [M+H$^+$]: 1108.4 found: 1108.4.

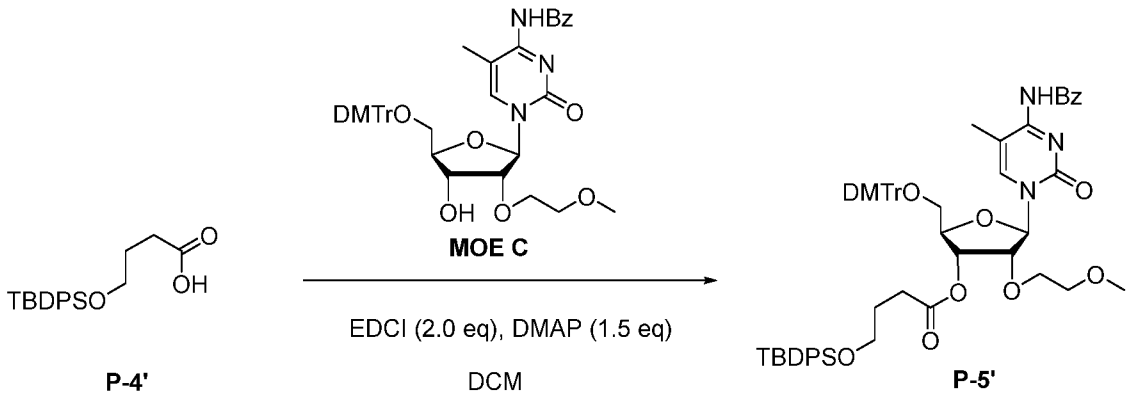
[0443] General procedure for preparation of Fragment P



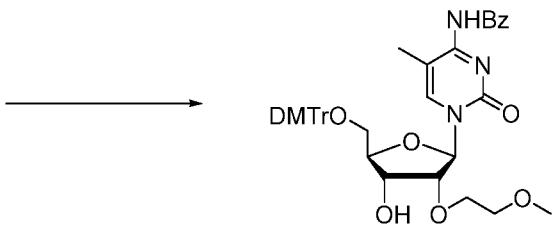
P-5 Fragment P

[0444] To a solution of compound **P-5** (0.5 g, 0.45 mmol, 1.00 eq) in THF (1.0 mL) was added solution of imidazole (614.2 mg, 9.02 mmol, 20.0 eq) and pyridine/hydrofluoride (128.9 mg, 4.5 mmol, 70% purity, 10.0 eq) in THF (2.0 mL). The mixture was stirred at 25°C for 20 hrs (Compound **P-5** was converted to Fragment **P** with > 95% conversion).

[0445] *c. Alternative Scheme for Synthesis of monomer Fragment P*



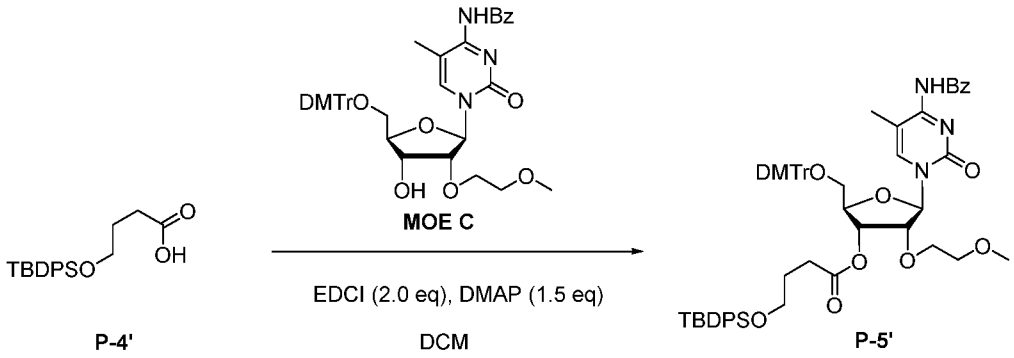
P-4' **P-5'**



Fragment P

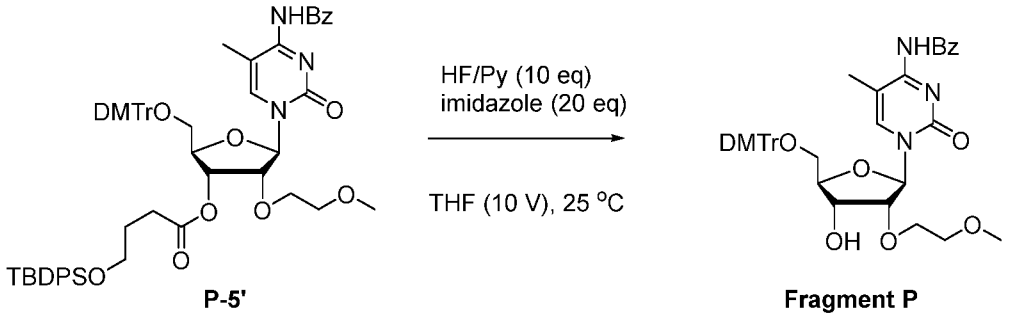
[0446] *d. Alternative Procedures for Synthesis of Fragment P*

[0447] General procedure for preparation of Compound P-5'



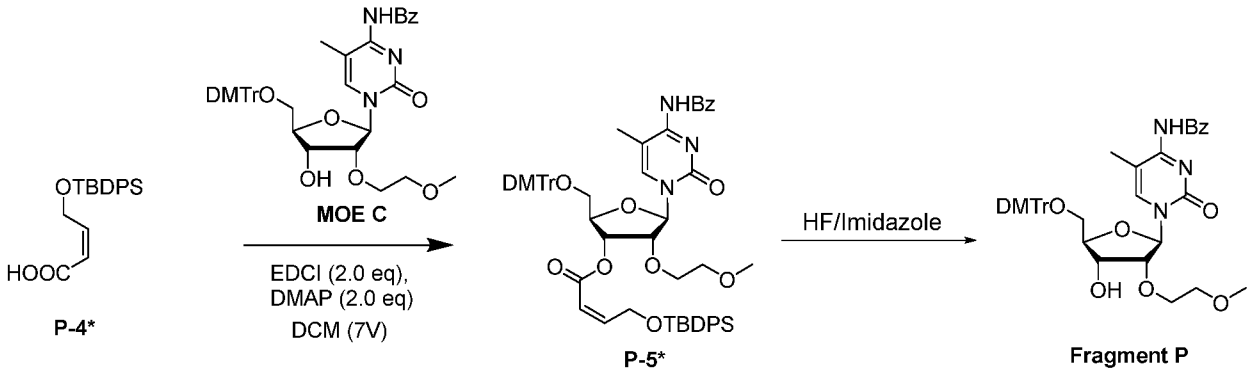
[0448] To a solution of compound P-4' (1.0 g, 2.92 mmol, 1.0 eq) and 5'-DMTr-MOE C-3'-OH (2.10 g, 2.92 mmol, 1.0 eq) in DCM (10.0 mL) was added EDCl (2.80 g, 5.0 eq) and DMAP (0.71 g, 2.0 eq) at 25°C under and stirred for 24 hours. The mixture was concentrated in vacuum and the residue was purified by silica gel column chromatography (Heptane/EtOAc 5:1 to 3:1). Compound P-5' was obtained as a white solid (1.97 g, 90% yield). Mass calcd for C61H67N3011Si+ [M+H+]: 1046.4 found: 1046.4.

[0449] General procedure for preparation of Fragment P

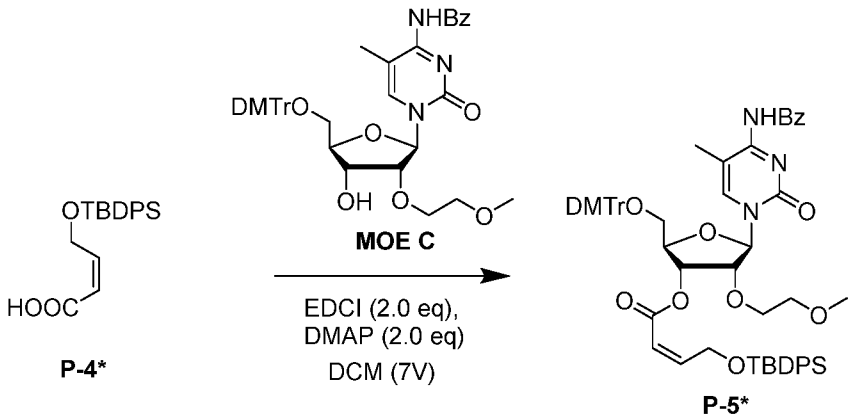


[0450] To a solution of compound **P-5'** (0.3 g, 0.29 mmol, 1.00 eq) in THF (1.0 mL) was added solution of imidazole (390.2 mg, 5.73 mmol, 20.0 eq) and pyridine/hydrofluoride (81.9 mg, 2.9 mmol, 70% purity, 10.0 eq) in THF (2.0 mL). The mixture was stirred at 25°C for 20 hours and Compound **P-5'** was converted to Fragment P with ~ 60% conversion.

[0451] *e. Alternative Scheme for Synthesis of monomer Fragment P*

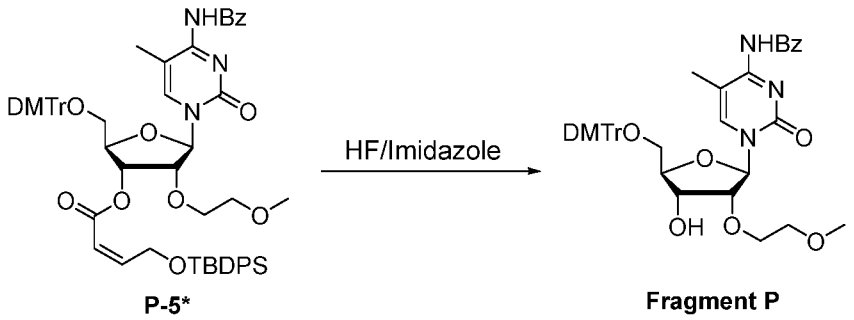


[0452] General procedure for preparation of Compound P-5*



[0453] To a solution of compound **P-4*** (2.12 g, 6.23 mmol, 1.5 eq) and 5'-DMTr-MOE C-3'-OH (3.0 g, 4.16 mmol, 1.0 eq) in DCM (20.0 mL) was added EDCl (1.59 g, 2.0 eq) and DMAP (1.02 g, 2.0 eq) at 25°C under and stirred for 24 hours. The mixture was concentrated in vacuum and the residue was purified by silica gel column chromatography (Heptane/EtOAc 5:1 to 3:1). Compound **P-5*** was obtained as a sticky oil (2.5 g, 93% yield). Mass calcd for $C_{61}H_{65}N_3O_{11}Si^+$ [M+H$^+$]: 1046.4 found: 1044.4.

[0454] General procedure for preparation of Fragment P



[0455] To a solution of compound P-5* (0.15 g, 0.145 mmol, 1.00 eq) in THF (1.0 mL) was added solution of imidazole (195.1 mg, 2.87 mmol, 20.0 eq) and pyridine/hydrofluoride (41 mg, 1.45 mmol, 70% purity, 10.0 eq) in THF (2.0 mL). The mixture was stirred at 50°C for 6 hrs and Compound P-5* was converted to Fragment P with ~ 90% conversion.

Example 22. Synthesis of Oligonucleotide Fragment B from M40

[0456] *a. Scheme for Synthesis of Oligonucleotide Fragment B from M40*

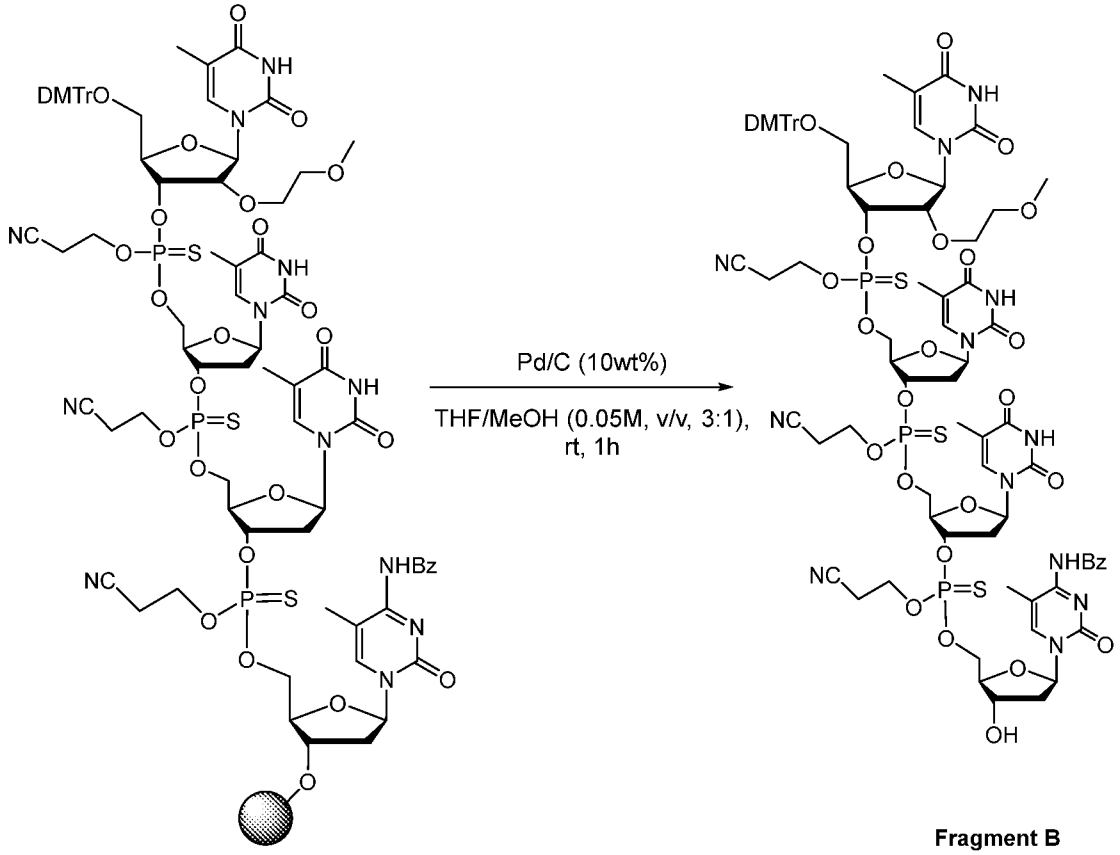
[0457] Fragment B was synthesized according to synthetic scheme depicted in FIG. 11.

[0458] *b. Procedures for Synthesis of Oligonucleotide Fragment B from reagent M40*

[0459] Procedures for synthesizing oligonucleotide fragment B from reagent M40 were similar those described for the synthesis of oligonucleotide fragment B from M19, except for

the last step of selective deprotection of M40. The procedure of selective deprotection of M40 is described as below.

[0460] The procedure of selective deprotection of M40



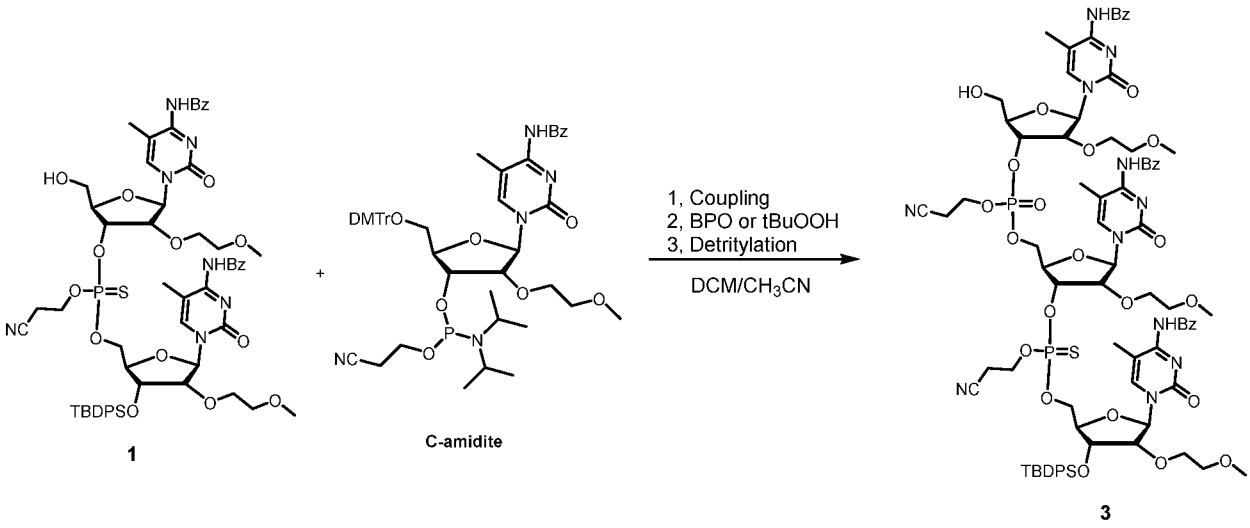
DMTrO-UTTC-OM40

Fragment B

[0461] Under a hydrogen atmosphere, stirred a mixture of DMTrO-UTTC-OM40 (1.0 eq) and palladium on carbon (10wt%) in tetrahydrofuran and methanol (0.05M, v/v, 3:1)) vigorously at room temperature for 1 hour. Filtered and concentrated the reaction mixture to residue which was precipitated in MTBE to yield desire product. Fragment B was obtained as a pale-yellow solid in 70.5% yield and 85.2% purity.

Example 23. One Pot Procedure for Preparation of P=O Linkage

[0462] *a. Scheme for One Pot Procedure for Preparation of P=O Linkage*



[0463] *b. Procedure for Preparation of P=O Linkage*

[0464] A mixture of **compound 1** (12 g, 10 mmol, 1.00 eq), MOE C amidite (11.16 g, 12 mmol, 1.20 eq) and 3Å MS (12.0 g) in CH_3CN/DCM (100 mL, v/v = 1:3) was stirred at 20-30 °C for 1 h, DCI (1.94 g, 15 mmol, 1.50 eq) was added, and the reaction mixture was stirred at 20-30 °C for 1.0 h (HPLC indicated the reaction conversion > 99.5%). H_2O (40 mg, 2 mmol, 0.2 eq) was added and the mixture was stirred at 25 °C for 30 min. NMI (1.35 g, 15 mmol, 1.5 eq), BPO (3.89 g, 11 mmol, 1.1 eq) and iodine (278 mg in DCM 6 mL, 1 mmol, 0.1 eq) were added to the reaction mixture at 0-5 °C and stirred at 0-5 °C for 1 h. Piperazine (652 mg, 7 mmol, 0.7 eq) was added and the mixture and stirred at 0-5 °C for 30 min. Dodecane-1-thiol (6.64 g, 3.0 eq) and 3Å MS (10.0 g) were added and the mixture was stirred at 0-10 °C for 60 min. TFA (13.7 g, 110 mmol, 11.00 eq) was added dropwise at 0-5 °C and stirred at 10-20 °C for 60 min. NMI (9.88 g, 110 mmol, 11.0 eq) was added at 0-5 °C and stirred at 0-5 °C for 10 min. The reaction mixture was filtered to remove 3 Å MS and added into 5% $NaHCO_3$ solution (120 mL) with vigorous stirring. EtOAc (120 mL) and MTBE (120 mL) were added and stirred vigorously for 10 min. The organic layer was separated, washed with 5% aqueous $NaHCO_3$ solution (120 mL), H_2O (120 mL), brine (120 mL), dried over $MgSO_4$ (24 g), filtered, and concentrated in vacuo. The crude product was dissolved in EtOAC (36 mL) slowly added to a mixture of heptane/TBME (216 L, 1:1, v/v). The precipitated product was filtered, washed with heptane/TBME (2 x 400 mL, 1:1, v/v) and dried under vacuum at 20-30 °C for 16 h to yield compound **3** as a white solid (14.3 g, 80.1% yield). HRMS calcd for $C_{82}H_{97}N11O_{24}P_2SSi^+$ $[M+H]^+$: 1742.5751, found: 1742.5732.

[0465] c. Comparison Among Different Oxidation Reagents

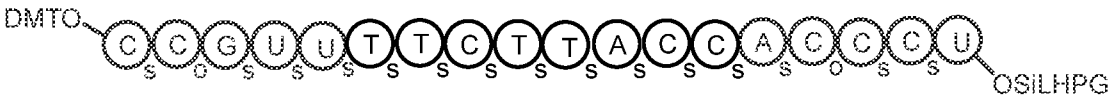
[0466] Several oxidation reagents, including iodine/pyridine, mCPBA, BPO, and tBuOOH, have been tested in the one pot procedure (coupling/oxidation/detritylation) for preparing

P=O linkage. The scheme, which shows the reaction product 3 and by-products 1 and 2, is depicted in FIG. 12. The performance of each oxidation reagent is summarized in Table 2 below.

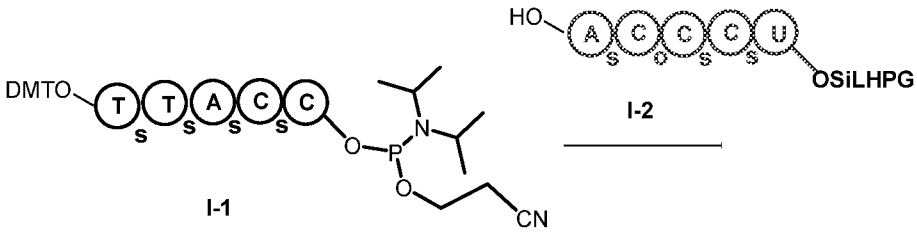
Oxidation reagent	By-product 1	By-product 2	Purification
iodine/pyridine	0.4-1.0%		Need to remove pyridine before detritylation
mCPBA		>10% PS was converted to PO	
BPO	<0.2%	<0.1%	
tBuOOH	<0.2%	<0.1%	

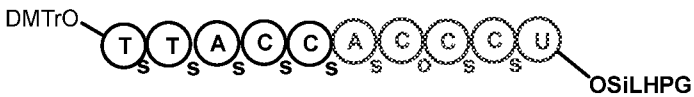
[0467] Oxidation reagents BPO and tBuOOH demonstrated superior oxidation performance than iodine/pyridine and mCPBA in the one-pot procedure for preparation of the P=O linkage in oligonucleotides. When BPO or tBuOOH was used in the one-pot procedure, both of them produced less by-products than iodine/pyridine and mCPBA did. In addition, the one-pot procedure was not successful for iodine/pyridine because it required an additional purification step to remove pyridine before carrying out the detritylation step.

Example 24. Synthesis of Oligonucleotide I



[0468] Step 1: synthesis of compound I-3





I-3

[0469] To a first round bottom flask (RBF) was added compound I-2 (1 eq) under Ar. It was dried three times by co-evaporating with (DCM/ACN=3:1,4 v) at 25-30°C. Then DCM/ACN=2:1 (6V) was added to the RBF, followed by 3A MS (5%) at 25-30 °C for 1h. Compound I-1 (1.5 eq) was then added to a second RBF under Ar and was dried three times

by co-evaporating with (ACN 4 v) at 25-30°C. DCM (2V) was added to the second RBF and the resulting solution in the second RBF was added to the first RBF dropwise at 20~25 °C, followed by the addition DCI (2 eq). The resulting mixture was stirred at 25-30°C for 1h. A sample was taken for analysis. Then to the reaction mixture was added DDTT (2eq). The mixture was stirred at 25-30°C for 0.5h. A sample was taken for analysis. Then the mixture was filtered to remove 3 Å MS, washed with DCM (2 V x 2). The resulted solution was slowly added into ACN (50 V) at 20 °C for 0.5 h. Solid was collected by filtration, washed by ACN (5 V x 2) to afford compound I-3 as a white solid.

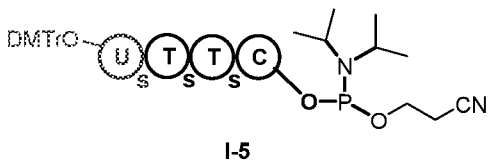
[0470] Step 2: detritylation of compound I-3

I-3

I-4

[0471] Compound I-3 (1 eq) was added into a RBF under Ar, followed by DCM (7 V) at 0-5°C under Ar and 3A MS (5%) at 20-25°C for 1h. Then to the mixture was added $C_{12}H_{25}SH$ (2 eq), followed by TCA (10 eq) dropwise at 0-5°C for 2h. A sample was taken for analysis. Py (12 eq) was added at 0-5°C. The mixture was filtered to remove 3 Å MS and washed by DCM (2 V x 2). The pH value was adjusted to 7~8 by adding $NaHCO_3$ (4% wt , 10 V). Then the mixture was extracted with DCM (2V x 2). The organic layer was dried over anhydrous $MgSO_4$, filtered, and washed by DCM (2 V x 2). The filtrate was concentrated to ~5V which was slowly added into ACN (50 V) at 20 °C for 0.5 h. Solid was collected by filtration and washed by ACN (5 V x 2) to give compound I-4 as a white solid.

[0472] Step 3: synthesis of compound I-6



I-5

I-4

I-6

[0473] Compound I-4 (1 eq) was added to a first RBF under Ar. It was dried three time by co-evaporating with DCM/ACN(3:1,4 v) at 25-30°C. Then DCM/ACN (2:1, 6V) was added to the RBF, followed by 3A MS (5%) at 25-30 for 1h. Compound I-5 was added (1.5 eq) to a second RBF under Ar. The mixture was dried three times by co-evaporating with ACN (4 v) at 25-30°C and then DCM (2V) was added. the resulting solution in the second RBF was transferred to the first RBF dropwise at 20~25 °C, followed by the addition of DCI (2 eq). The resulting mixture was stirred at 25-30°C for 1h. A sample taken out for analysis. DDTT (2eq) was added to the RBF . The resulting mixture was stirred at 25-30°C for 0.5h. A sample was taken out for analysis. Then the reaction mixture was filtered to remove 3 Å MS, washed by DCM (2 V x 2). The resulting solution was slowly added into ACN (50 V) at 20 °C for 0.5 h. Solid was collected by filtration and washed by ACN (5 V x 2) to give compound I-6 as a white solid.

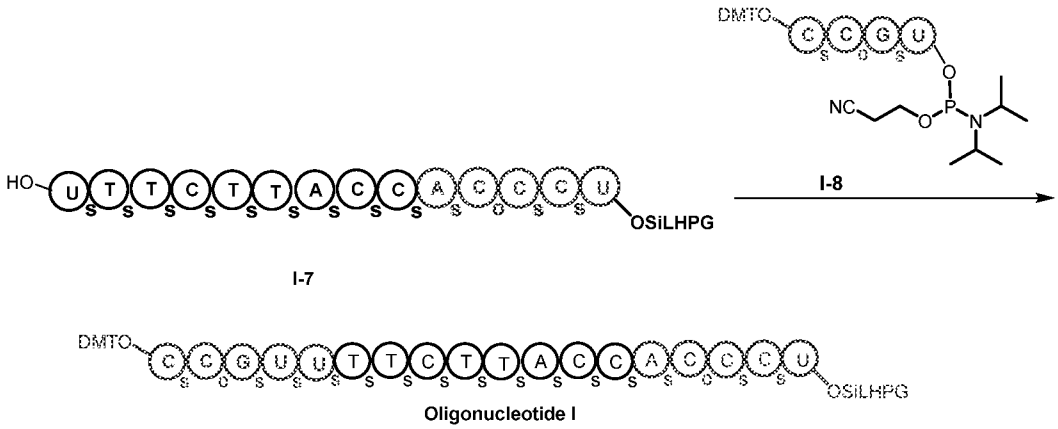
[0474] Step 4: detritylation of compound I-6

I-6

I-7

[0475] Compound I-6 (1 eq) was added to a round bottom flask (RBF) under Ar, followed by DCM (7 V) at 0-5°C under Ar and 3A MS (5%) at 20-25°C for 1h. Then to the mixture was added $C_{12}H_{25}SH$ (3 eq), followed by TCA (12 eq) dropwise at 0-5°C for 2h. A sample was taken out for analysis. Then Py (15 eq) was added to the RBF at 0-5°C. The mixture was filtered to remove 3 Å MS and washed with DCM (2 V x 2). The pH value was adjusted to 7~8 by adding $NaHCO_3$ (4% wt , 10 V). Then it was extracted with DCM (2V x 2). The organic layer was dried over anhydrous $MgSO_4$, filtered, and washed by DCM (2 V x 2). The filtrate was concentrated to ~5V which was slowly added into ACN (50 V) at 20 °C, for 0.5 h. Solid was collected by filtration and washed with ACN (5 V x 2) to give compound I-7 as a white solid.

[0476] Step 5: synthesis of oligonucleotide I



[0477] Compound I-7 (1 eq) was added to a first RBF under Ar. It was dried three time by co-evaporating with DCM/ACN(3:1,4 v) at 25-30°C. Then DCM/ACN (3:1, 10V) was added to the RBF, followed by 3A MS (5%) at 25-30 for 1h. Compound I-8 was added (1.7 eq) to a second RBF under Ar. The mixture was dried three times by co-evaporating with ACN (4 v) at 25-30°C and then DCM (2V) was added. the resulting solution in the second RBF was transferred to the first RBF dropwise at 20~25 °C, followed by the addition of DCI (2.5 eq). The resulting mixture was stirred at 25-30°C for 1h. A sample was taken out for analysis. DDTT (2eq) was added to the RBF. The resulting mixture was stirred at 25-30°C for 0.5h. A sample was taken out for analysis. Then the reaction mixture was filtered to remove 3 Å MS, washed by DCM (2 V x 2). The resulting solution was slowly added into ACN (50 V) at 20 °C for 0.5 h. Solid was collected by filtration and washed by ACN (5 V x 2) to give oligonucleotide I in 3.1 g (76.4% yield, 69% UV purity) as a white solid.

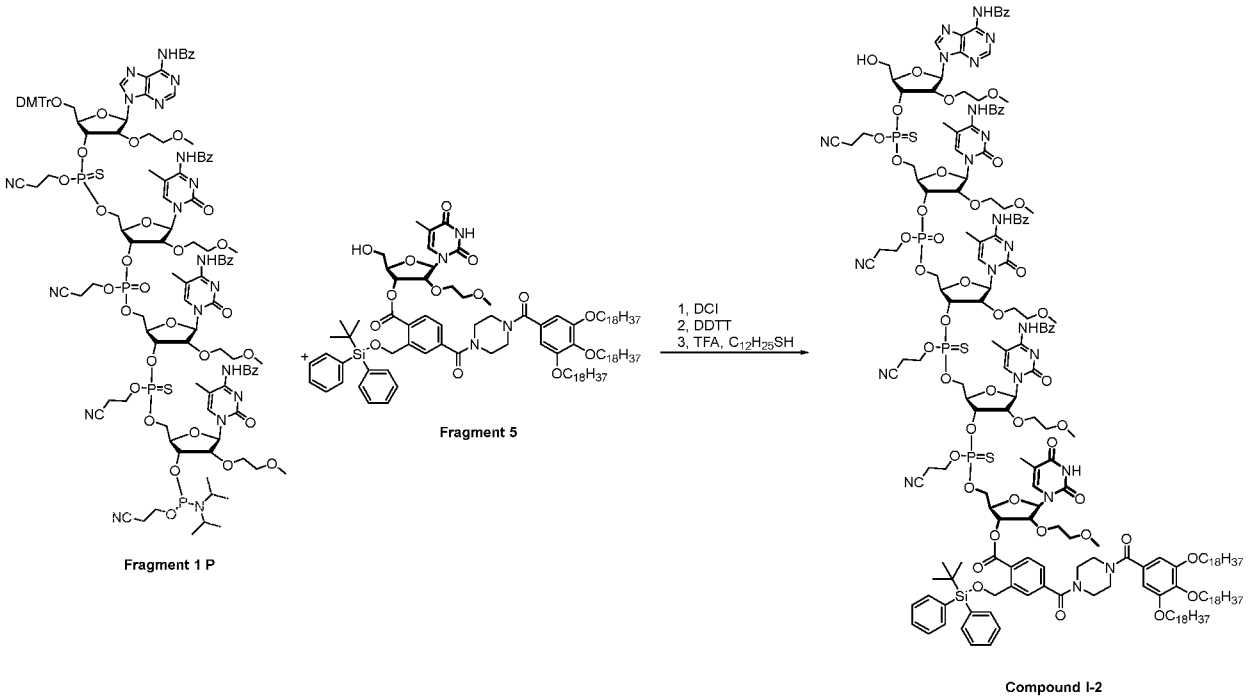
[0478] Example 25. Scale up Synthesis of Oligonucleotide I

[0479] *a. Scheme for Synthesis of Oligonucleotide I*

[0480] Oligonucleotide I was synthesized at a 50 gram scale according to synthetic scheme depicted in FIG. 13.

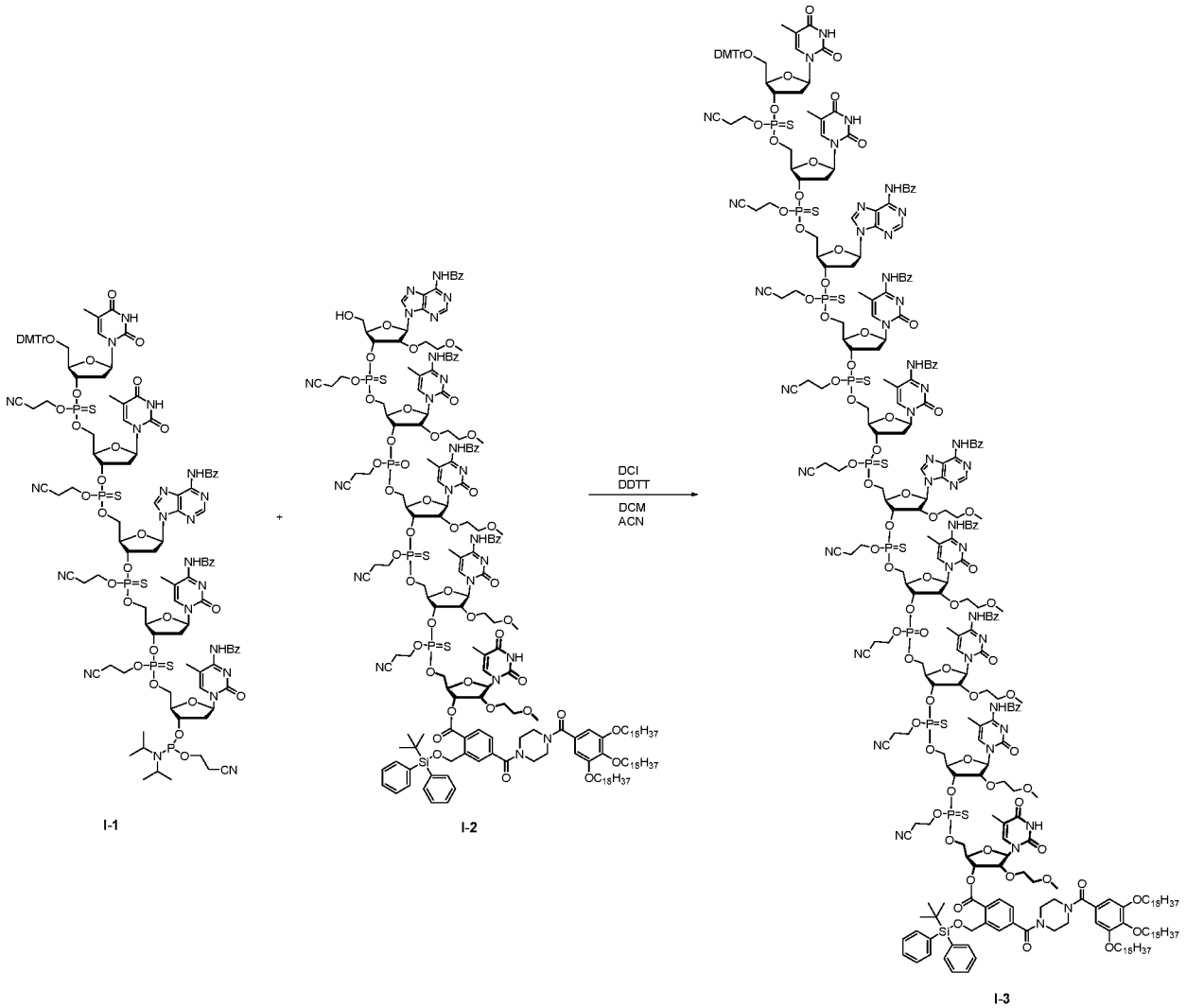
[0481] *b. Procedures for Synthesis of Oligonucleotide I*

[0482] General Procedure for Preparation of Compound I-2



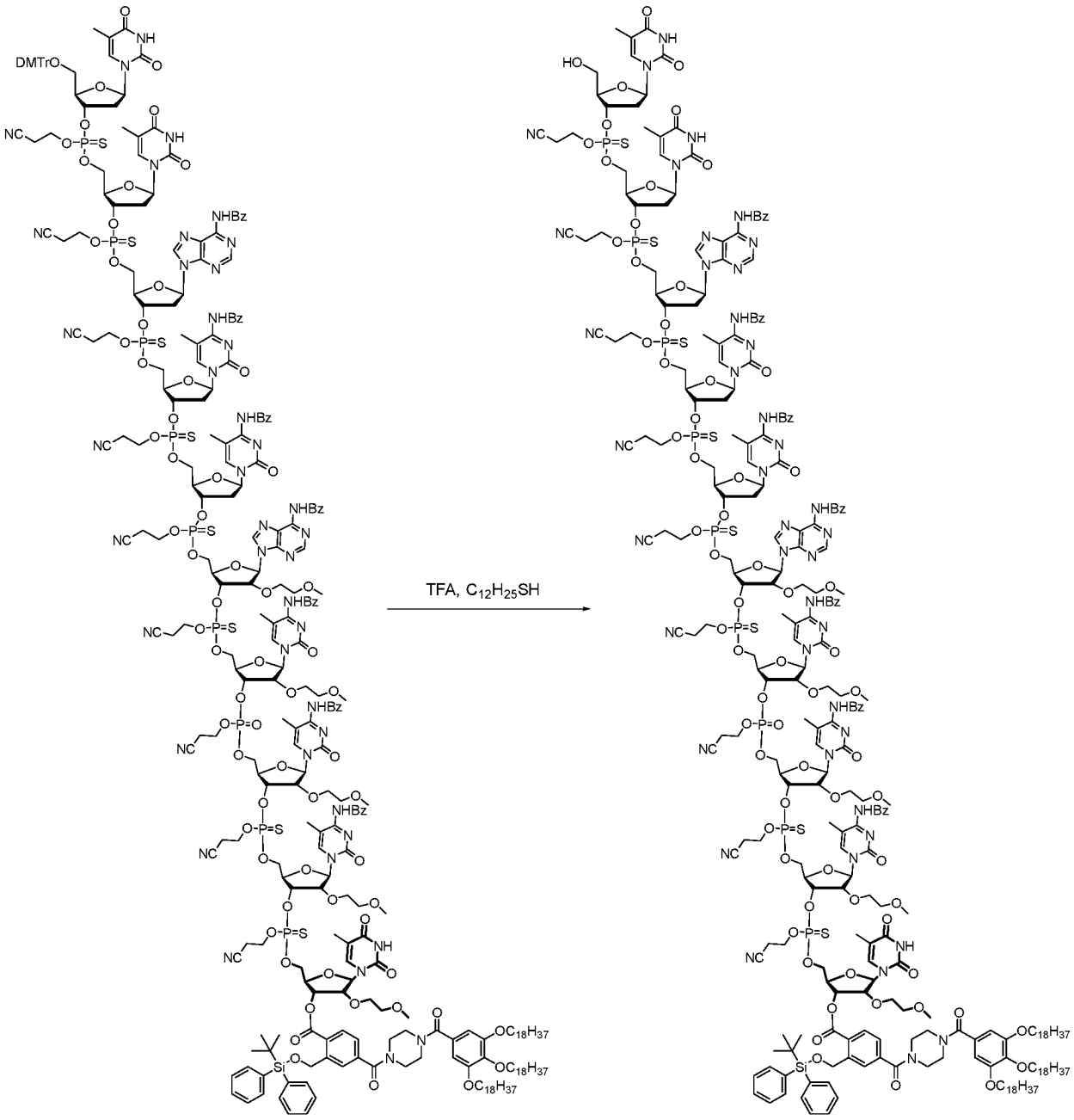
[0483] A mixture of **Fragment 1P** (24.0 g, 14.1 mmol), **Fragment 5** (44.1 g, 17.2 mmol), 3Å MS (5 g/100mL, 12 g), and DCM (240 mL) under N_2 atmosphere was stirred at 20-25 °C for 1.0 h. DCI (6.6 g, 56.4 mmol) was added and the reaction mixture was stirred for 1.0 h at 20-30 °C. DDTT (7.2 g, 35.25 mmol) was added and the reaction mixture was stirred at 20-25 °C for 30 min. Dodecane-1-thiol (9.94 g, 49.3 mmol) was added and the reaction mixture was stirred at 0±5 °C for 10 min. TFA (14.4 g, 126.9 mmol) was added slowly and the reaction mixture was stirred at 0±5 °C for 1.0 h. NMI (12.7 g, 155.1 mmol) was added in 10 min, and the reaction mixture was filtered to remove 3Å MS, concentrated to about 100 mL by rotovap and added to CH_3CN (2.6 L) in 30 min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with ACN (2 x 100 mL) and dried under vacuum at 20-30 °C for 16 h to yield **compound I-2** as a white solid (46.7 g, 85.1% yield).

[0484] General Procedure for Preparation of Compound I-3



[0485] A mixture of **compound I-2** (44.0 g, 11.3 mmol), **compound I-1** (40.8 g, 15.9 mmol), 3Å MS (44 g) and ACN/ DCM (440 mL, 1:3, v/v) was stirred at 20-25 °C for 1.0 h under N_2 atmosphere. DCI (6.66 g, 56.5 mmol) was added, and the reaction mixture was stirred for 1.0 h. DDTT (5.1 g, 34.9 mmol) was added and the reaction mixture was stirred at 20-25 °C for 30 min. The reaction mixture was filtrated and the 3Å MS filter cake was washed with DCM (2 x 50 mL). The combined filtrate was concentrated to about 200 mL on a rotary evaporator and added to ACN (2 L) in 30 min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with ACN (2 x 100 mL) and dried under vacuum at 20-30 °C for 12 h to yield **compound I-3** as a slightly yellow solid (71.4 g, 98.6% yield).

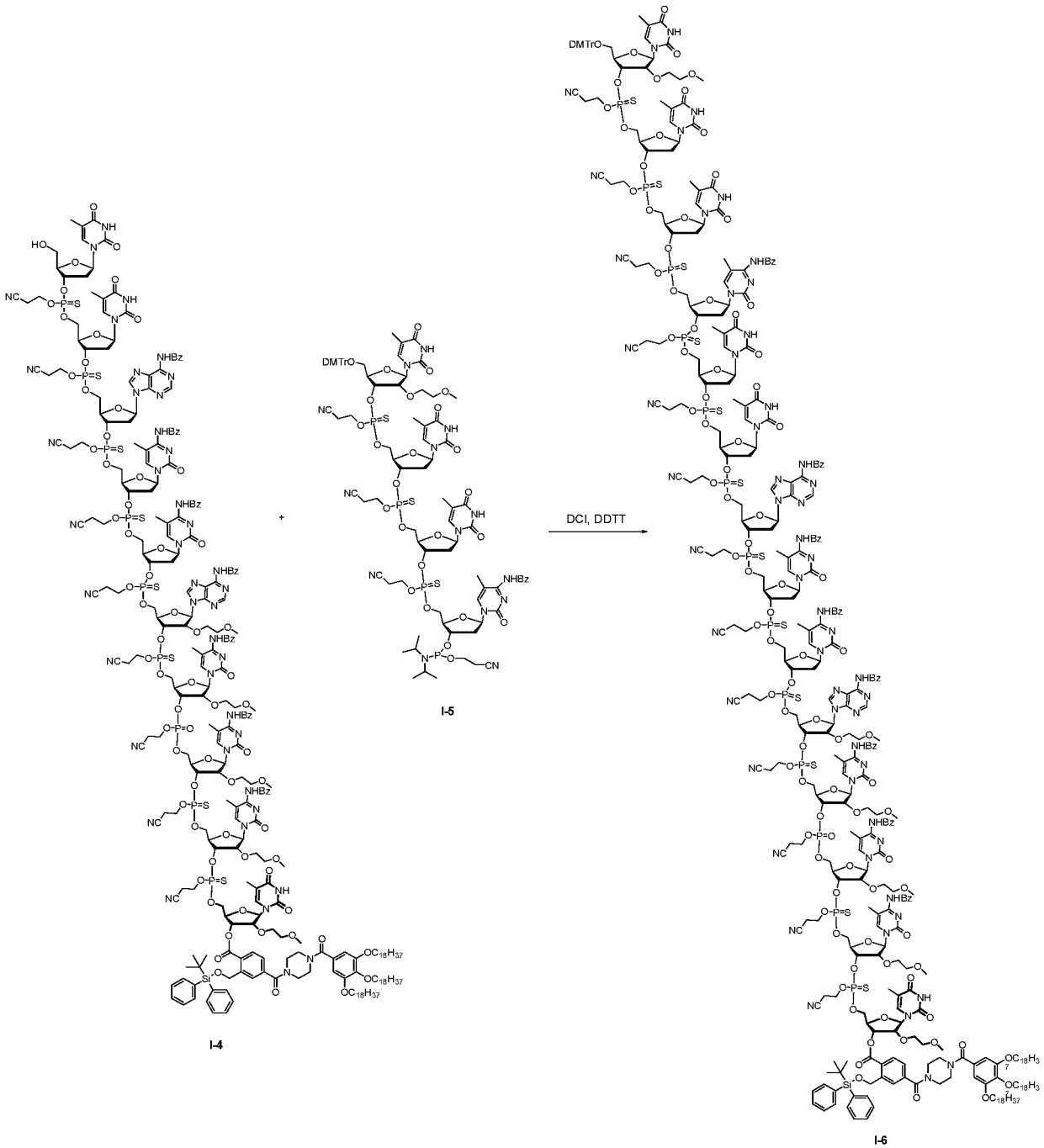
[0486] General Procedure for Preparation of Compound I-4



I-3 I-4

[0487] A mixture of **compound I-3** (69.0 g, 10.8 mmol), 3Å MS (71.0 g) and DCM (480 mL) was stirred at 20-25 °C for 1.0 h under N_2 atmosphere and cooled to 0±5 °C. Dodecane-1-thiol (5.6 g, 27 mmol) was added and the reaction mixture was stirred at 0 °C for 10 min. TFA (13.6 g, 118.8 mmol) was added slowly and the reaction mixture was stirred at 0±5 °C for 1.5. NMI (11.5 g, 140.4 mmol) was added in 10 min, the reaction mixture was filtered to remove 3Å MS, concentrated to about 200 mL on rotary evaporator and added to ACN (2.5 L) in 30 min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with ACN (2 x 172 mL) and dried under vacuum at 20-30 °C for 14 h to yield **compound I-4** as a white solid (55.86 g, 85.0% yield).

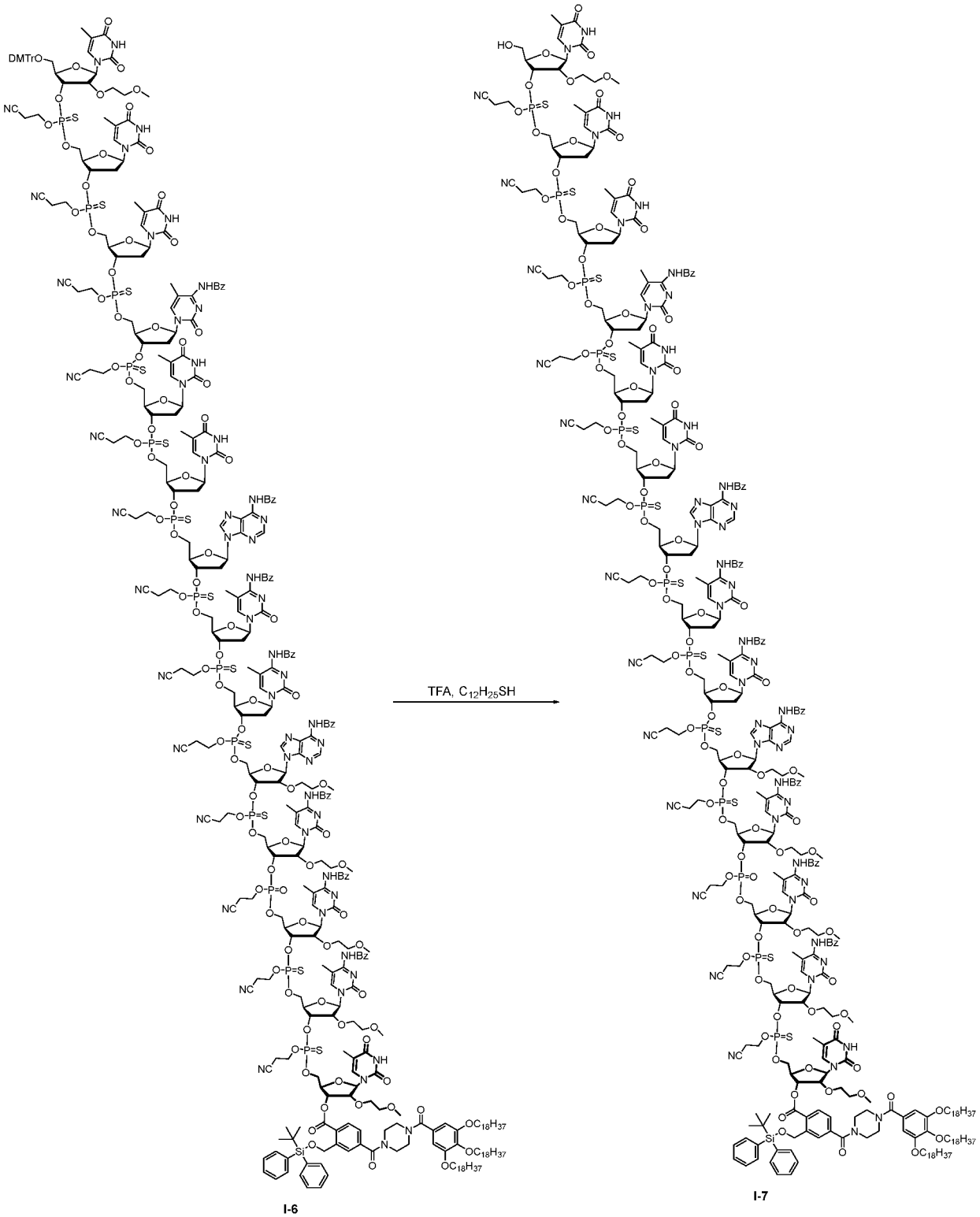
[0488] General Procedure for Preparation of Compound I-6



[0489] A mixture of **compound I-4** (39.9 g, 6.55 mmol), **compound I-5** (18.8 g, 9.17 mmol), 3Å MS (40 g) and ACN/DCM (400 mL, 1:3, v/v) was stirred at 20-25 °C for 1.0 h under N_2 atmosphere. DCI (3.87 g, 32.8 mmol) was added and the reaction mixture was stirred for 1.0 h. DDTT (2.96 g, 14.4 mmol) was added and the reaction mixture was stirred at 20-25 °C for 30 min. DCM (100 mL) was added and the reaction mixture was filtrated and the 3Å MS filter cake was washed with DCM (2 x 30 mL). The combined filtrate was concentrated to about 200 mL on a rotary evaporator and slowly added to ACN (1.36 L) in 30 min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with

ACN (3 x 100 mL) and dried under vacuum at 20-30 ºC for 12 h to yield **compound I-6** as a light-yellow solid (49.8 g, 94.3% yield).

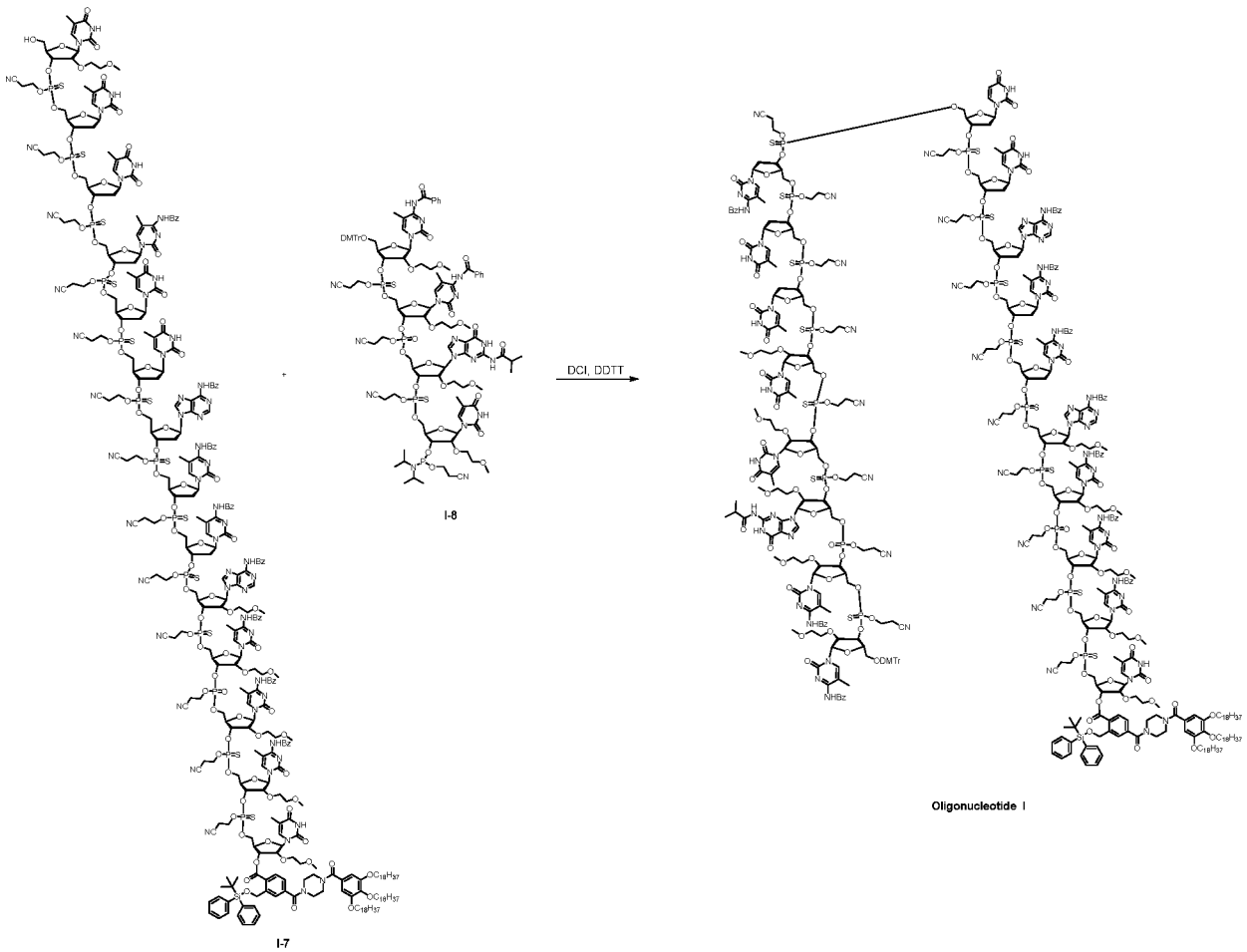
[0490] General Procedure for Preparation of Compound I-7



[0491] A mixture of **compound I-6** (50.0 g, 6.20 mmol), 3Å MS (18.0 g) and DCM (350 mL) was stirred at 20-25 ºC for 1.0 h under N_2 atmosphere and cooled down to 0±5 ºC.

Dodecane-1-thiol (3.77 g, 18.6 mmol) was added, and the reaction mixture was stirred at 0 °C for 10 min. TFA (9.19 g, 80.6 mmol) was added slowly and the reaction mixture was stirred at 0±5 °C for 1.5 h. NMI (8.14 g, 99.2 mmol) was added in 10 min, and the reaction mixture was filtered, and the 3Å MS filter cake was washed with DCM. The combined filtrate was concentrated to about 200 mL on a rotary evaporator and added to ACN (2.65 L) in 30 min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with ACN (3 x 150 mL) and dried under vacuum at 20-30 °C for 14 h to yield **compound I-7** as a white solid (45.9 g, 95.3% yield).

[0492] General Procedure for Preparation of Oligonucleotide I



[0493] A mixture of **compound I-7** (42.0 g, 5.41 mmol), **compound I-8** (23.9 g, 9.74 mmol), 3Å MS (42 g) and DCM/CH_3CN (400 mL, 3:1, v/v) was stirred at 20-25 °C for 1.0 h under N_2 atmosphere. DCI (3.2 g, 27.05 mmol) was added, and the reaction mixture was stirred for 1.0 h. DDTT (2.45 g, 11.9 mmol) was added and the reaction mixture was stirred at 20-25 °C for 30 min. DCM (200 mL) was added and the reaction mixture was filtrated, and 3Å MS filter cake was washed with DCM (2 x 50 mL). The combined reaction mixture was concentrated to about 300 mL on a rotary evaporator and slowly added to ACN (2.50 L) in 30

min with vigorous agitation at 0±5 °C. The precipitated product was filtered, washed with ACN (2 x 150 mL) and dried under vacuum at 20-30 °C for 14 h to yield oligonucleotide I as a light-yellow solid (51.6 g, 94.1% yield).

[0494] Characterization of oligonucleotide I: The mixture of oligonucleotide I (100.0 mg) and 30% NH_4OH (2 mL) in a 4 mL pressure flask was stirred at 65 °C for 4 h. The resulting compound was checked in LCMS. The structure of oligonucleotide I was confirmed by LCMS. HRMS calcd for $C_{231}H_{318}N_{53}O_{118}P_{17}S_{15}/4^-$ [M]/4: 1682.0, found: 1682.1.

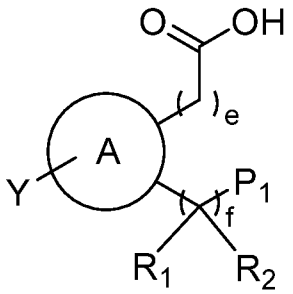
[0495] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0496] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

CLAIMS

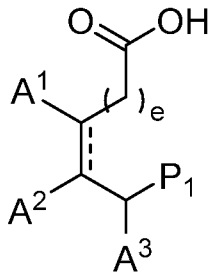
What is claimed is:

1. A compound of formula I' or B:



I'

or



B

or a salt thereof, wherein:

one of A^1, A^2 and A^3 is Y^A and the others are H;

$\equiv$ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

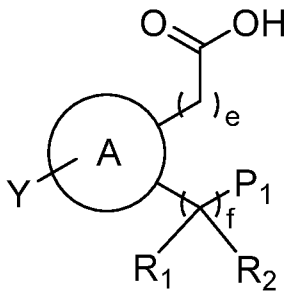
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

2. The compound of claim 1, wherein the compound is of formula I':



I'

or a salt thereof, wherein:

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, $-OC(O)R^{8A}$, $-C(O)OR^{8A}$, $NR^{8A}R^{9A}$, $-NR^{8A}COR^{9A}$, $-CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

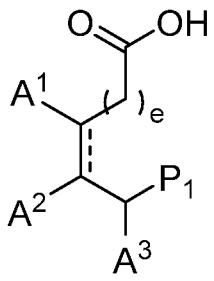
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

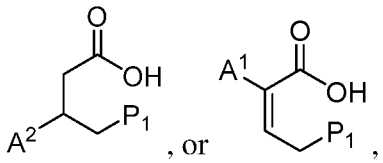
3. The compound of claim 1, wherein the compound is of formula B:



B

or a salt thereof.

4. The compound of claim 3, wherein the compound is represented by one of the following formula:



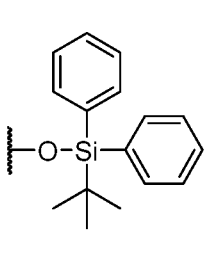
B-1 B-2

or a salt thereof.

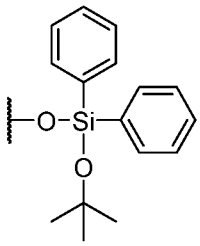
5. The compound of any one of claims 1-4 or a salt thereof, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

6. The compound of any one of claims 1, 2 and 5 or a salt thereof, wherein ring A is phenyl or naphthalenyl.

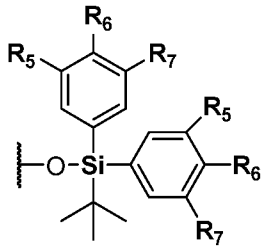
7. The compound of any one of claims 1 to 6, or a salt thereof, wherein P_1 is a silyl hydroxyl protecting group selected from the following:



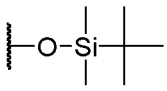
-O-TBDPS ,



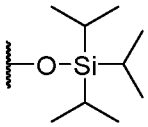
-O-TBoDPS ,



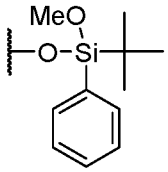
-O-TBDAS ,



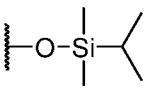
-O-TBDMS ,



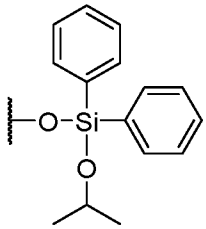
-O-TIPS ,



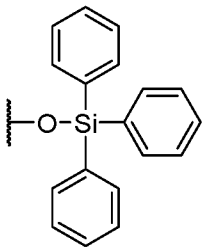
-O-TBMPS ,



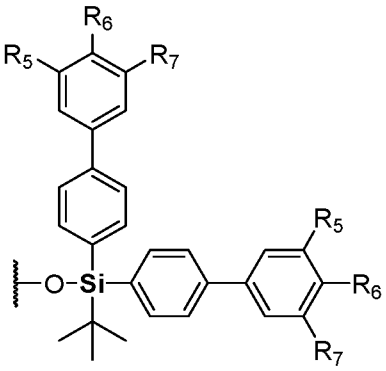
-O-DMIPS ,



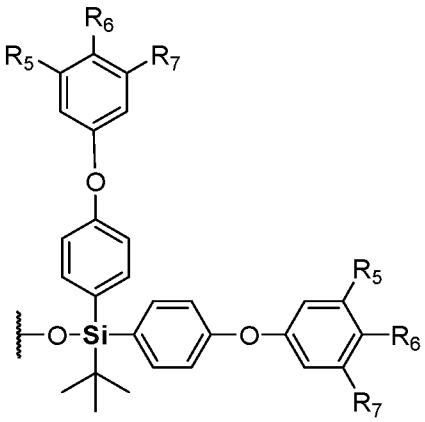
-O-IPoDPS ,



-O-TPS ,



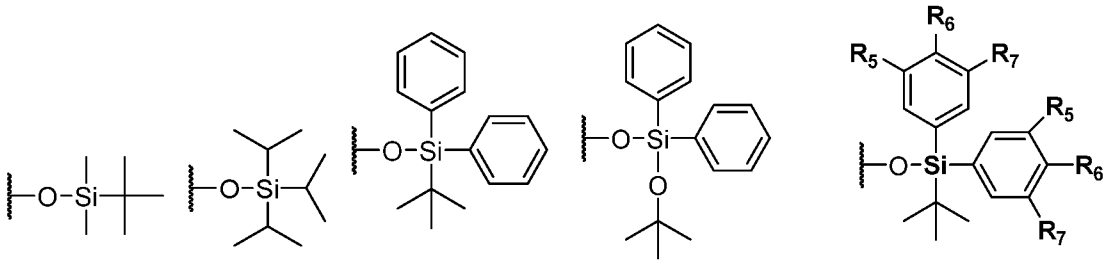
-O-TBDAS-1 ,



and -O-TBDAS-2 ;

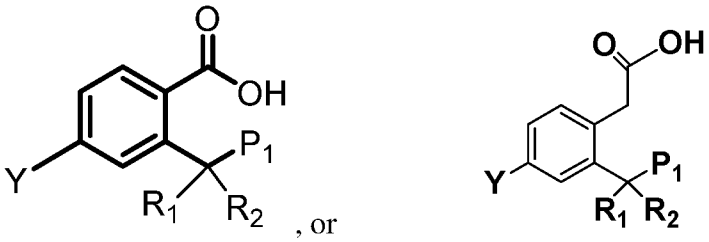
wherein ⊢ represents the point of attachment for P_1 and R_5, R_6 and R_7 are each independently H, $C_{1\text{-}30}$alkyl, or $C_{1\text{-}30}$alkoxy.

8. The compound of any one of claims 1 to 7, or a salt thereof, wherein P_1 is selected from the group consisting of –O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , and -O-TBDAS .

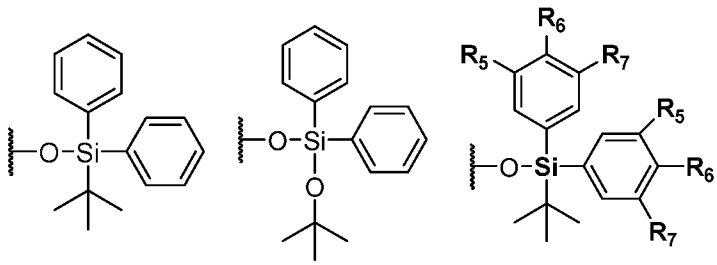
9. The compound of any one of claims 1, 2, and 5 to 8 represented by Formula I or Ia:



I Ia

or a salt thereof;

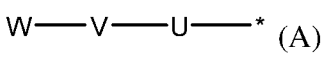
wherein P_1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDPS , -O-TBoDPS , -O-TBDAS ; and

R_5, R_6 and R_7 are each independently H, $C_{1\text{-}30}$alkyl, or $C_{1\text{-}30}$alkoxy.

10. The compound of any one of claims 1 to 9 or a salt thereof, wherein Y is represented by Formula A:

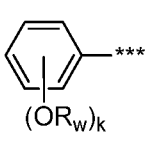
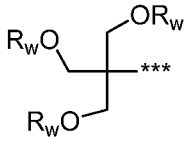
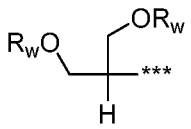
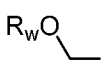


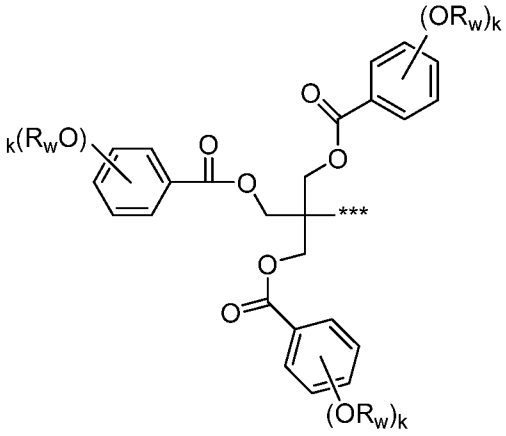
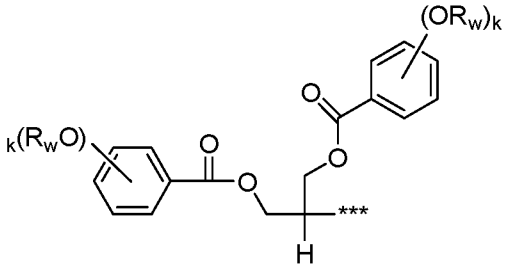
(A)

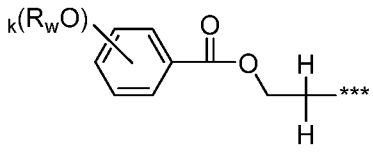
wherein:

—* represents the point of attachment for Y;

W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:


(A1),

(A2),

(A2-1),

(A2-2),


(A3),

(A3-1),

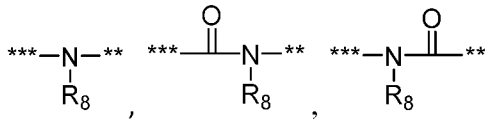
or

(A3-2),

wherein

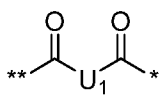
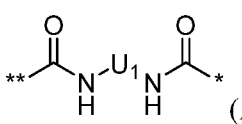
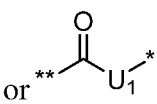
—*** represents the point where W and V connect;

each R_w is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

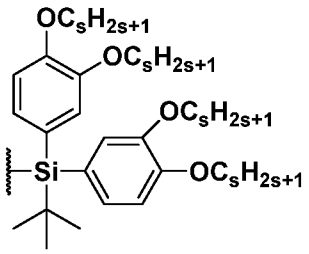
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:


(A4),

(A5), or

(A6),

wherein U_1 is $C_{1\text{-}6}$alkylene, $C_{1\text{-}6}$alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

11. The compound of any one of claims 7 to 10 or a salt thereof, wherein the TBDAS group is:

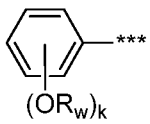


,

wherein s is an integer from 1 to 30.

12. The compound of any one of claims 1 to 11 or a salt thereof, wherein P_1 is –O-TBDPS.

13. The compound of any one of claims 10-12 or a salt thereof, wherein W is represented by Formula A1:

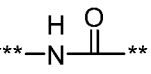


A1,

wherein R_w is C_nH_{2n+1};

n is an integer from 1 to 30.

14. The compound of any one of claims 10-13 or a salt thereof, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and $C_{28}H_{57}$.

15. The compound of any one of claims 10-14 or a salt thereof, wherein V is a bond, CH_2, CH_2CH_2, C(=O), ***-C(=O)-O-**, or 

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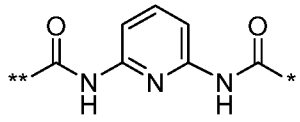
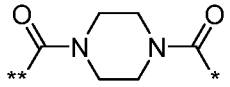
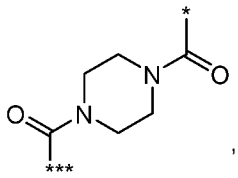
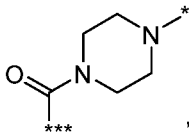
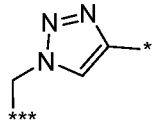
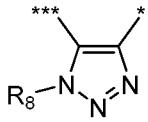
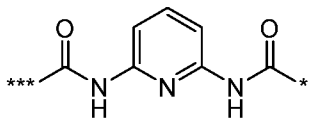
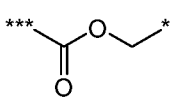
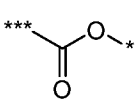
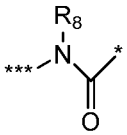
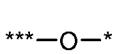
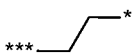
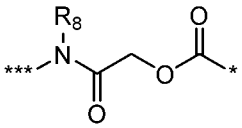
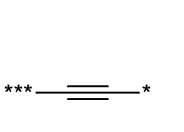
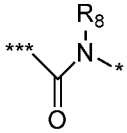
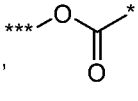
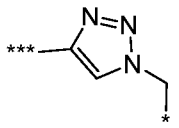
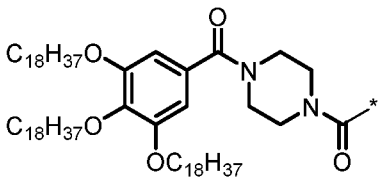
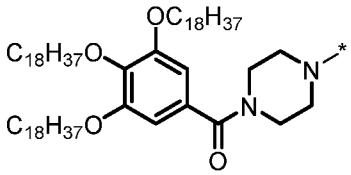
16. The compound of any one of claims 10-15 or a salt thereof, wherein U is a bond, CH_2, CH_2CH_2, carbonyl, triazolylene, piperazinylene,  , or  .

17. The compound of any one of claims 10-14 or a salt thereof, wherein U-V is selected from the group consisting of

 ,  ,  ,  ,  , ,  ,  ,  ,  ,  ,  ,  ,  ,  , , and  ;

wherein R_8 is H or $C_{1\text{-}6}$alkyl.

18. The compound of any one of claims 1-12 or a salt thereof, wherein Y is selected from the groups consisting of


,

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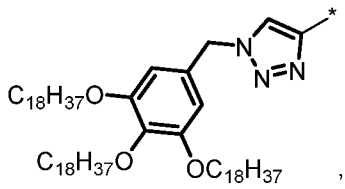
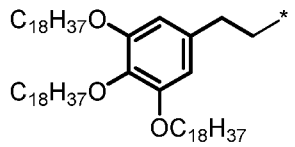

,

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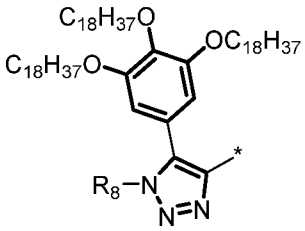
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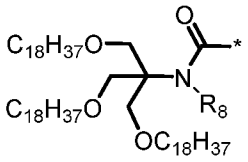
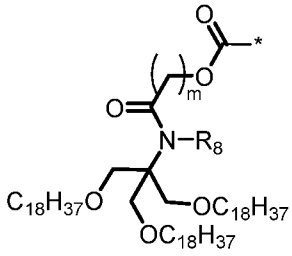
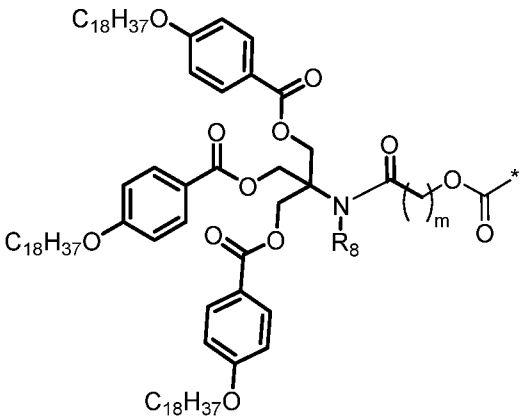


, and

;

wherein

R_8 is H or $C_{1\text{-}6}$alkyl; and

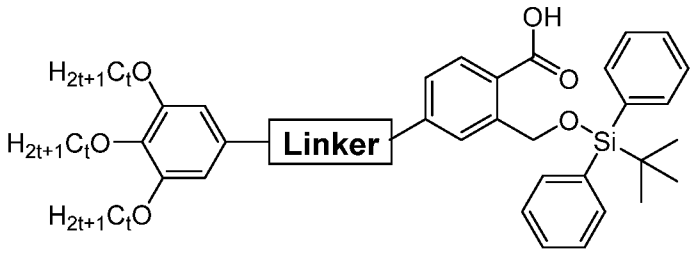
m is an integer from 1 to 5.

19. The compound of any one of claims 1, 2, and 5-18 or a salt thereof, wherein R_1 and R_2 are independently H or CH_3.

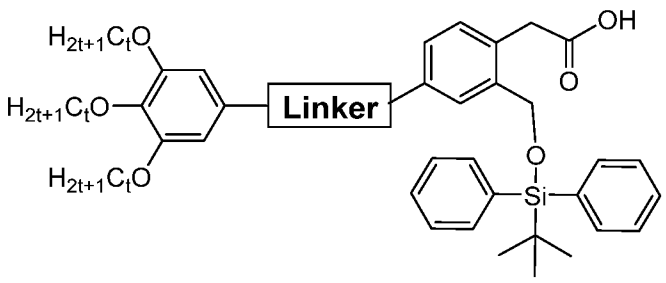
20. The compound of any one of claims 1, 2, and 5-19 or a salt thereof, wherein e is 0, 1, or 2; and f is 0, 1, or 2.

21. The compound of any one of claims 10-20 or a salt thereof, wherein R_8 is H or $C_{1\text{-}4}$alkyl.

22. The compound of claim 1 or a salt thereof represented by Formula II or IIa



(II), or

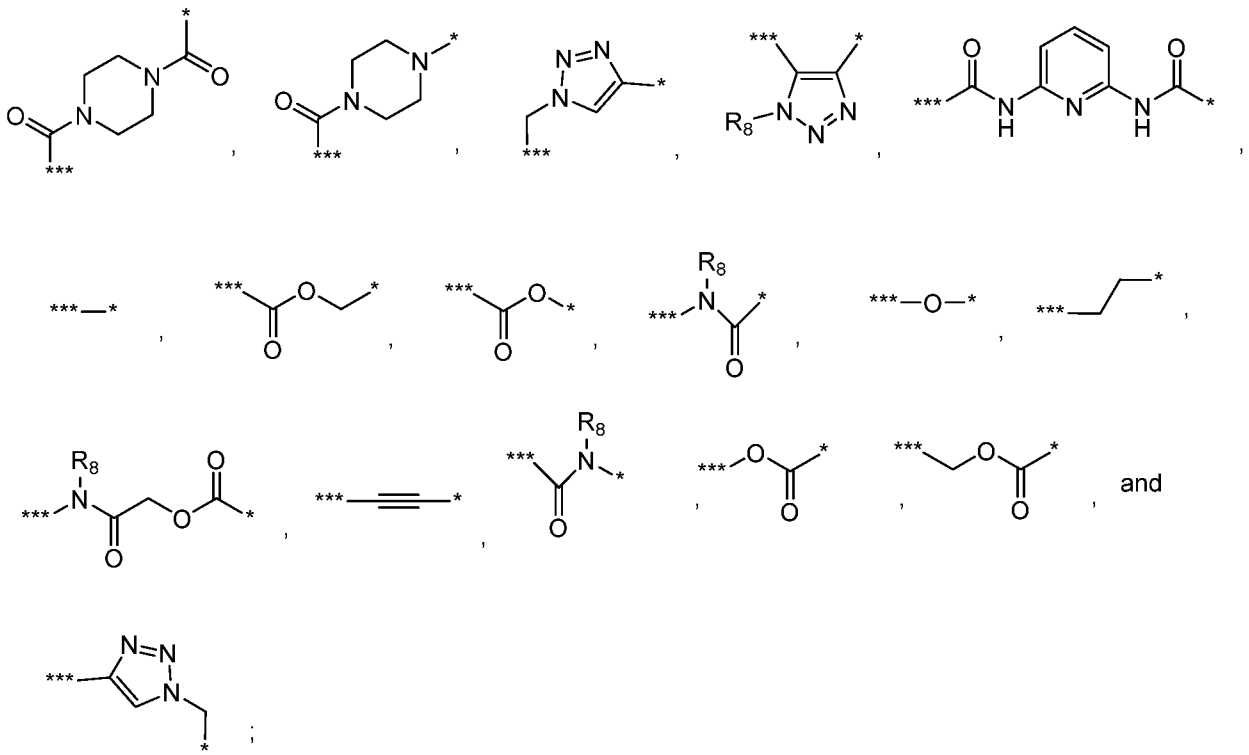


(IIa),

wherein

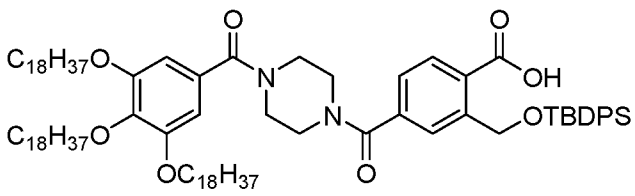
t is an integer from 10 to 30;

Linker is selected from the group consisting of

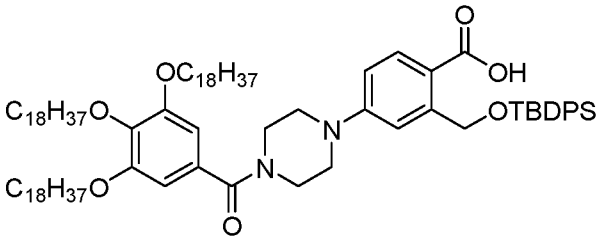


wherein R_8 is H or $C_{1\text{-}6}$alkyl.

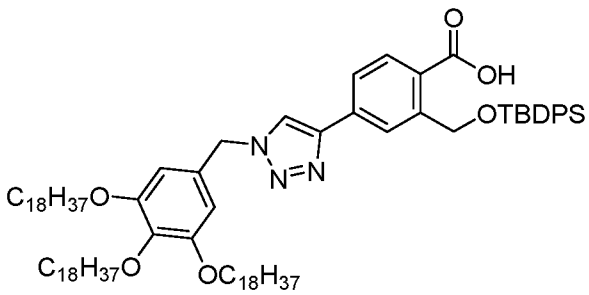
23. The compound of claim 22 is selected from the group consisting of



,



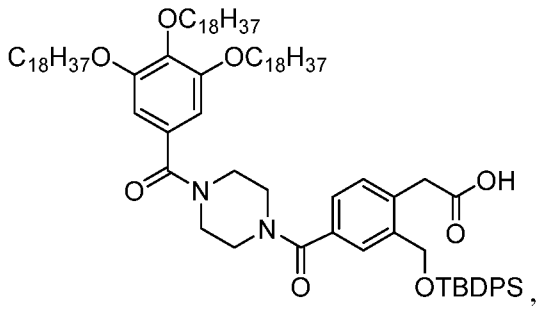
, and



;

or a salt thereof.

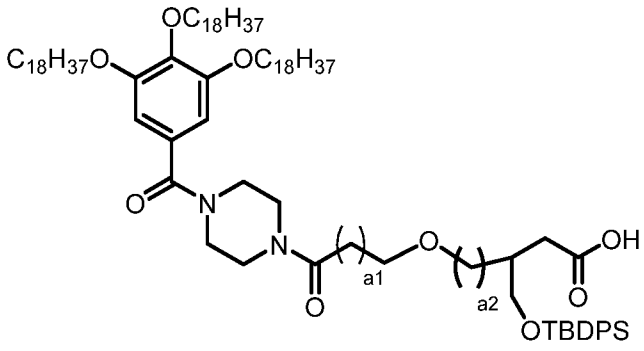
24. The compound of claim 1, wherein the compound is



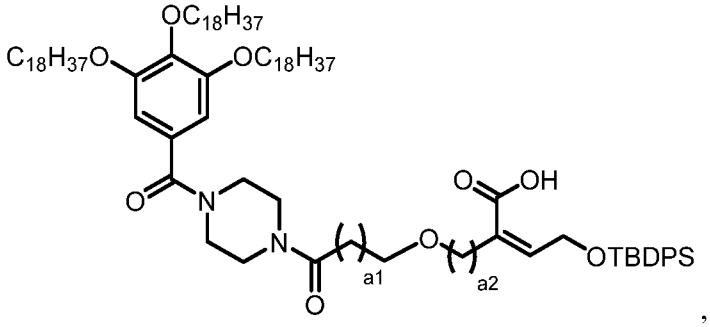
,

or a salt thereof.

25. The compound of claim 1, wherein the compound is selected from one of the following formulae:



, and

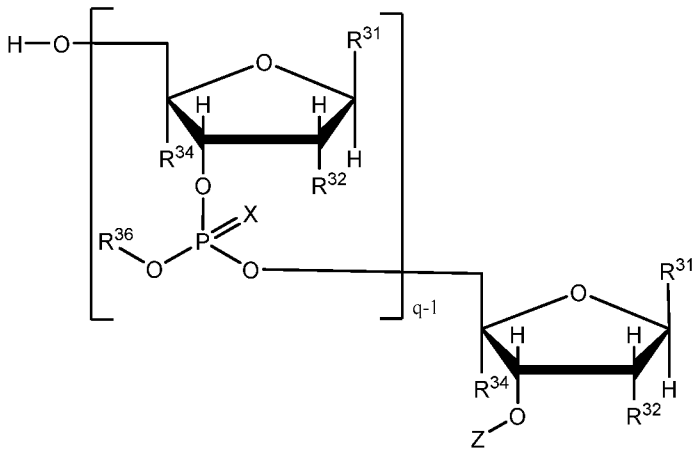


,

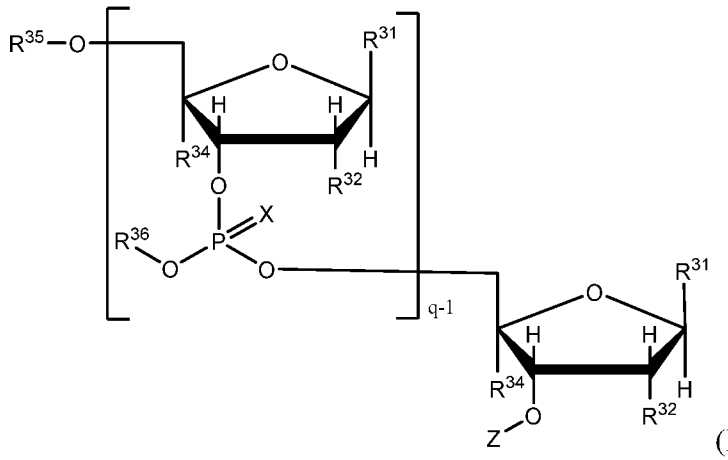
or a salt thereof.

26. A compound of Table 1 or a salt thereof.

27. A nucleotide or oligonucleotide represented by Formula III or IIIP,



(III), or



(IIIP);

or a salt thereof, wherein

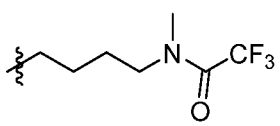
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is optionally protected by an amine protecting group;

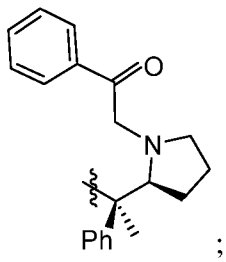
R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently H, C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is 

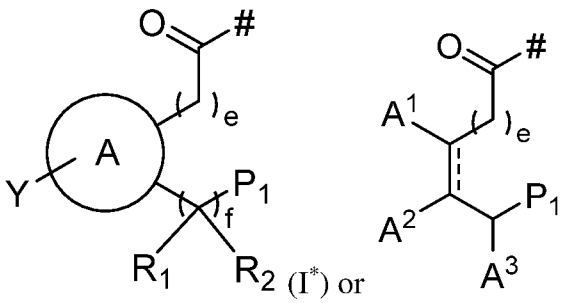
, or 

;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B*,



(I*) or (B*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

═══ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

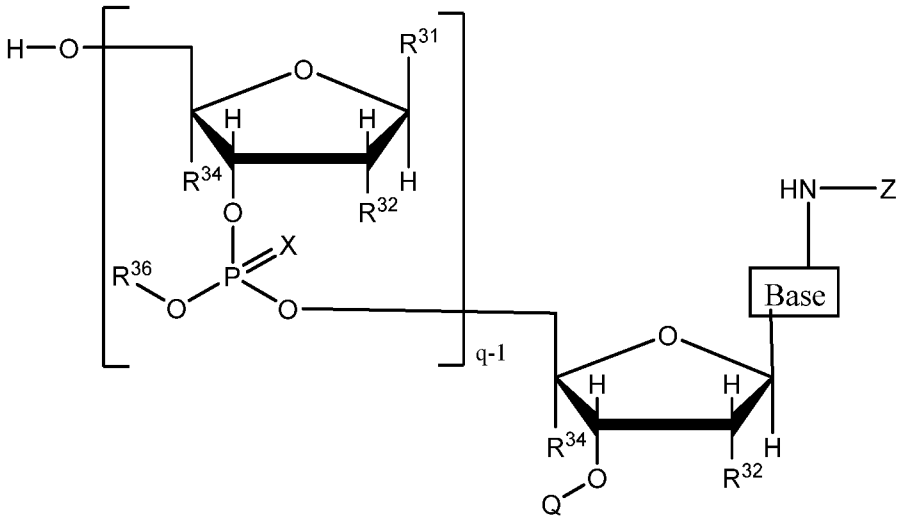
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

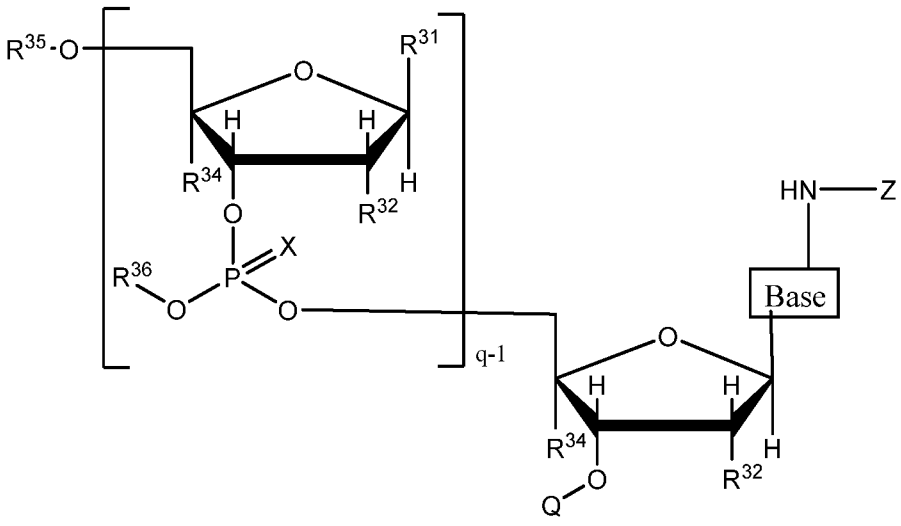
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

28. A nucleotide or oligonucleotide represented by Formula III' or IIIP',



(III'), or



(IIIP');

or a salt thereof, wherein

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

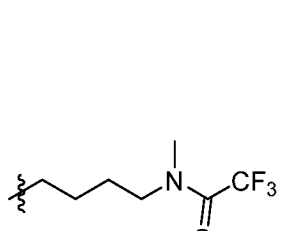
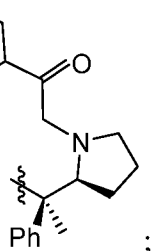
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is optionally protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

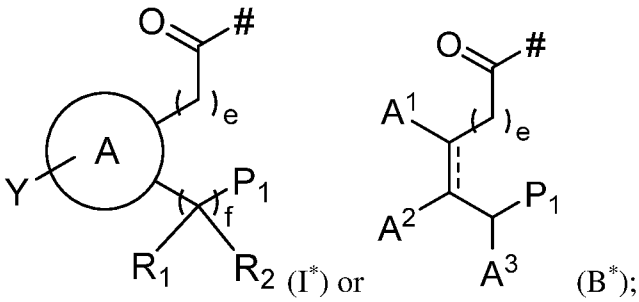
R^{36}, for each occurrence, is independently H, C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, -NO_2 or halogen; or

R^{36} is  , or  ;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms

independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

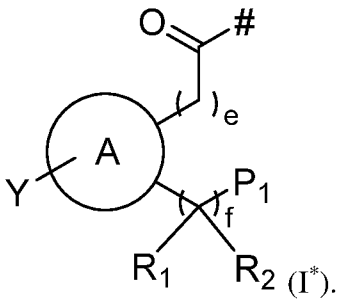
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

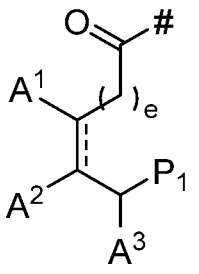
f is an integer from 0 to 6.

29. The nucleotide or oligonucleotide of claim 27 or 28 or a salt thereof, wherein Z is a group represented by Formula I^*,



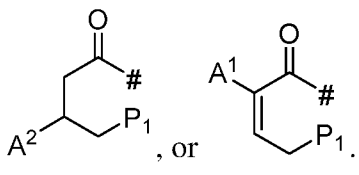
(I^*).

30. The nucleotide or oligonucleotide of claim 27 or 28 or a salt thereof, wherein Z is a group represented by Formula B^*,



(B^*).

31. The nucleotide or oligonucleotide of claim 30, wherein Z is a group represented by Formula B-1^* or B-2^*:

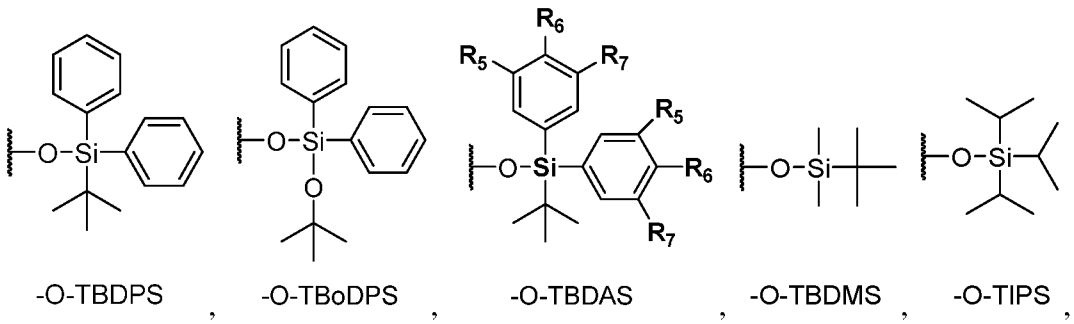


B-1^* B-2^*.

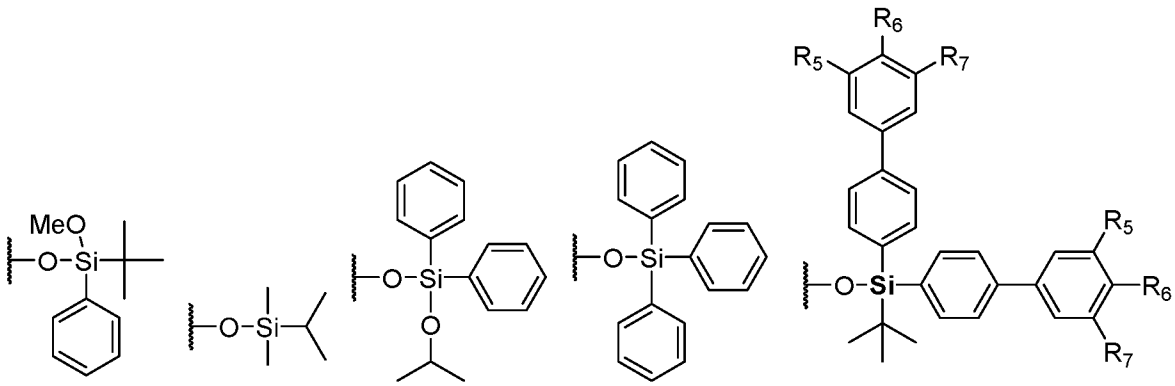
32. The nucleotide or oligonucleotide of any one of claims 27-31 or a salt thereof, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

33. The nucleotide or oligonucleotide of claim 27, claim 28, or claim 32 or a salt thereof, wherein ring A is phenyl or naphthalenyl.

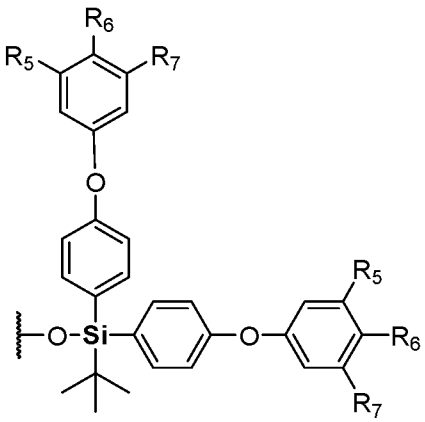
34. The nucleotide or oligonucleotide of any one of claims 27 to 33 or a salt thereof, wherein P_1 is a silyl hydroxyl protecting group selected from the following:



-O-TBDPS , -O-TBoDPS , -O-TBDAS , -O-TBDMS , -O-TIPS ,

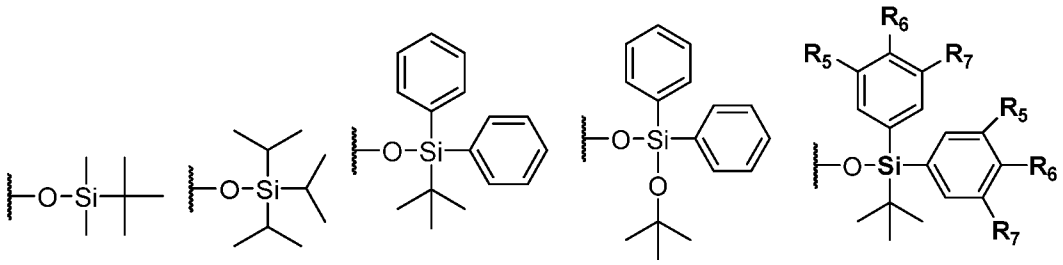


-O-TBMPS , **-O-DMIPS** , **-O-IPoDPS** , **-O-TPS** , -O-TBDAS-1 ,



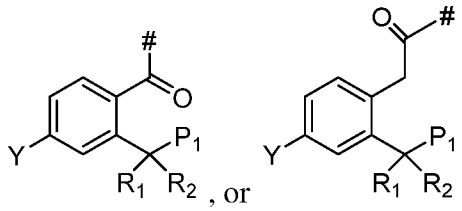
and -O-TBDAS-2 ; wherein ⊢ represents the point of attachment for P_1 and R_5, R_6 and R_7 are each independently H, $C_{1\text{-}30}$alkyl, or $C_{1\text{-}30}$alkoxy.

35. The compound of any one of claims 27 to 34, or a salt thereof, wherein P_1 is selected from the group consisting of –O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , -O-TBDAS .

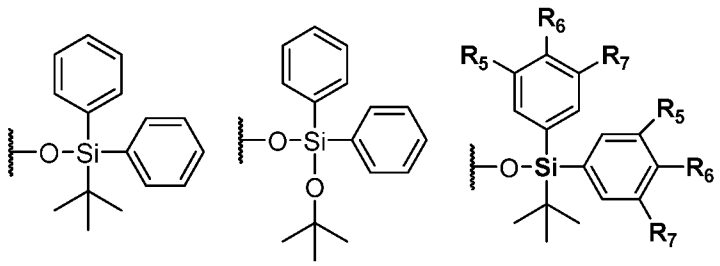
36. The nucleotide or oligonucleotide of any one of claims 27-35 or a salt thereof, wherein Z is a group represented by Formula I^{**} or Ia^{**}:



I^{**} , or Ia^{**}

or a salt thereof;

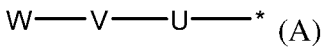
wherein P_1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDPS , -O-TBoDPS , -O-TBDAS ; and

R_5, R_6 and R_7 are each independently H, C1-30alkyl, or C1-30alkoxy;.

37. The nucleotide or oligonucleotide of any one of claims 27-36 or a salt thereof, wherein Y is represented by Formula A:

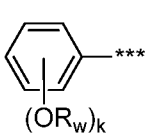


(A)

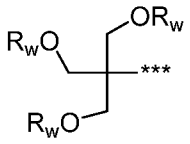
wherein:

—* represents the point of attachment for Y;

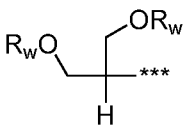
W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:



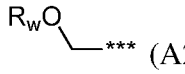
(A1),



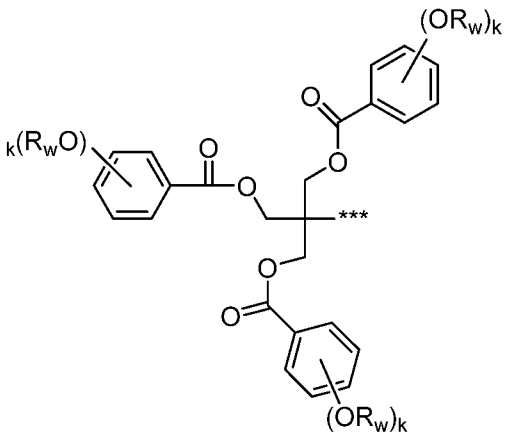
(A2),



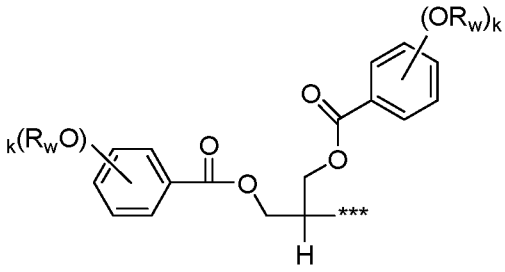
(A2-1),



(A2-2),

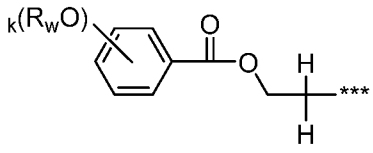


(A3),



(A3-1),

or



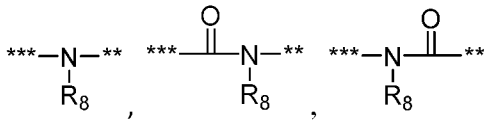
(A3-2),

wherein

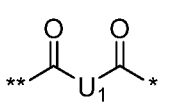
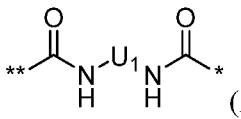
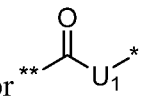
—*** represents the point where W and V connect;

each R_w is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

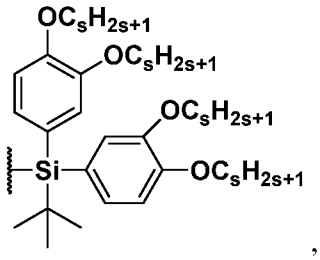
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:

 (A4),  (A5), or  (A6),

wherein U_1 is C_{1-6}alkylene, C_{1-6}alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

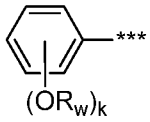
38. The nucleotide or oligonucleotide of any one of claims 34-37 or a salt thereof, wherein the TBDAS group is:



wherein s is an integer from 1 to 30.

39. The nucleotide or oligonucleotide of any one of claims 27-37 or a salt thereof, wherein P_1 is TBDPS.

40. The nucleotide or oligonucleotide of any one of claims 37-39 or a salt thereof, wherein W is represented by Formula A1:

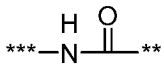


A1,

wherein R_w is C_nH_{2n+1};

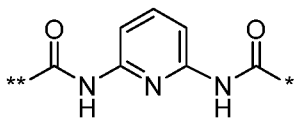
n is an integer from 1 to 30.

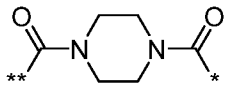
41. The nucleotide or oligonucleotide of any one of claims 37-40 or a salt thereof, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and $C_{28}H_{57}$.

42. The nucleotide or oligonucleotide of any one of claims 37-41 or a salt thereof, wherein V is a bond, CH_2, CH_2CH_2, C(=O)-, ***-C(=O)-O-**, or 

.

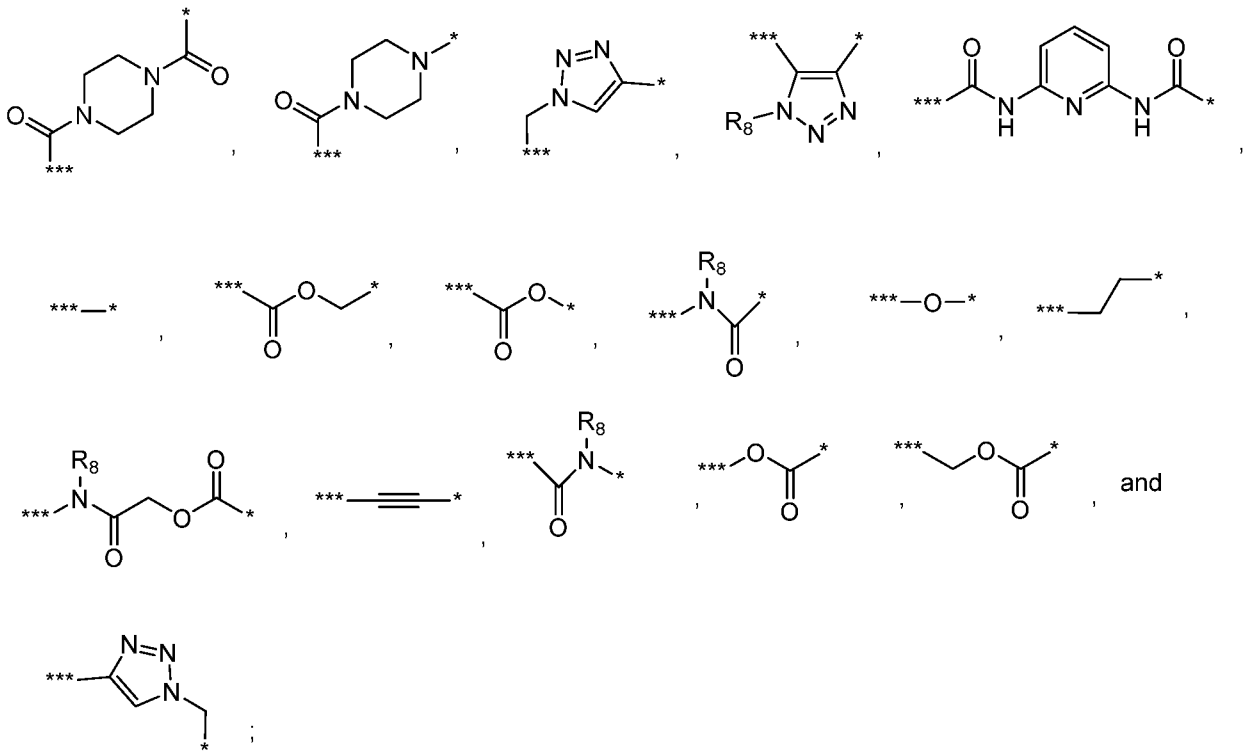
43. The nucleotide or oligonucleotide of any one of claims 37-42 or a salt thereof, wherein U is a bond, CH_2, CH_2CH_2, carbonyl, triazolylene, piperazinylene,



, or 

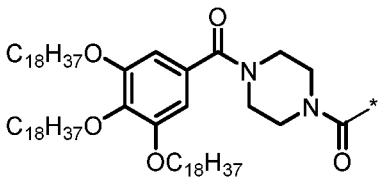
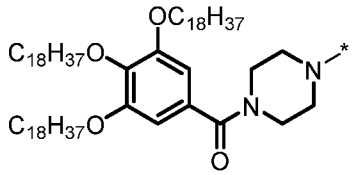
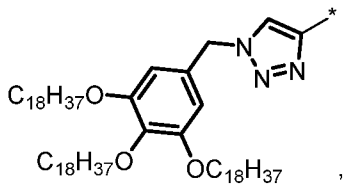
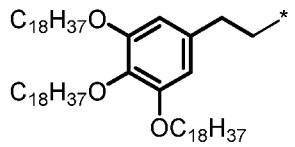
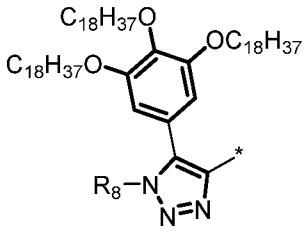
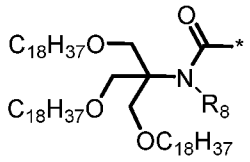
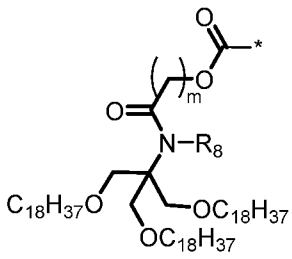
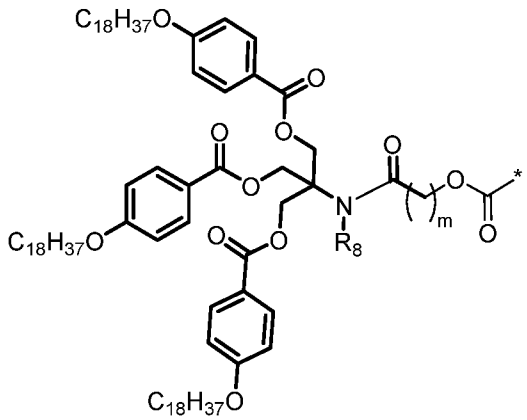
.

44. The nucleotide or oligonucleotide of any one of claims 37-41 or a salt thereof, wherein U-V is selected from the group consisting of



wherein R_8 is H or $C_{1\text{-}6}$alkyl.

45. The nucleotide or oligonucleotide of any one of claims 27-39 or a salt thereof, wherein Y is selected from the groups consisting of


,

,

,

,

,


, and

;

wherein

R_8 is H or $C_{1\text{-}6}$alkyl; and

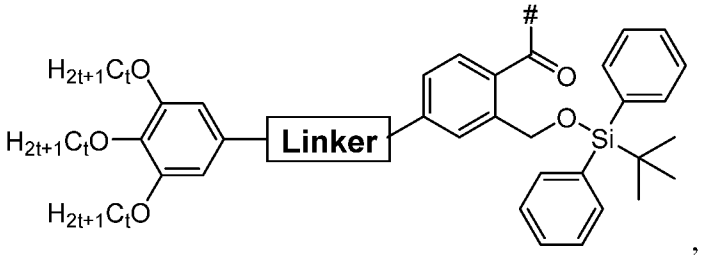
m is an integer from 1 to 5.

46. The nucleotide or oligonucleotide of any one of claims 27-45 or a salt thereof, wherein R_1 and R_2 are independently H or CH_3.

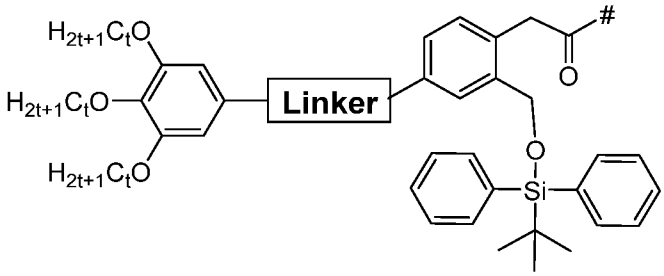
47. The nucleotide or oligonucleotide of any one of claims 27-46 or a salt thereof, wherein e is 0, 1, or 2; and f is 0, 1, or 2.

48. The nucleotide or oligonucleotide of any one of claims 37-47 or a salt thereof, wherein R_8 is H or $C_{1\text{-}4}$alkyl.

49. The nucleotide or oligonucleotide of claim 27 or claim 28 or a salt thereof, wherein Z is represented by Formula II* or IIa*,



II*

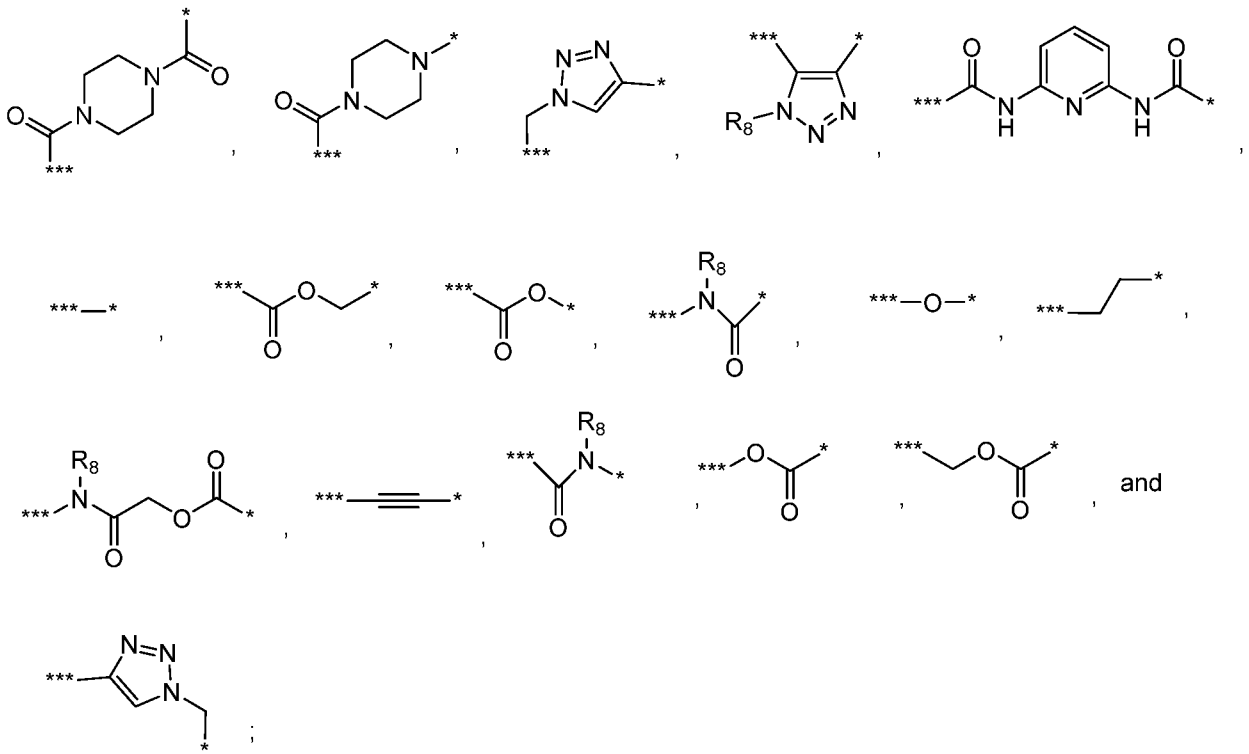


IIa*

wherein

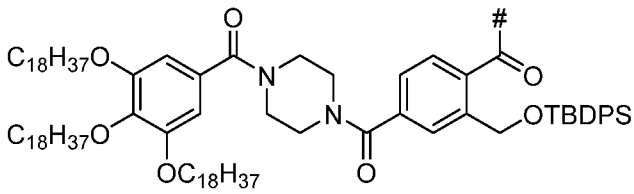
t is an integer from 10 to 30;

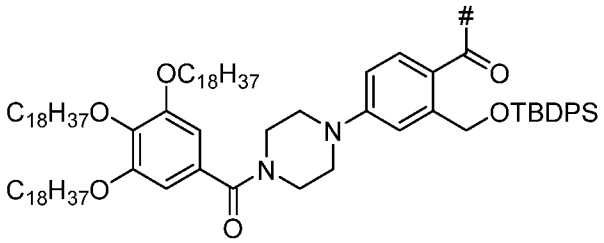
Linker is selected from the group consisting of



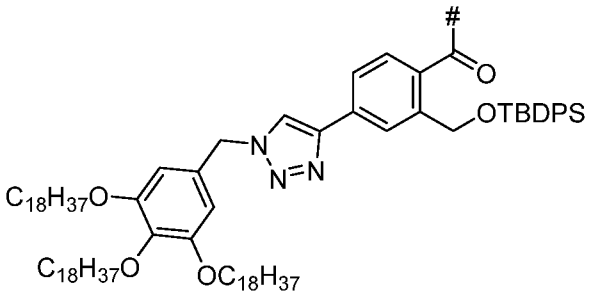
wherein R_8 is H or $C_{1\text{-}6}$alkyl.

50. The nucleotide or oligonucleotide of claim 49 or a salt thereof, wherein Z is

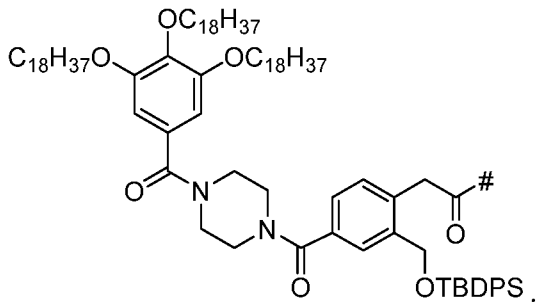




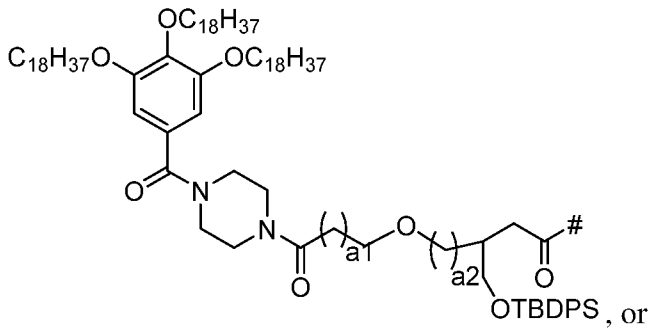
or

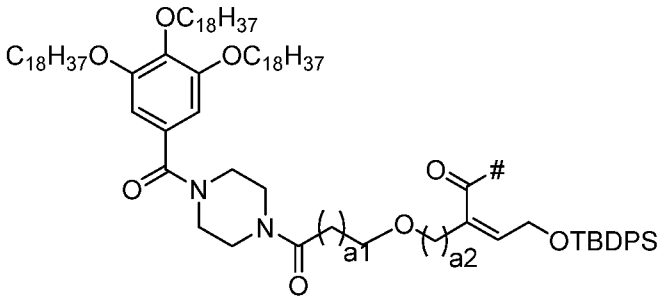


51. The nucleotide or oligonucleotide of claim 27 or 28, or a salt thereof, wherein Z is

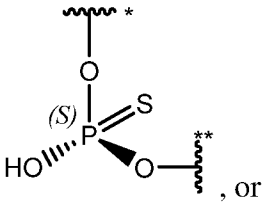


52. The nucleotide or oligonucleotide of claim 27 or 28, or a salt thereof, wherein Z is



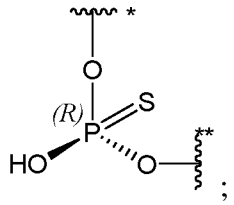


53. The nucleotide or oligonucleotide of any one of claims 27-52 or a salt thereof, wherein when X is S, the phosphorothiolate group has *S*-configuration as shown below:



, or

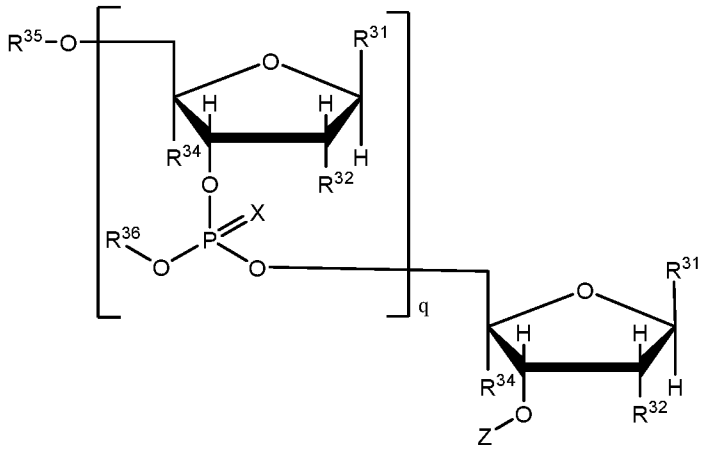
R-configuration as shown below:



;

wherein ~~~~ * indicates the connection point to 3'-OH group and ** indicates the connection point to 5'-OH group.

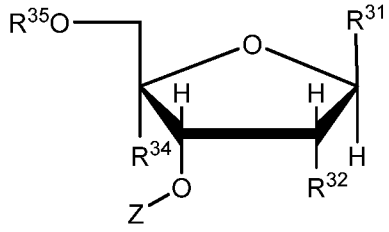
54. A process for preparing an oligonucleotide fragment of formula (V),



(V),

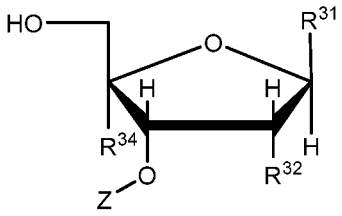
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



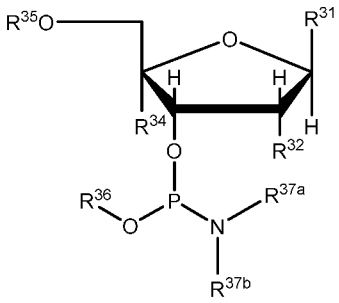
(VA),

or a salt thereof, to form a compound of formula (VB):



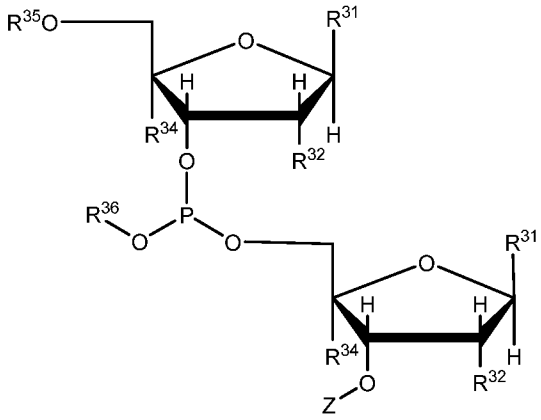
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC):



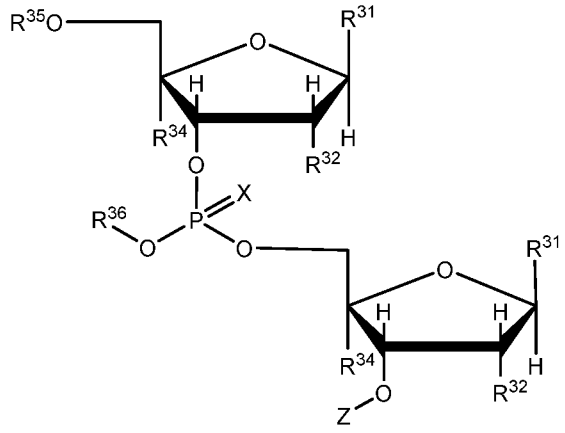
(VC),

or a salt thereof, to form a compound of formula (VD),



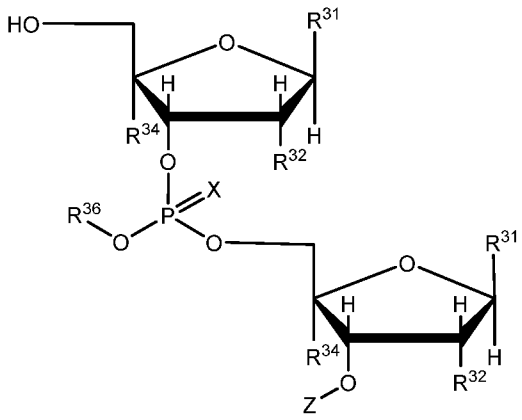
(VD), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE):



(VE), or a salt thereof;

4) deprotecting the compound of formula (VE), or a salt thereof to form a compound of formula (VF):



(VF), or a salt thereof;

5) when q is equal or greater than 2, starting with the compound of formula (VF), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V), or a salt thereof, wherein:

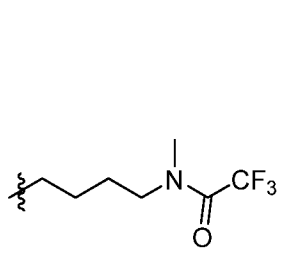
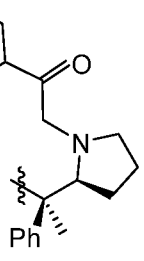
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

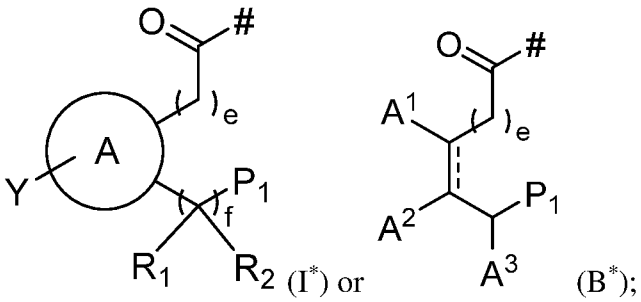
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

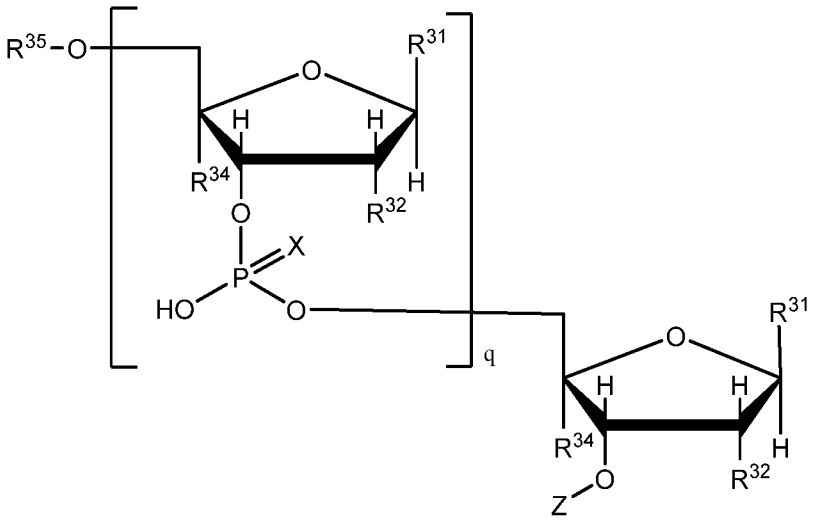
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

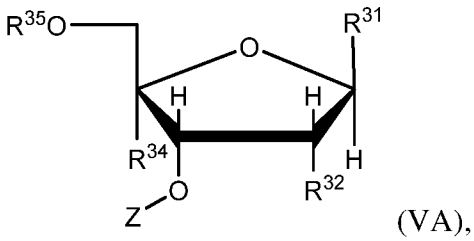
55. A process for preparing an oligonucleotide fragment of formula (V'),



(V'),

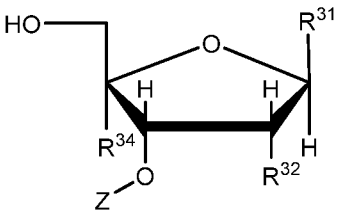
or a salt thereof, comprising the steps of:

1) deprotecting a compound of formula (VA):



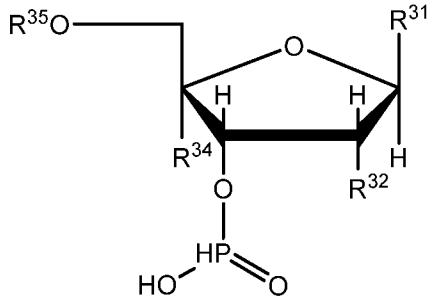
(VA),

or a salt thereof, to form a compound of formula (VB):



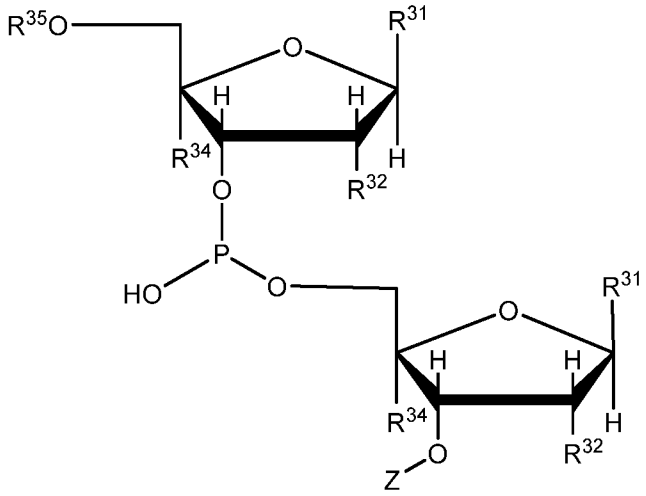
(VB), or a salt thereof;

2) reacting the compound of formula (VB), or a salt thereof, with a compound of formula (VC'):



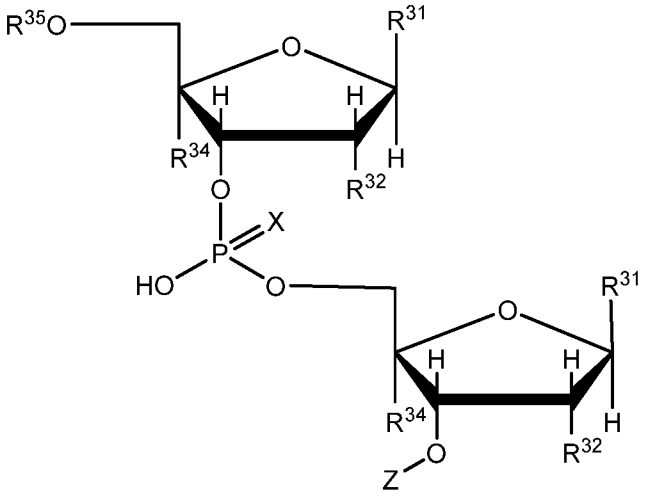
(VC'),

or a salt thereof, to form a compound of formula (VD'),



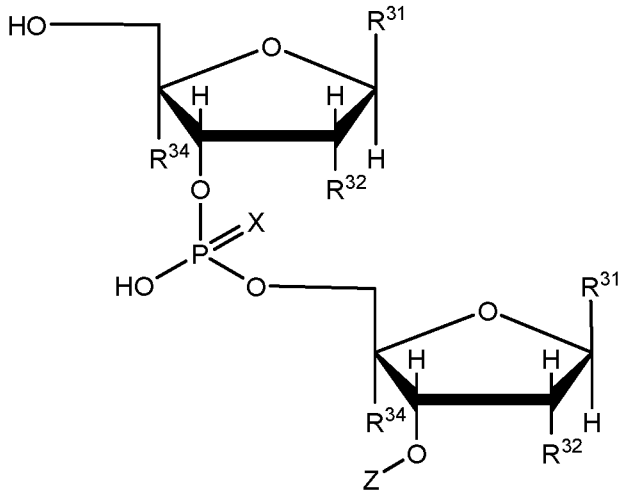
(VD'), or a salt thereof;

3) sulfurizing or oxidizing the compound of formula (VD'), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VE'):



(VE'), or a salt thereof;

4) deprotecting the compound of formula (VE'), or a salt thereof to form a compound of formula (VF'):



(VF'), or a salt thereof;

5) when q is equal or greater than 2, starting with the compound of formula (VF'), repeating steps 2), 3) and 4) for q-2 times, followed by steps 2) and 3) to yield the fragment of formula (V'), or a salt thereof, wherein:

R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

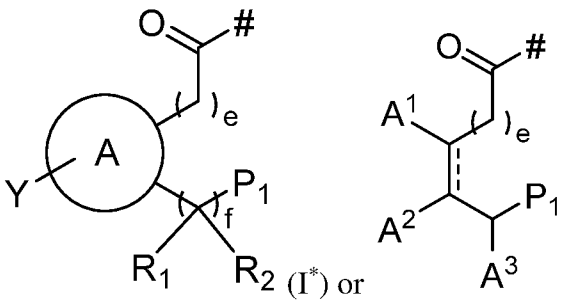
R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,



(I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

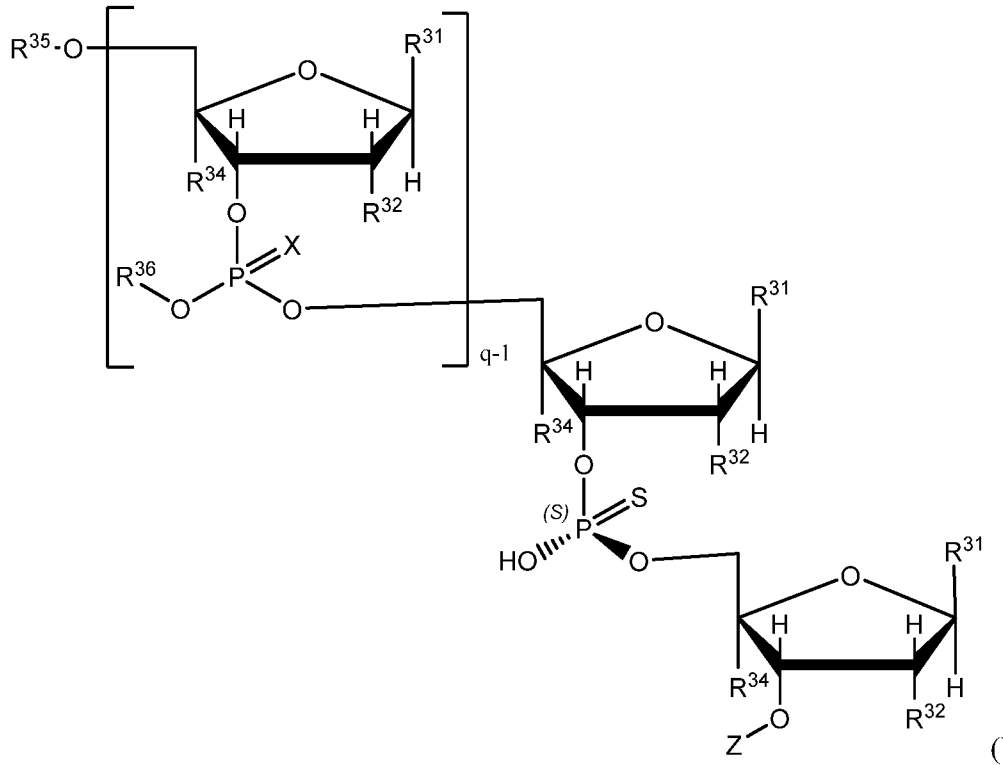
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

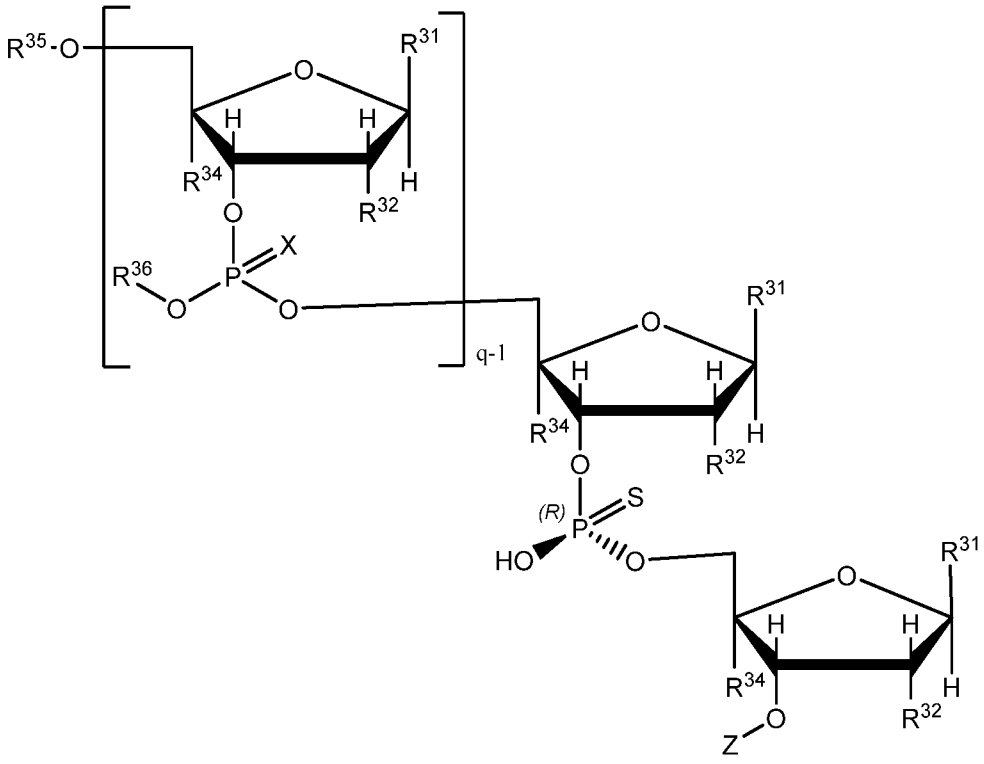
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

56. A process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



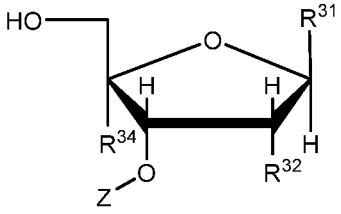
(V-C1), or



(V-C2)

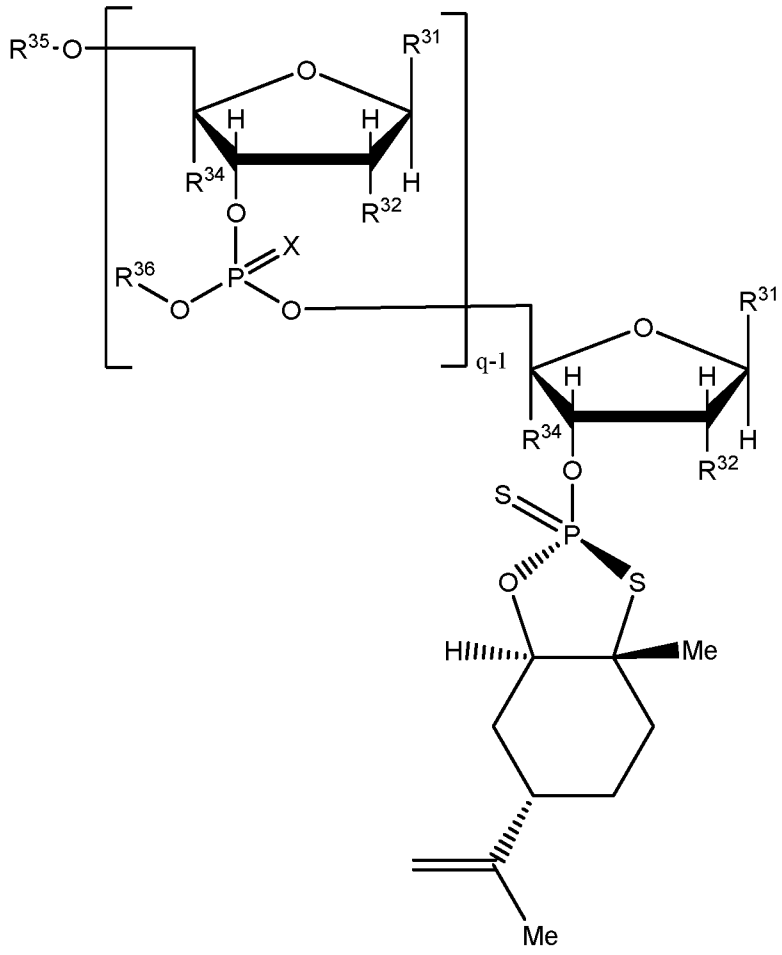
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

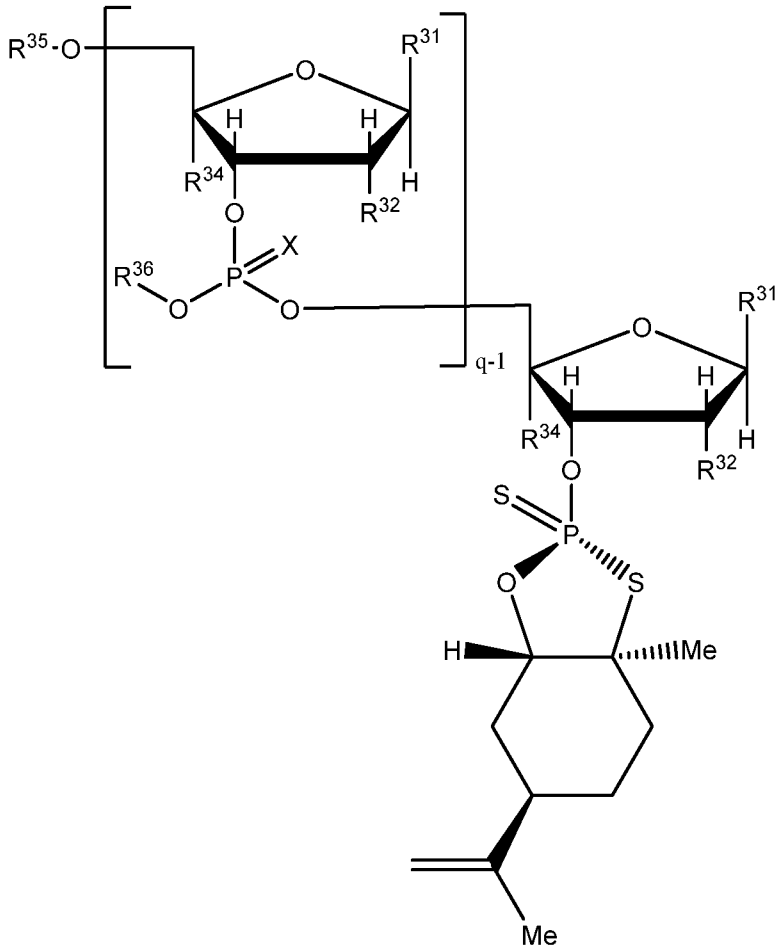


(VB),

or a salt thereof, with a compound of formula (V-CR1) or (V-CR2),



(V-CR1),



(V-CR2)

or a salt thereof, and a base, to form a compound of formula (V-C1) or (V-C2), wherein:

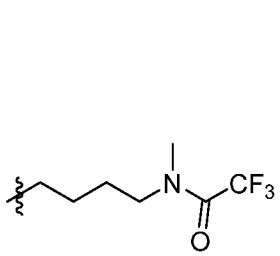
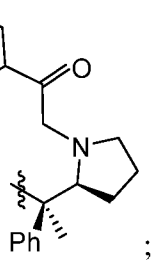
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

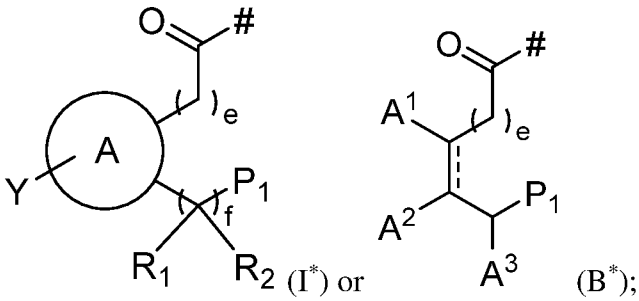
R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is  , or  ;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S, provided when X is S, the phosphorothiolate group has *S*-configuration, *R*-configuration or a mixture thereof (*e.g.*, a racemic mixture);

Z is a group represented by Formula I^* or B^*,



wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently $C_{1\text{-}6}$alkyl, $C_{1\text{-}6}$alkenyl, $C_{1\text{-}6}$alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or $C_{1\text{-}6}$alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

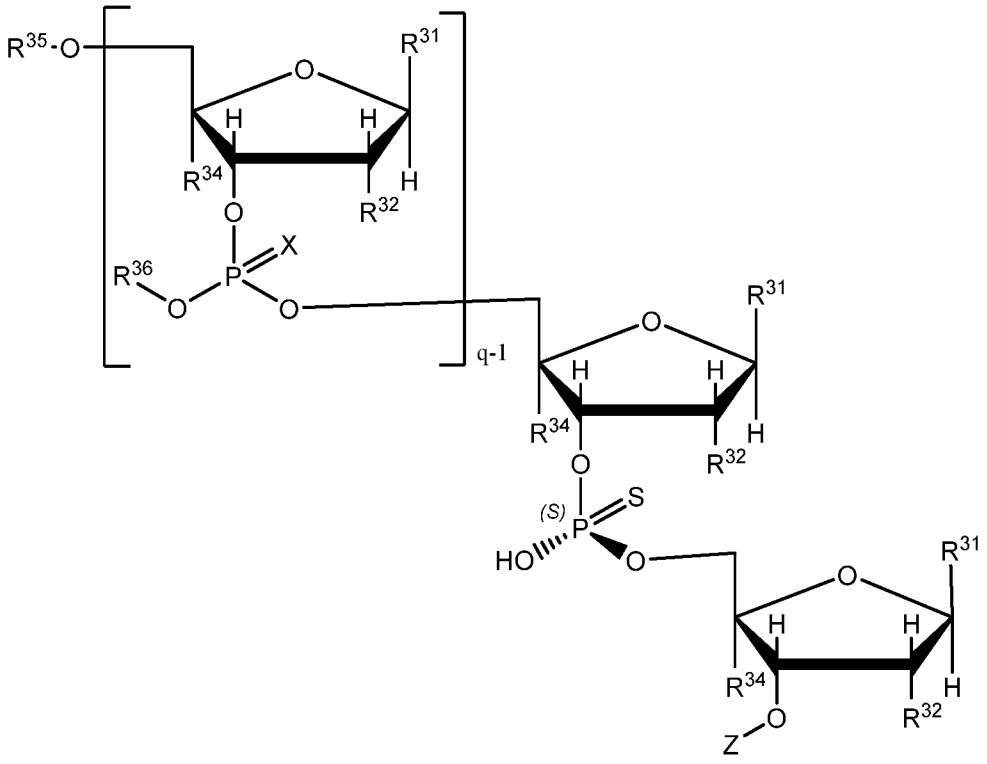
R_1 and R_2 are independently H, $C_{1\text{-}6}$alkyl, or phenyl; wherein $C_{1\text{-}6}$alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is $C_{1\text{-}30}$alkoxy;

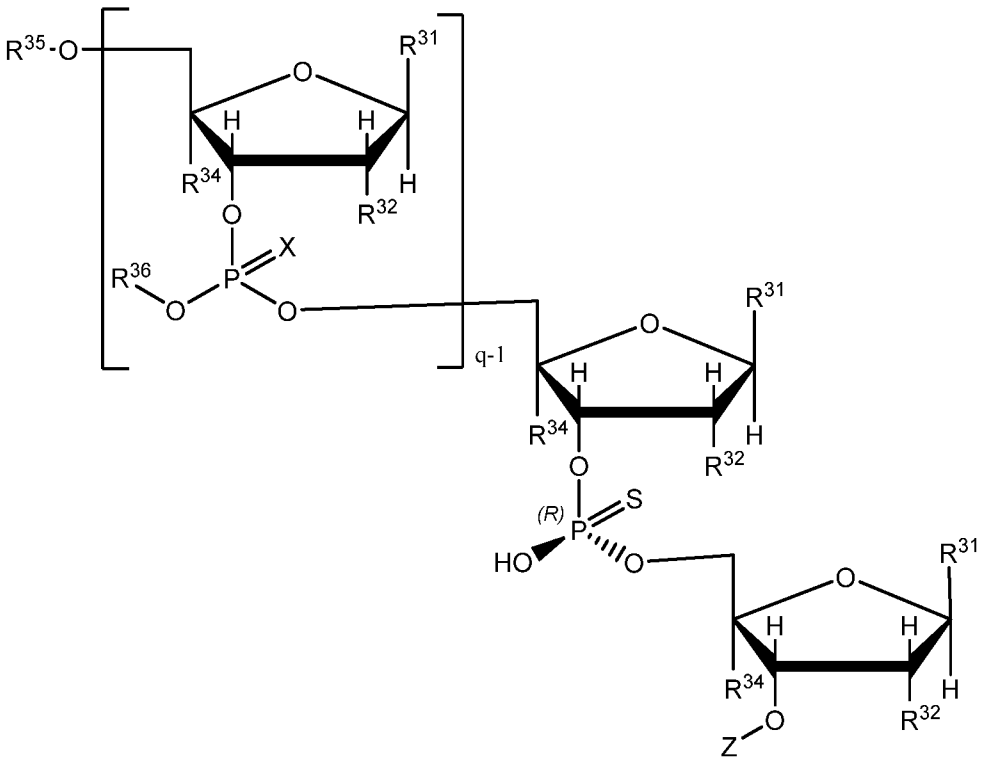
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

57. A process for preparing an oligonucleotide fragment of formula (V-C1) or (V-C2),



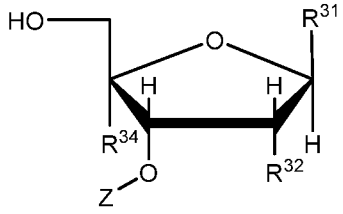
(V-C1), or



(V-C2)

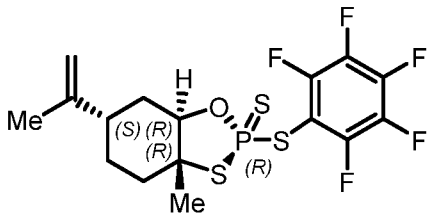
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VB),

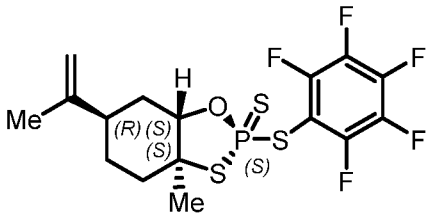


(VB),

or a salt thereof, with a reagent of formula (VR1) or (VR2),

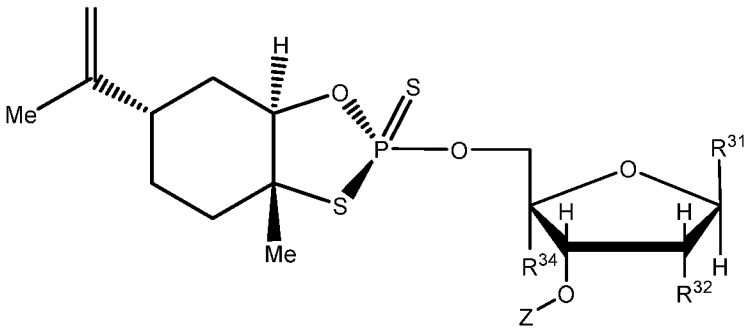


(VR1), or

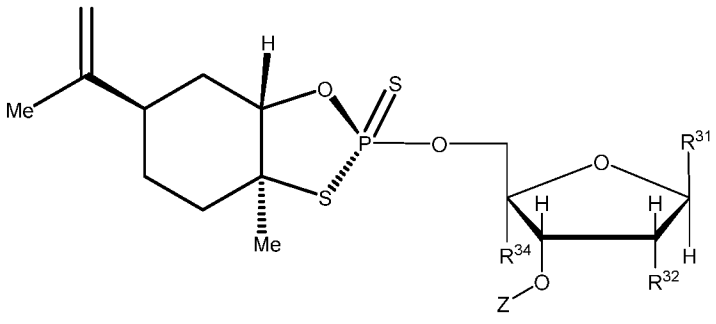


(VR2)

to form a compound of formula (V-CR3) or (V-CR4),



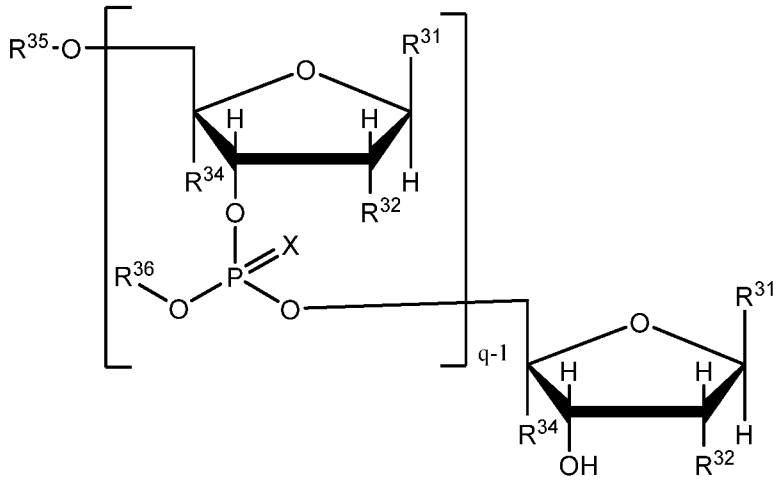
(V-CR3), or



(V-CR4)

or a salt thereof;

2) reacting the compound of formula (V-CR3) or (V-CR4), or a salt thereof, with a compound of formula (VG):



(VG),

or a salt thereof, and a base, to form a compound of formula (V-C1) or (V-C2), wherein:

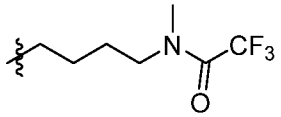
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

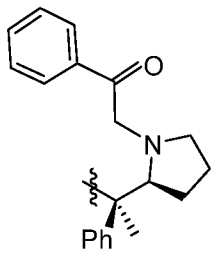
R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

R^{36} is 

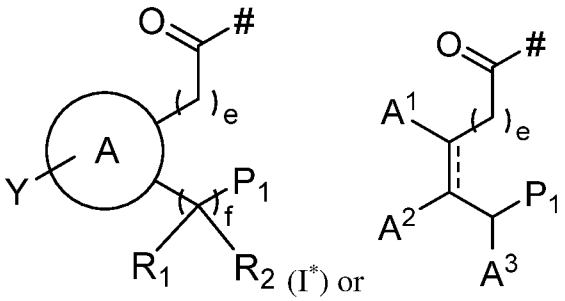
, or 

;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S, provided when X is S, the phosphorothiolate group has *S*-configuration, *R*-configuration or a mixture thereof (*e.g.*, a racemic mixture);

Z is a group represented by Formula I^* or B^*,



(I*) or (B*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

═══ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

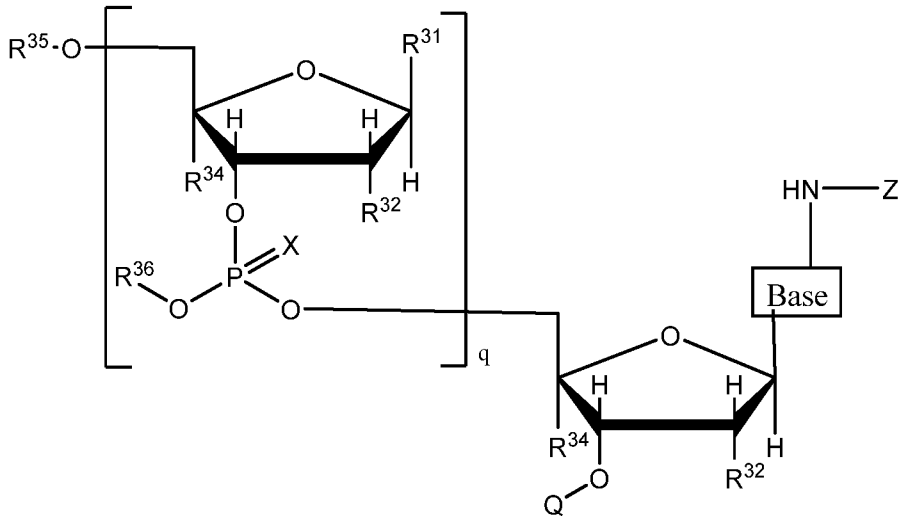
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

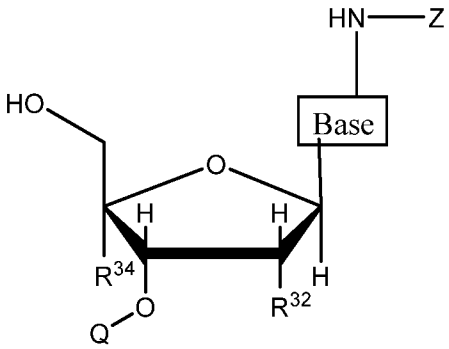
58. A process for preparing an oligonucleotide fragment of formula (VBZ),



(VBZ),

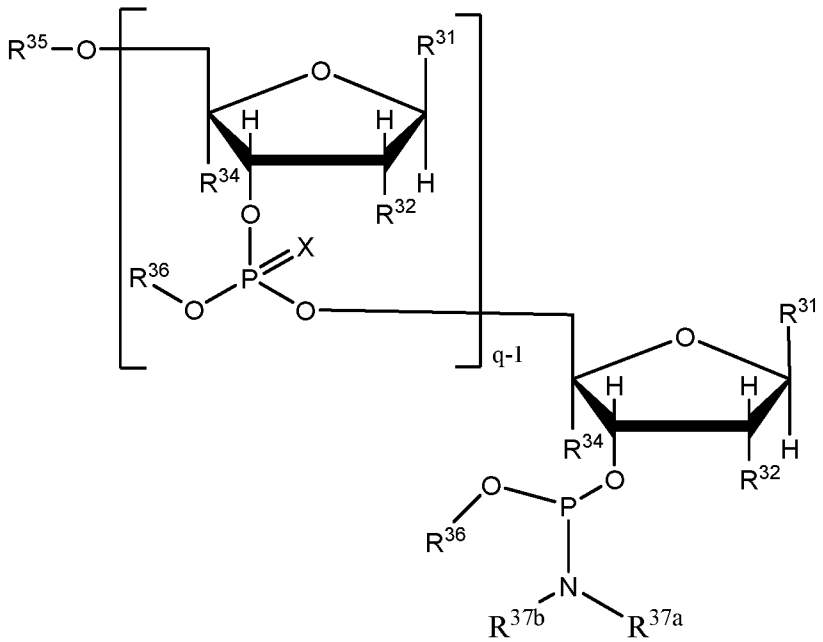
or a salt thereof, comprising the steps of:

1) reacting the compound of formula (VBZ-1),



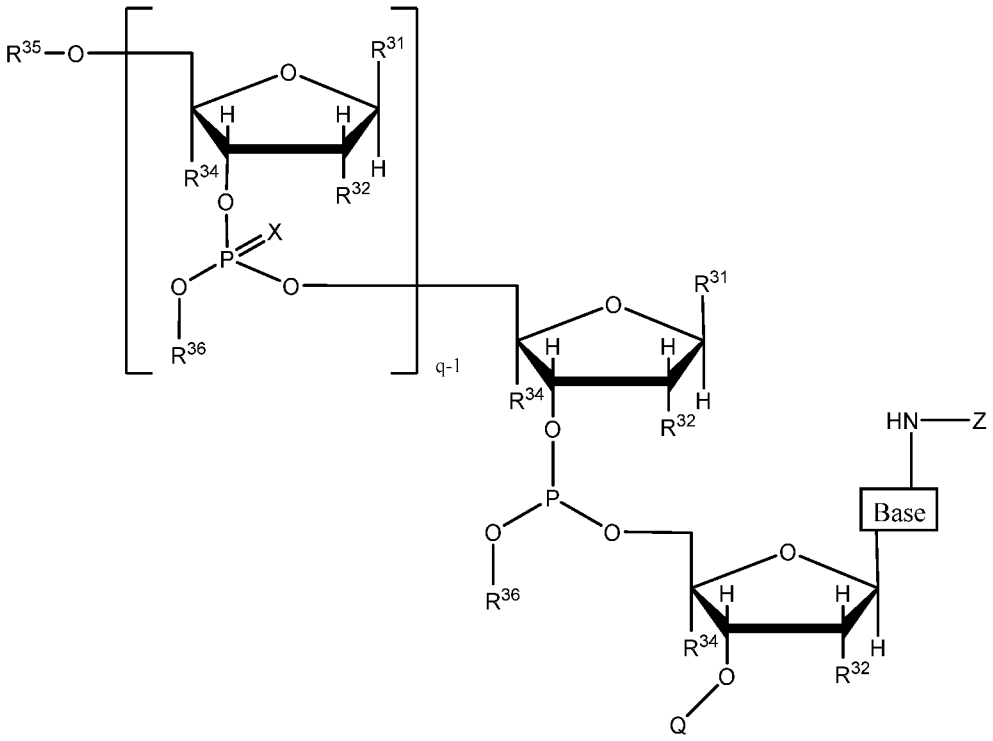
(VBZ-1),

or a salt thereof, with a compound of formula (VBZ-2):



(VBZ-2),

or a salt thereof, to form a compound of formula (VBZ-3),



(VBZ-3), or a salt thereof;

2) sulfurizing or oxidizing the compound of formula (VBZ-3), or a salt thereof, with a sulfurization or oxidation agent to form a compound of formula (VBZ), or a salt thereof;

wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

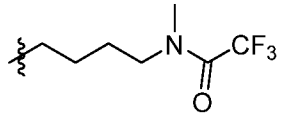
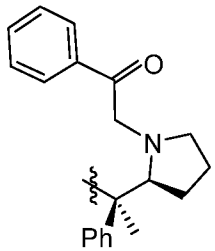
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, -NO_2 or halogen; or

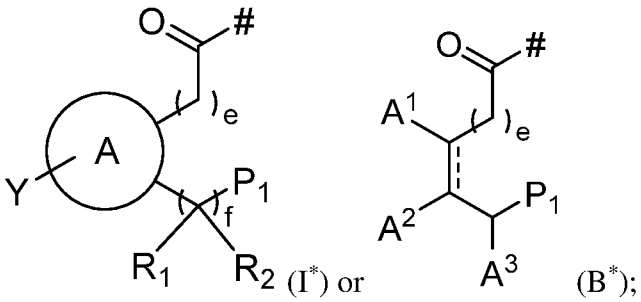
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

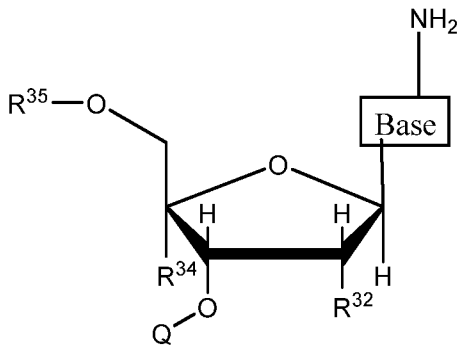
R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

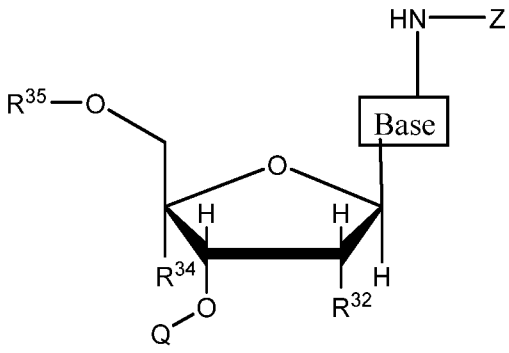
59. The process of claim 58, wherein the compound of formula VBZ-1 is prepared by

i) reacting the compound of formula (VBZ-4),



(VBZ-4),

or a salt thereof, with Z-OH to form a compound of formula VBZ-5,



(VBZ-5),

or a salt thereof; and

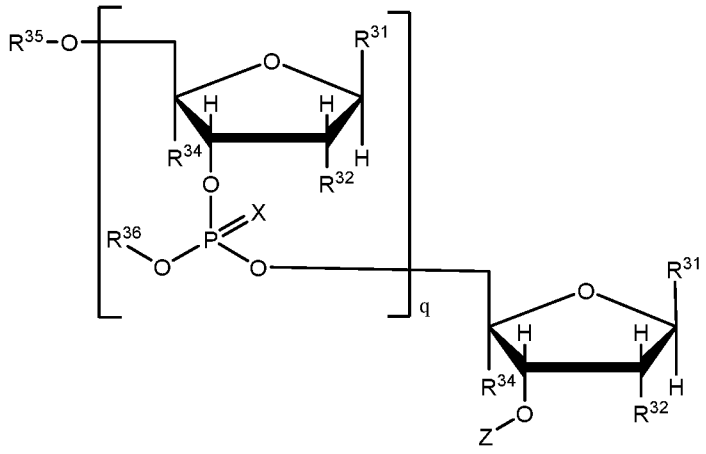
ii) deprotecting the compound of formula (VBZ-5) to form the compound of formula (VBZ-1).

60. The process of any one of claims 54-59, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

61. The process of claim any one of claims 54-60, wherein no chromatography is used for purifying the reaction product of any one of steps 1), 2), 3) and 4).

62. The process of any one of claims 54 to 61, wherein the reaction product of any one of steps 1), 2), 3) and 4) is purified by selective precipitation.

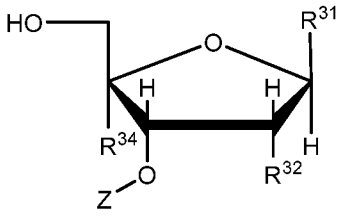
63. A process for preparing an oligonucleotide fragment of formula (V),



(V),

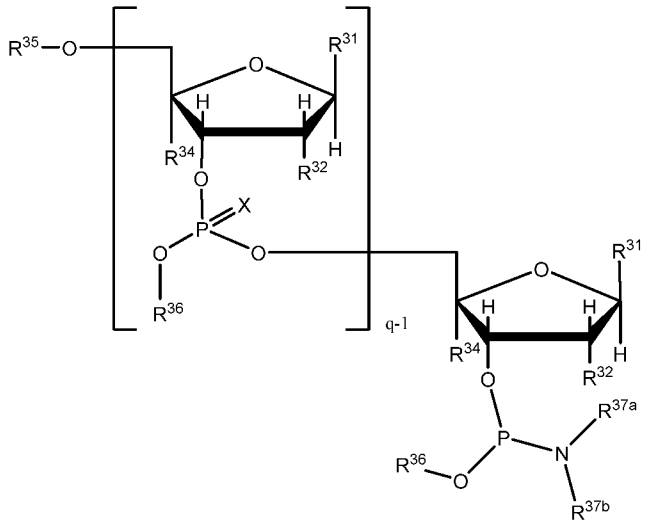
or a salt thereof, comprising the steps of:

c) coupling a nucleotide of formula (V-1):



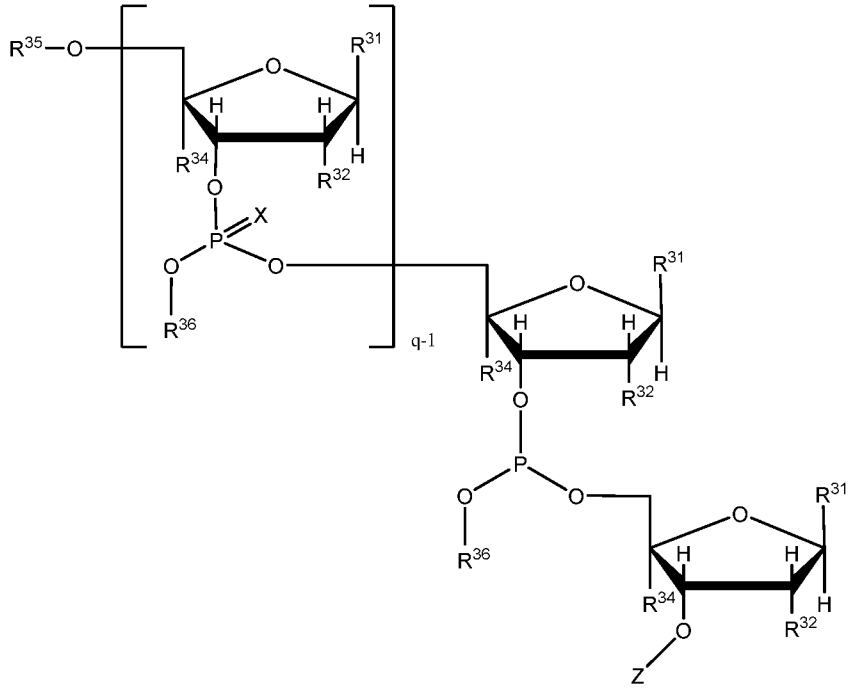
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2):



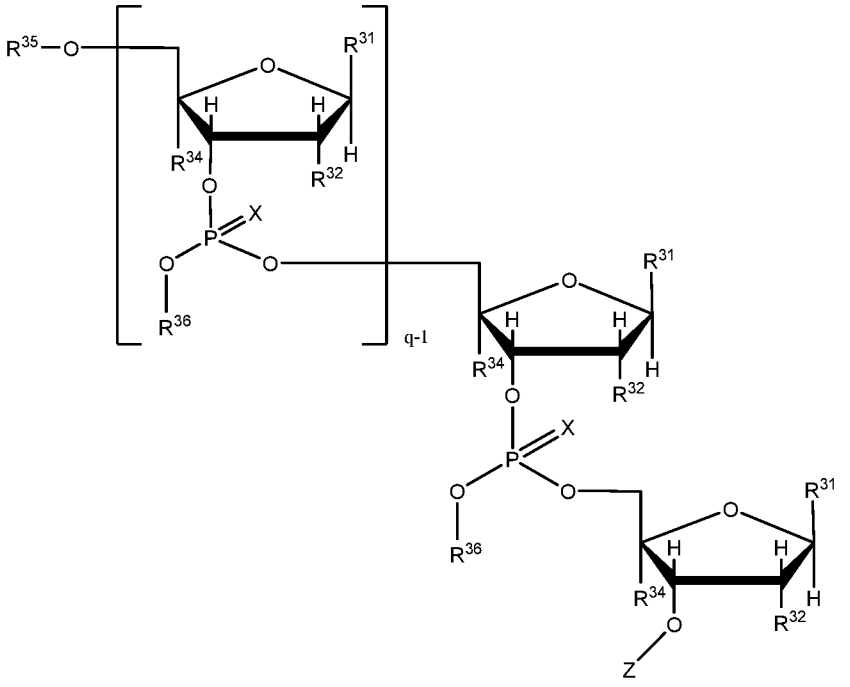
(V-2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3),



(V-3) or a salt thereof; and

d) sulfurizing or oxidizing the oligonucleotide of formula (V-3), or a salt thereof, to form an oligonucleotide of formula (V):



(V) or a salt thereof;

wherein:

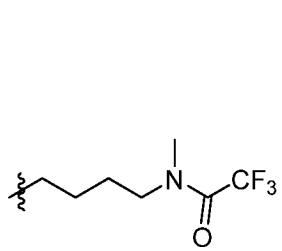
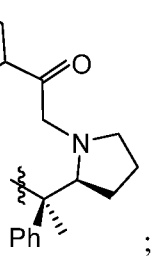
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, -NO_2 or halogen; or

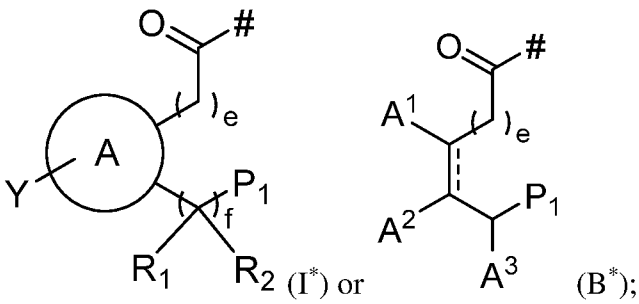
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms;

wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$,

3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

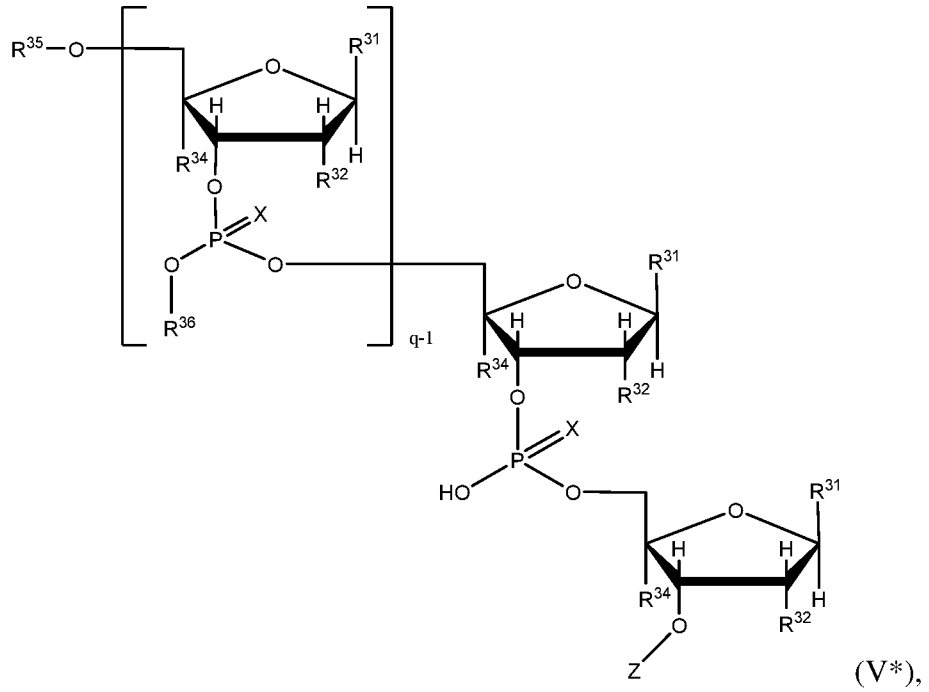
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

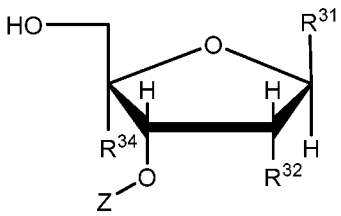
64. A process for preparing an oligonucleotide fragment of formula (V*),



(V*),

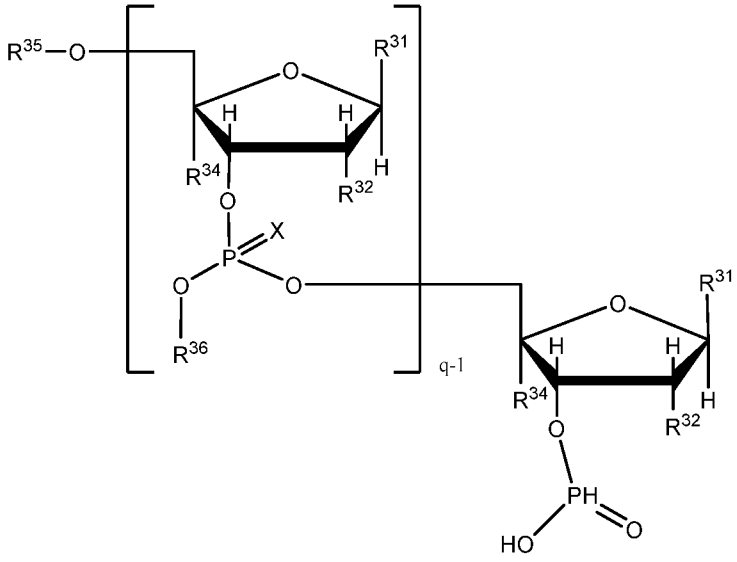
or a salt thereof, comprising the steps of:

a) coupling a nucleotide of formula (V-1):



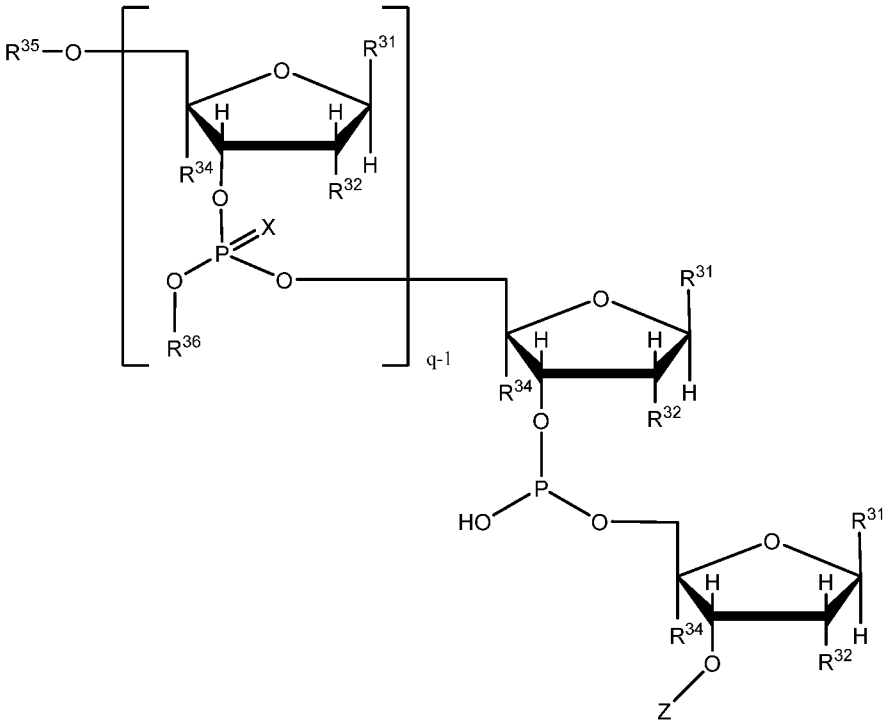
(V-1), or a salt thereof,

with an oligonucleotide fragment of formula (V-2'):



(V-2'), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (V-3'),



(V-3') or a salt thereof;

and

b) sulfurizing or oxidizing the oligonucleotide of formula (V-3'), or a salt thereof, to form the oligonucleotide of formula (V*) or a salt thereof;

wherein:

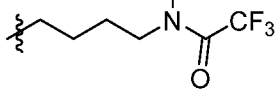
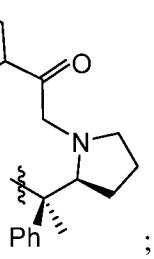
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently H, C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, $-NO_2$ or halogen; or

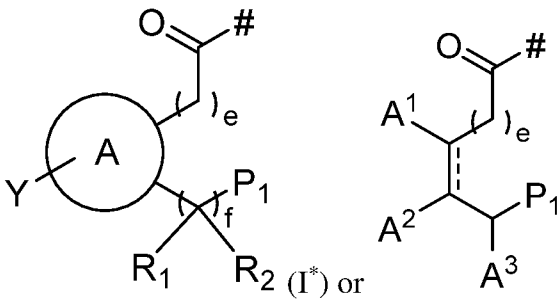
R^{36} is  , or  ;

R^{37a} and R^{37b} are independently C_{1-6}alkyl;

q is an integer from 1 to 20;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms;

wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl,

C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

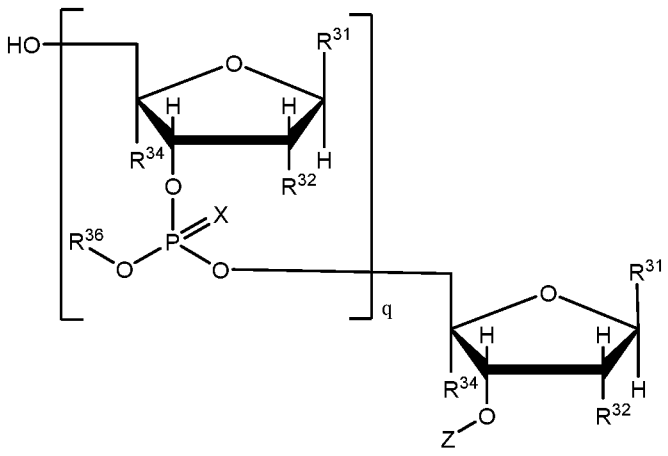
R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

f is an integer from 0 to 6.

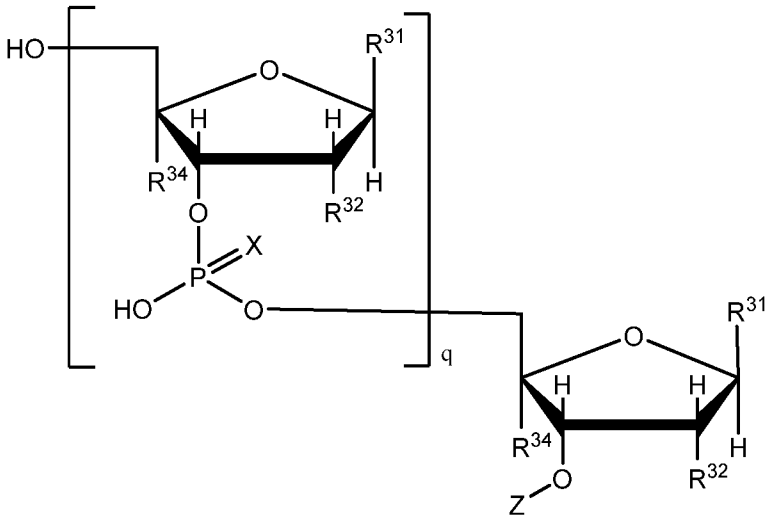
65. The process of claim 63 or 64, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

66. The process of claim 54 or 63, further comprising deprotecting the fragment of formula (V) to form deprotected fragment of formula (VH):



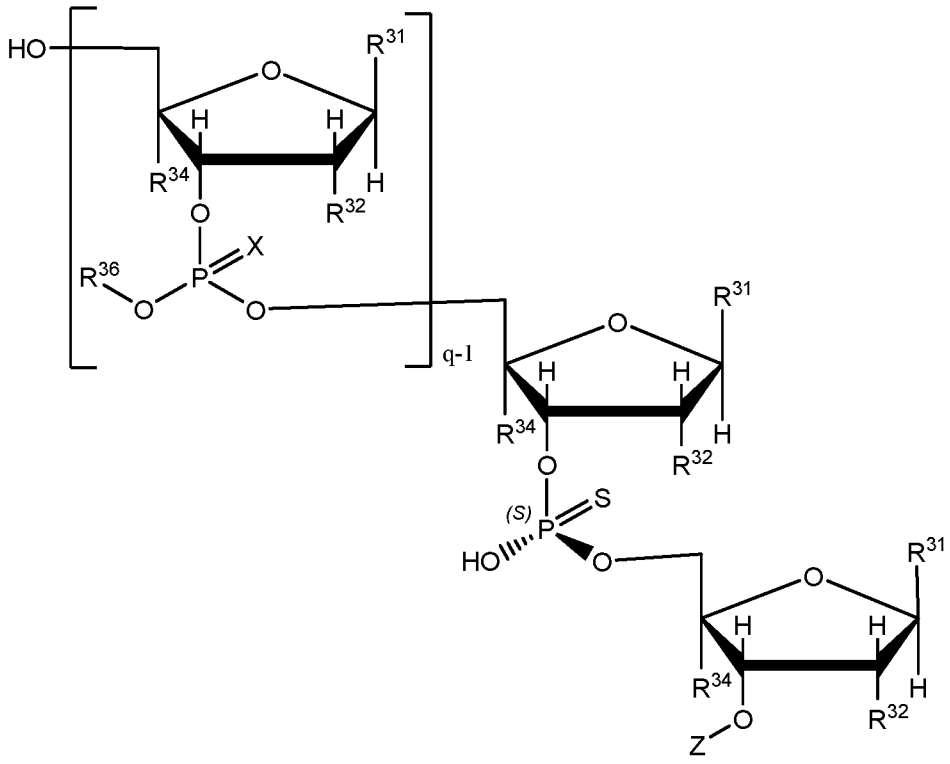
(VH), or a salt thereof.

67. The process of claim 55, further comprising deprotecting the fragment of formula (V') to form deprotected fragment of formula (VH'):

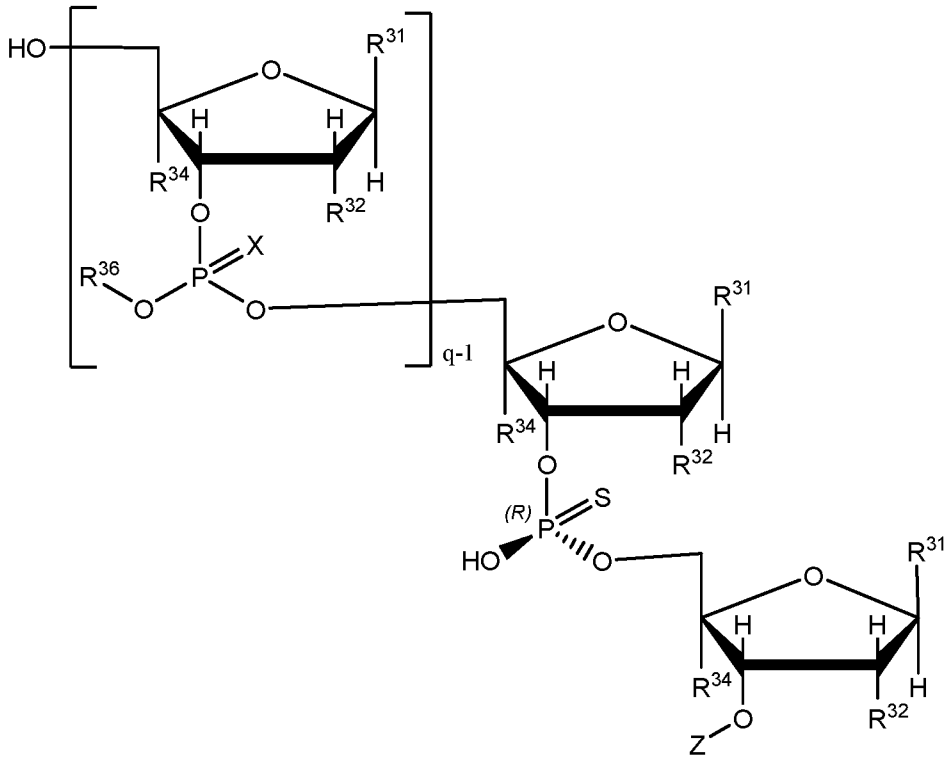


(VH'), or a salt thereof.

68. The process of claim 56 or 57, further comprising deprotecting the fragment of formula (V-C1) or (V-C2) to form deprotected fragment of formula (V-C3) or (V-C4):

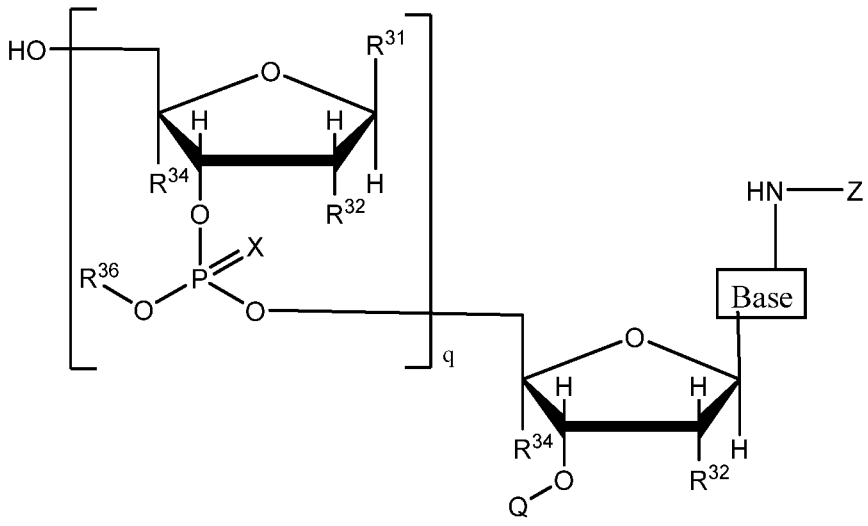


(V-C3), or a salt thereof, or



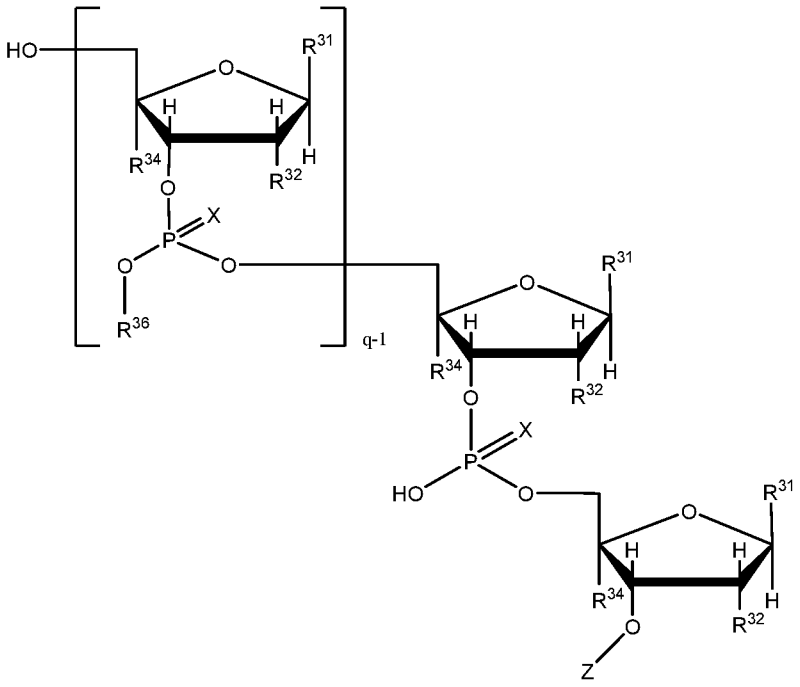
(V-C4), or a salt thereof.

69. The process of claim 58, further comprising deprotecting the fragment of formula (VBZ) to form deprotected fragment of formula (VBZ-6):



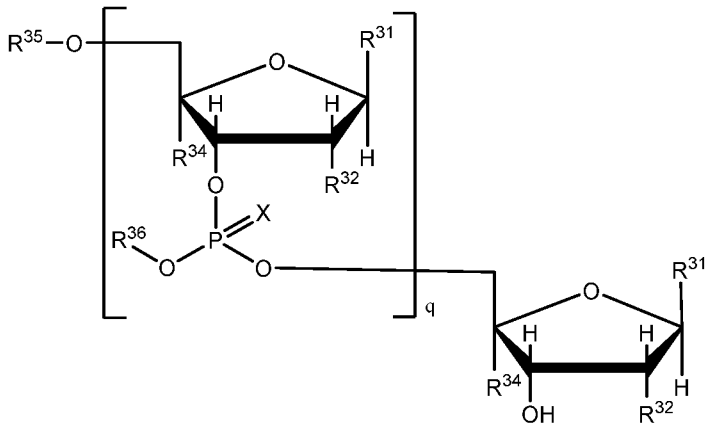
(VBZ-6), or a salt thereof.

70. The process of claim 64, further comprising deprotecting the fragment of formula (V*) to form deprotected fragment of formula (V*-1):

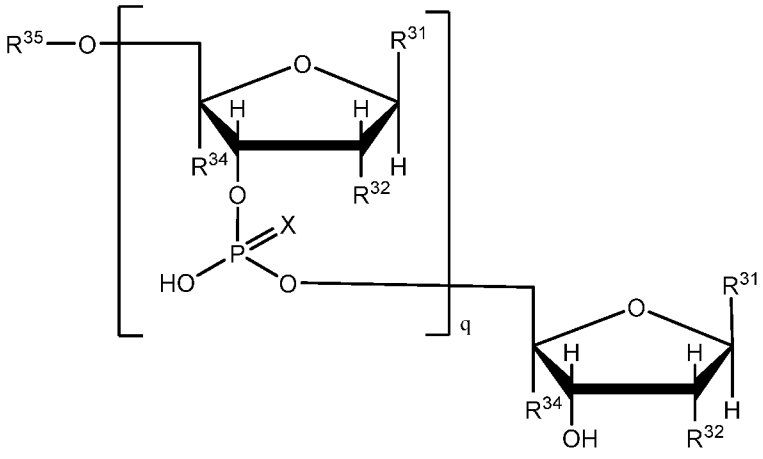


(V*-1), or a salt thereof.

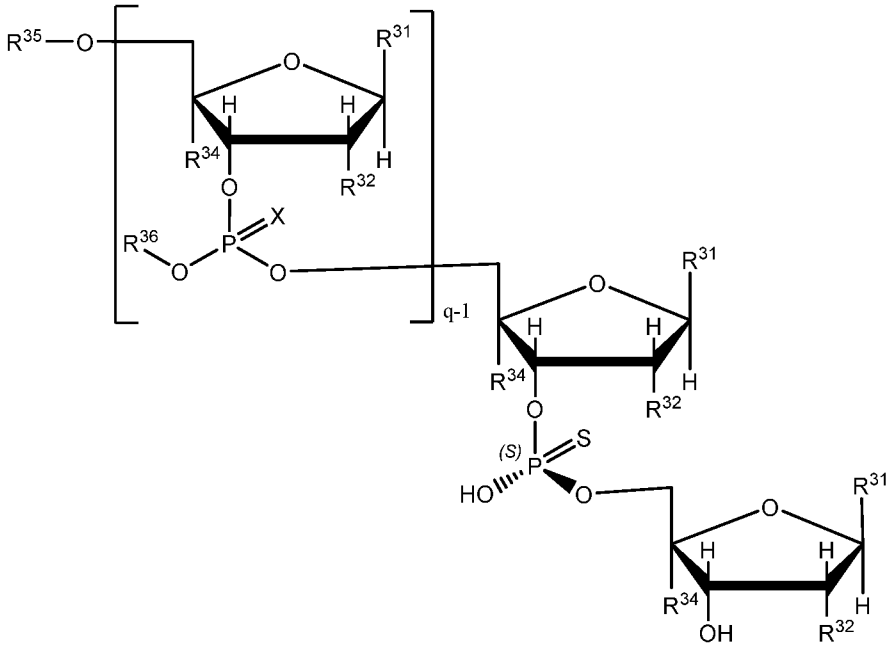
71. The process of any one of claims 54-58, 63, and 64, wherein further comprising desilylation of the fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) to form fragment of formula (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), or (V*-2):



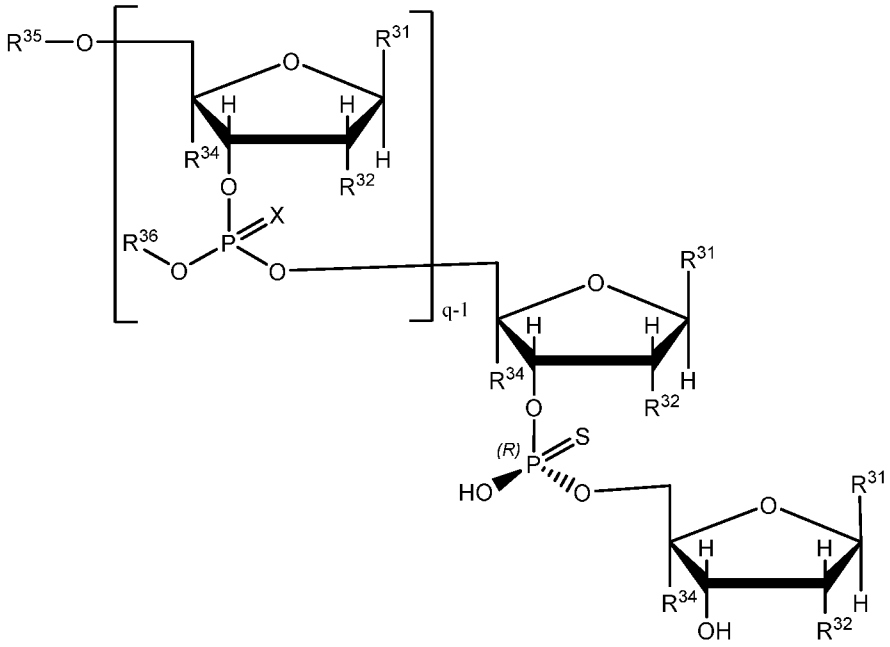
(VJ), or a salt thereof,



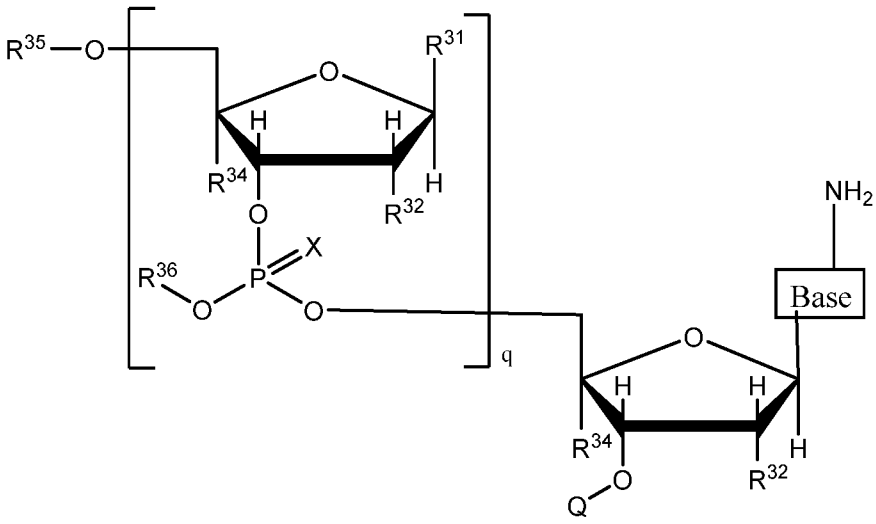
(VJ'), or a salt thereof,



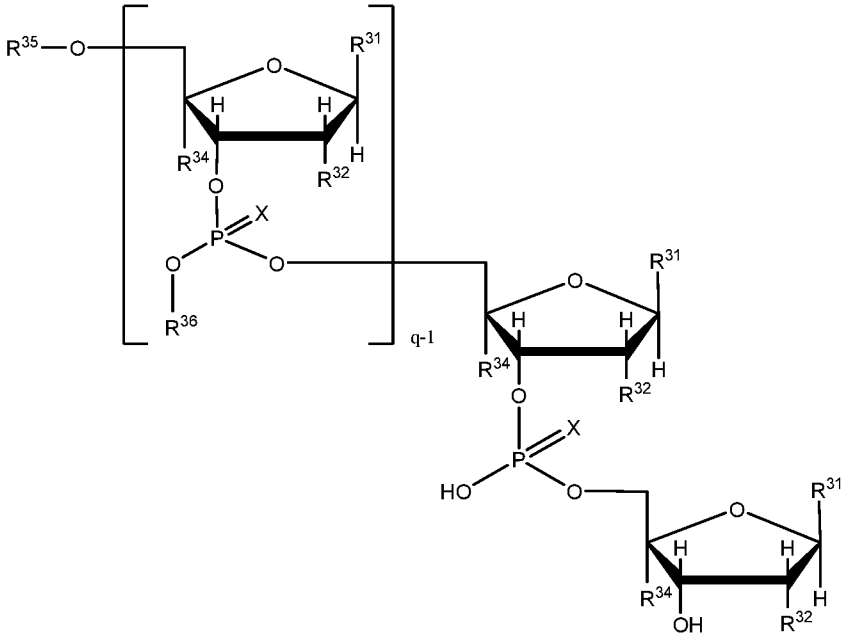
(V-C5), or a salt thereof,



(V-C6), or a salt thereof,

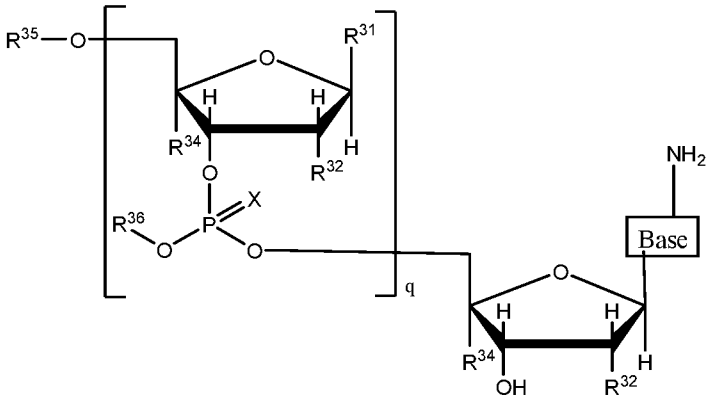


(VBZ-7), or a salt thereof, or



(V*-2), or a salt thereof,

provided when Q and P_1 are the same, the desilylation of the fragment of (VBZ) forms a fragment of formula (VBZ-7'):



(VBZ-7').

72. The process of claim 71, wherein the desilylation reaction is carried out by reacting the fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) with HF in the presence of a base.

73. The process of claim 72, wherein the base is imidazole or pyridine, wherein the imidazole or pyridine are optionally substituted.

74. The process of 71, wherein the desilylation reaction is carried out by reacting the fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), or (V*) with HF in the presence of pyridine and imidazole.

75. The process of claim 74, wherein the molar ratio of imidazole to HF is in the range of 0.5:1 to 10:1.

76. The process of claim 75, wherein the molar ratio of imidazole to HF is in the range of 1.1:1 to 5:1.

77. The process of claim 76, wherein the molar ratio of imidazole to HF is 2:1.

78. The process of any one of claims 74 -77, wherein the molar ratio of pyridine to HF is in the range of 100:1 to 1:1.

79. The process of any one of claims 74 -77, wherein the molar ratio of pyridine to HF is 1:1.

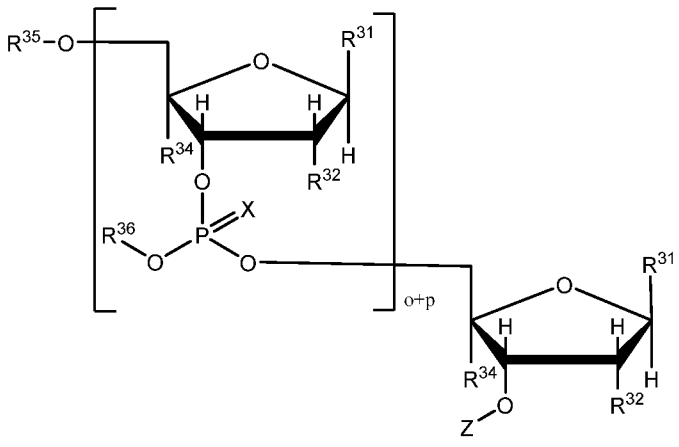
80. The process of any one of claims 54-71, wherein the fragment for formula (V), (V'), (V-C1), (V-C2), (VBZ), (V*), (VH), (VH'), (V-C3), (V-C4), (VBZ-6), (V*-1), (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), (VBZ-7') or (V*-2) is not purified by chromatography.

81. The process of claim 80, wherein the fragment of formula (V), (V'), (V-C1), (V-C2), (VBZ), (V*), (VH), (VH'), (V-C3), (V-C4), (VBZ-6), (V*-1), (VJ), (VJ'), (V-C5), (V-C6), (VBZ-7), (VBZ-7') or (V*-2) is purified by selective precipitation and/or extraction.

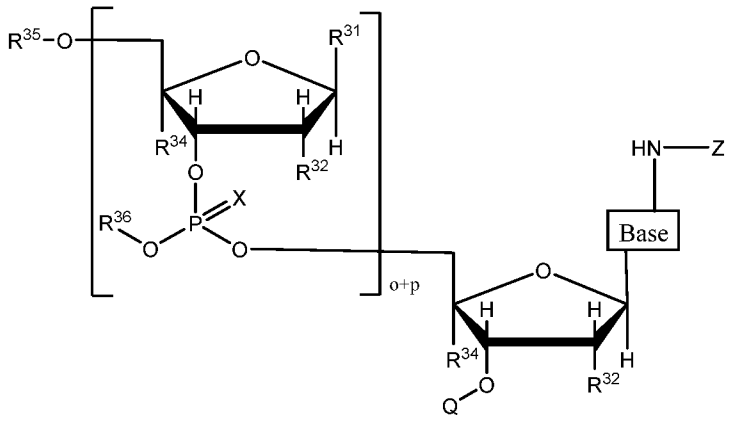
82. The process of any one of claims 54-81, wherein q is 2 to 5.

83. The process of claim 82, wherein q is 4.

84. A process for preparing an oligonucleotide of formula (VI) or (VI-1),



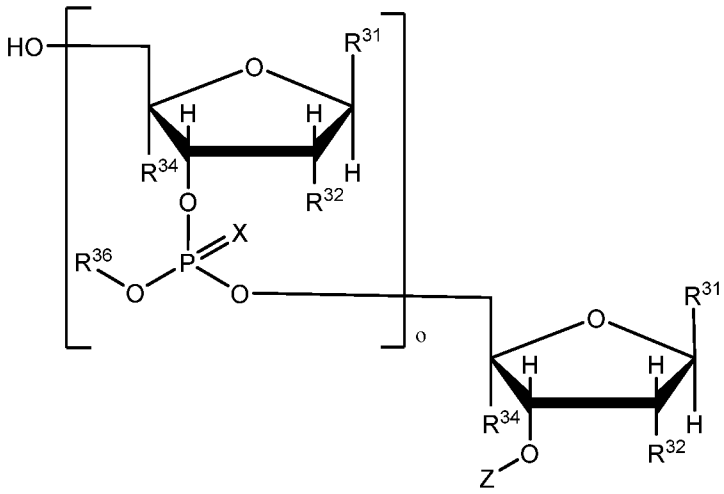
(VI),



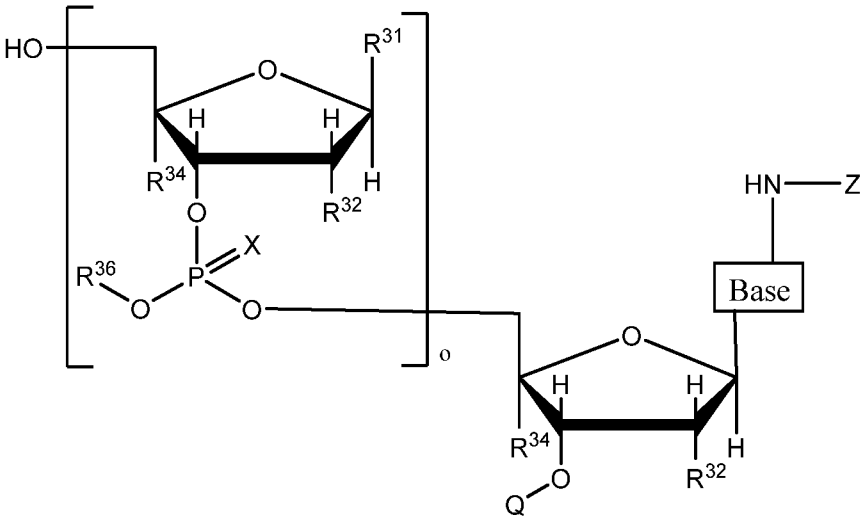
(VI-1)

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):

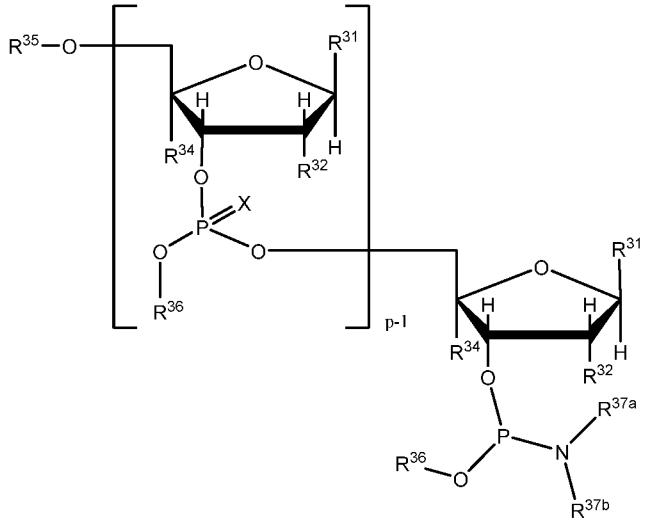


(F1),



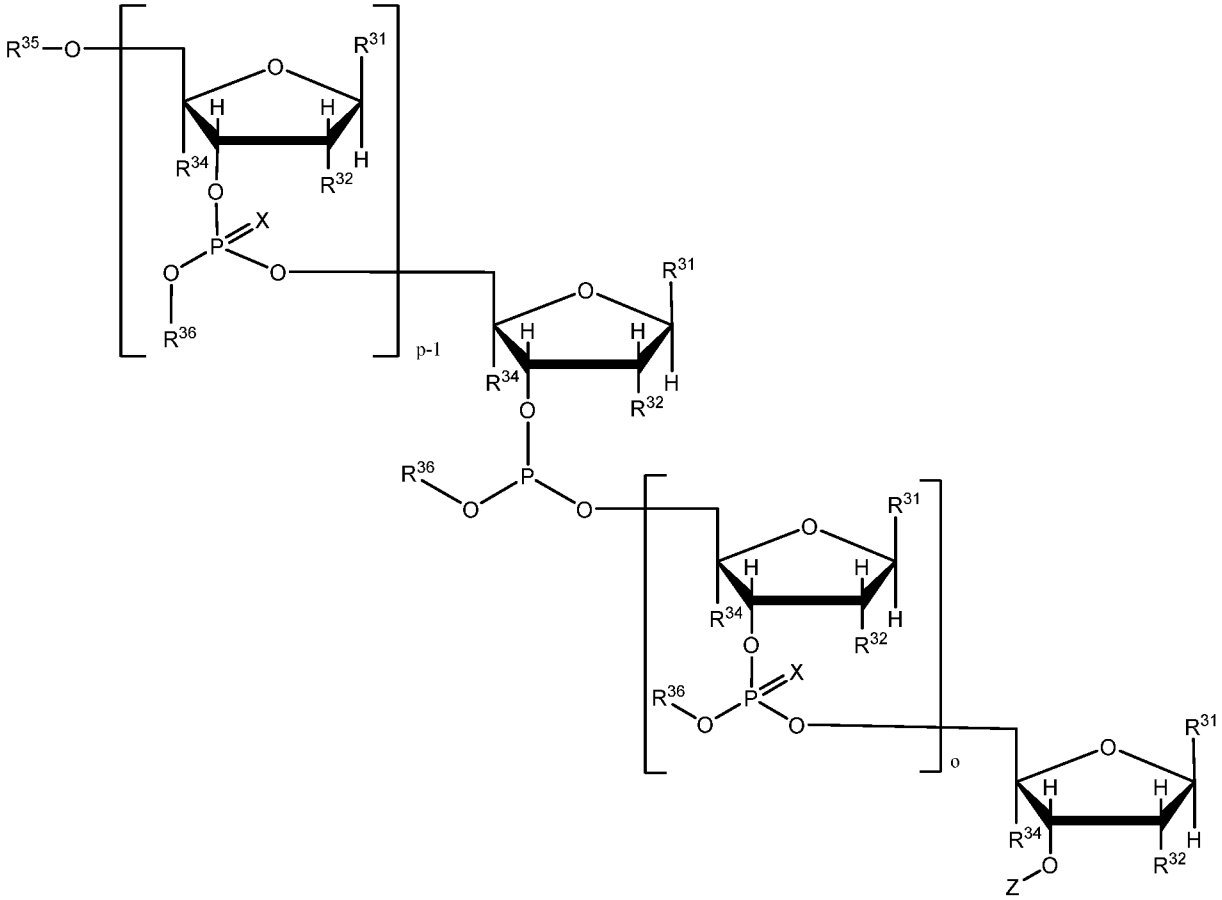
(F1-1),

or a salt thereof, with an oligonucleotide fragment of formula (F2):

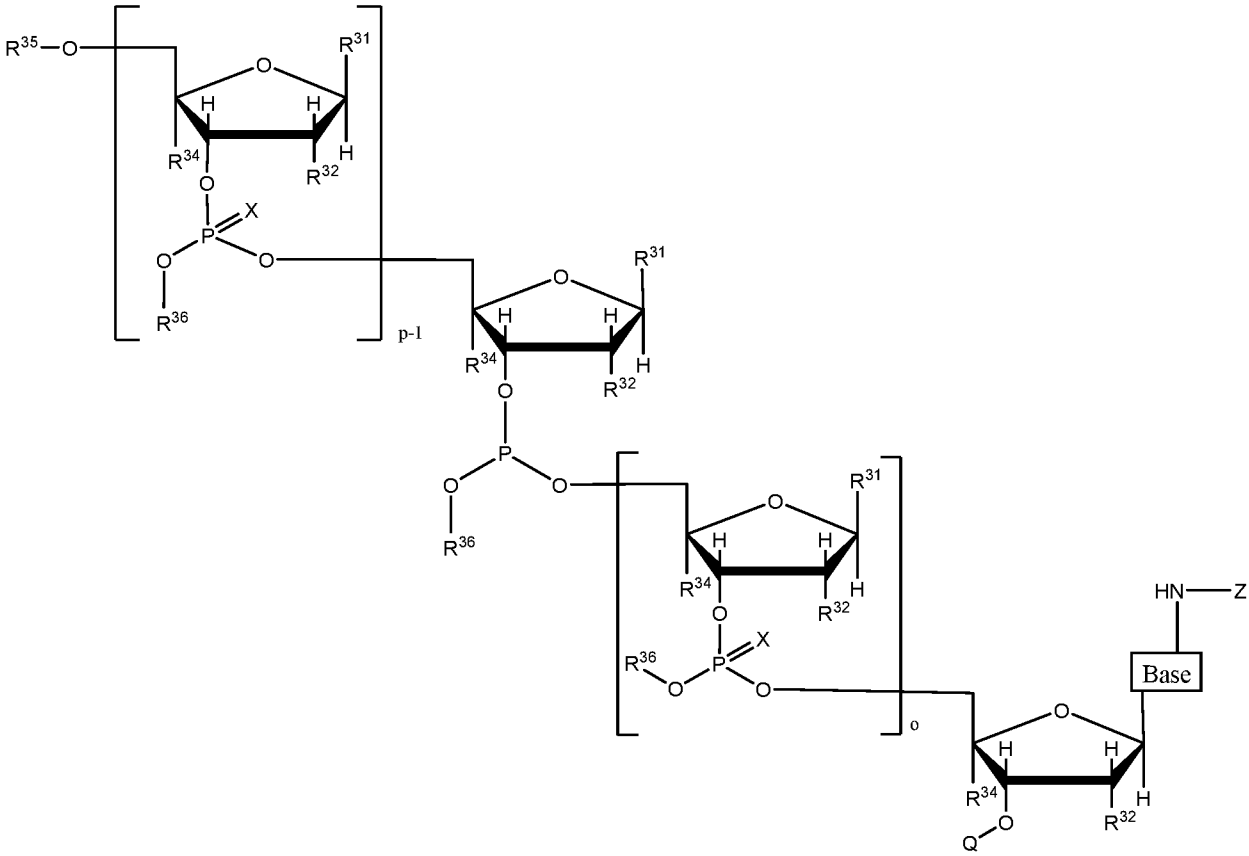


(F2), or a salt thereof,

in a solution to form an oligonucleotide fragment of formula (F3) or (F3-1),



(F3),



(F3-1),

or a salt thereof; and

b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3) or (F3-1), or a salt thereof, to form the oligonucleotide of formula (VI) or (VI-1) or a salt thereof,

wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

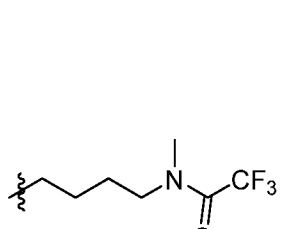
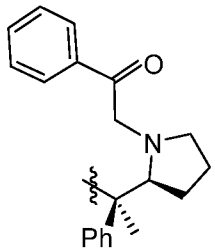
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, -NO_2 or halogen; or

R^{36} is  , or  ;

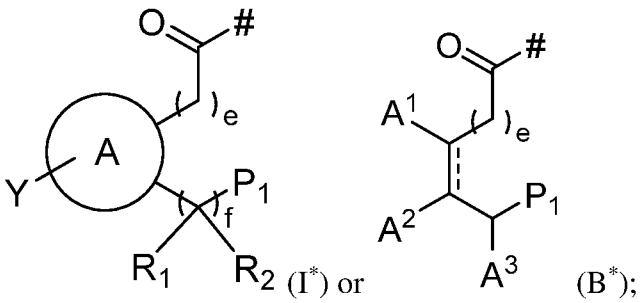
R^{37a} and R^{37b} are independently C_{1-6}alkyl;

p is an integer from 2 to 20;

o is an integer from 1 to 200;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

=== is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

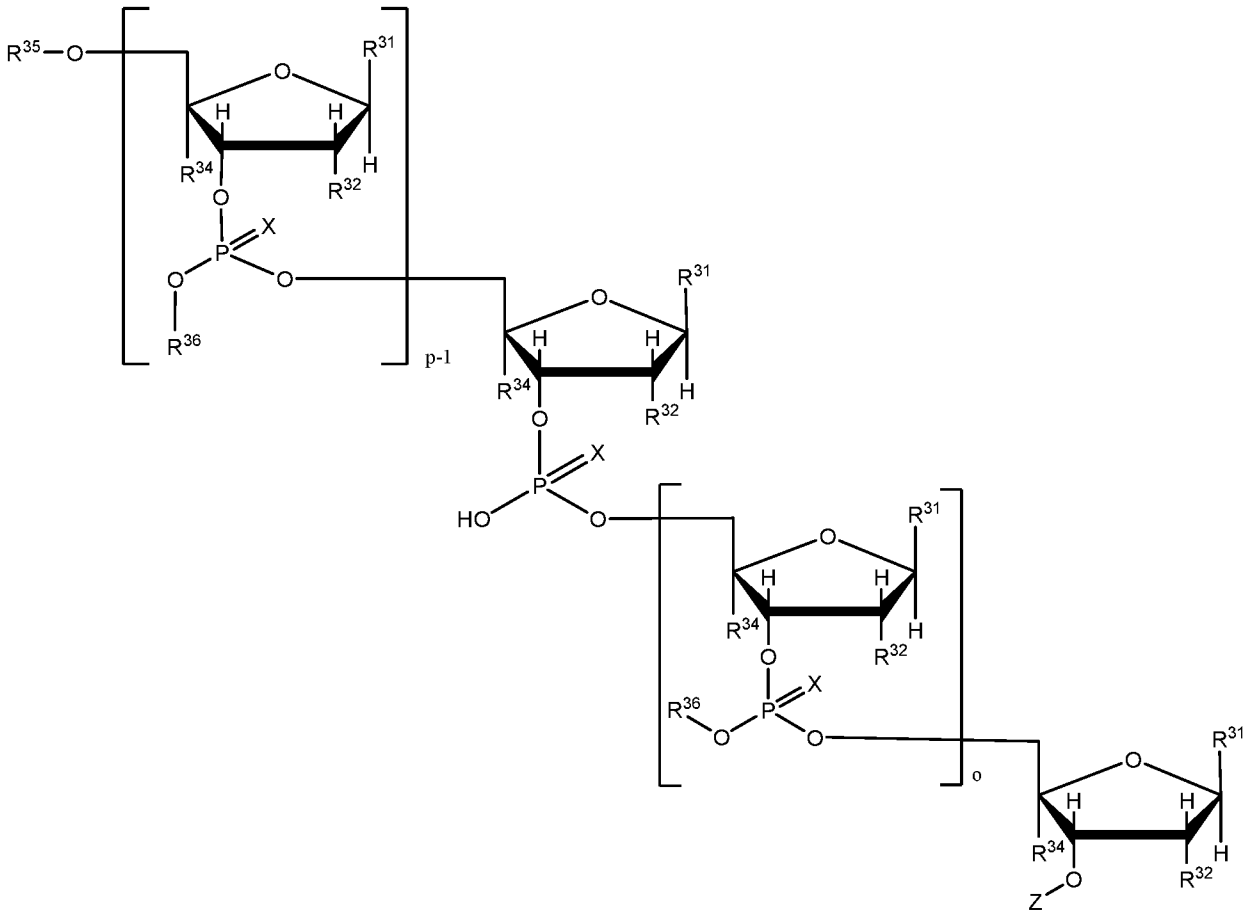
R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

R_3 is C_{1-30}alkoxy;

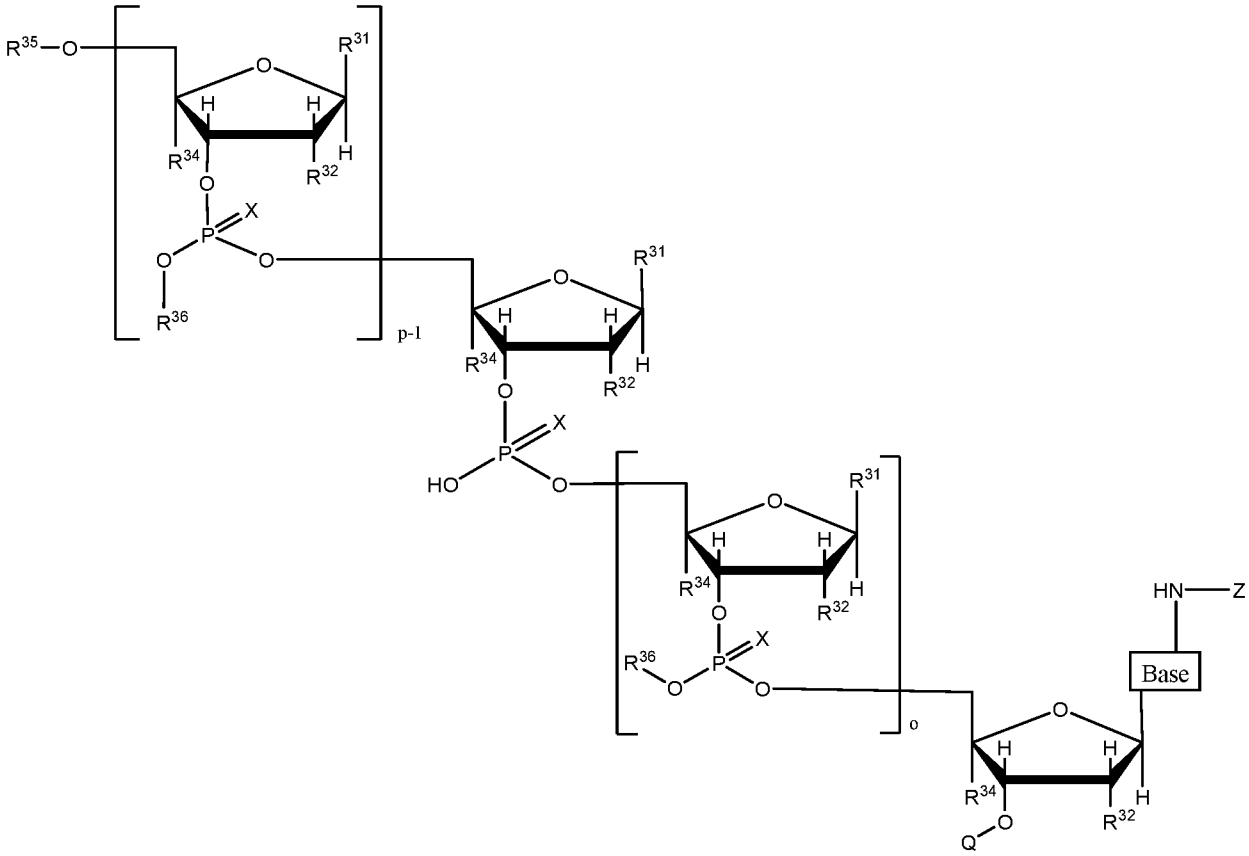
e is an integer from 0 to 6; and

f is an integer from 0 to 6.

85. A process for preparing an oligonucleotide of formula (VI') or (VI'-1),



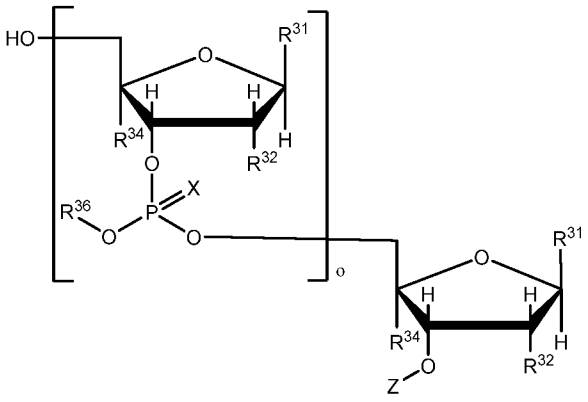
(VI'),



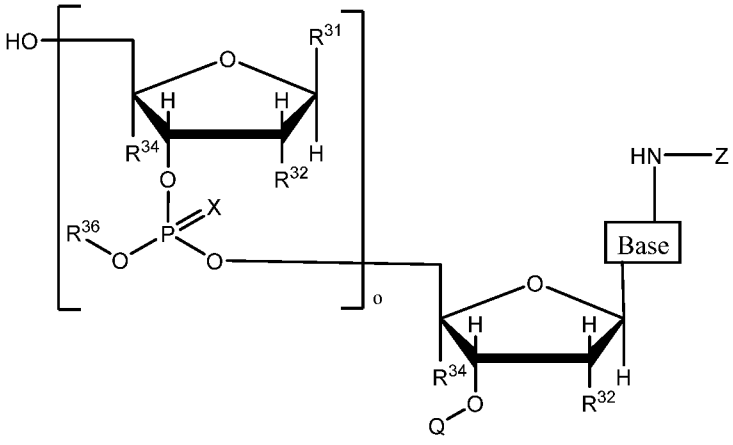
(VI'-1)

or a salt thereof, comprising

a) coupling an oligonucleotide fragment of formula (F1) or (F1-1):

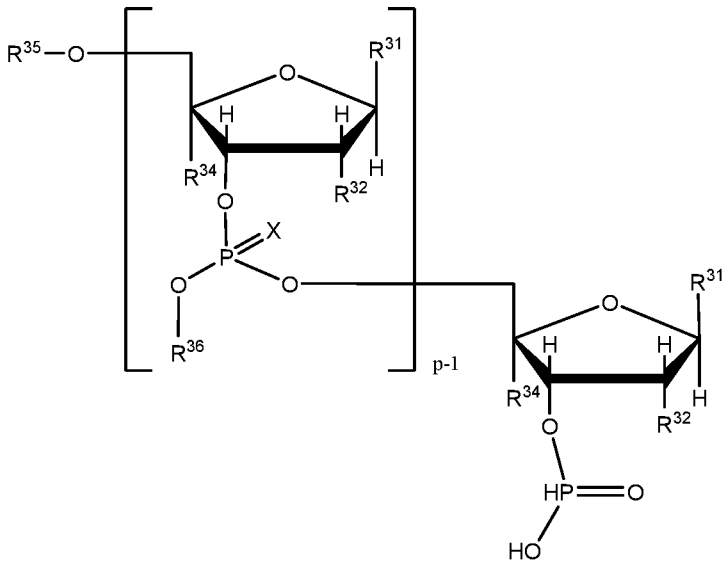


(F1),



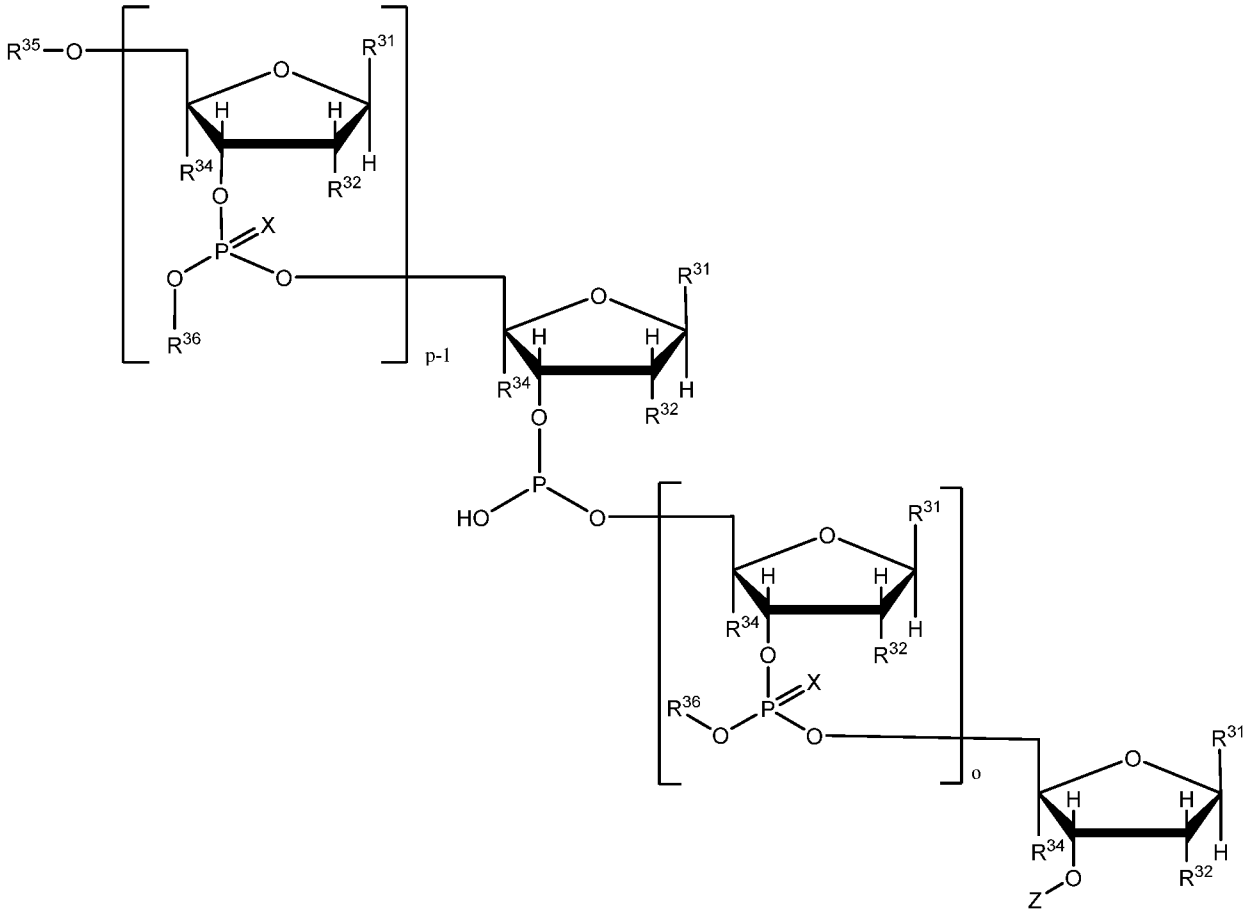
(F1-1),

or a salt thereof, with an oligonucleotide fragment of formula (F2'):

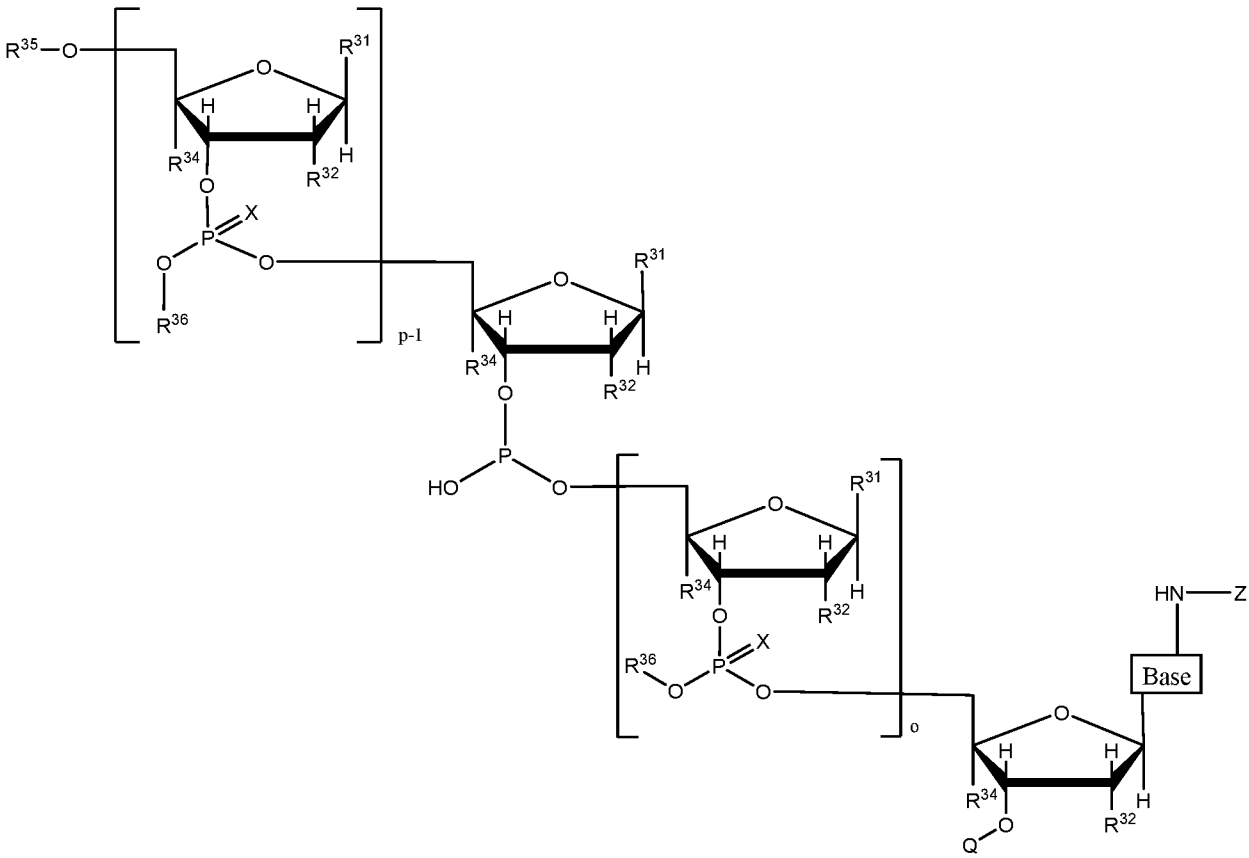


(F2'),

or a salt thereof, in a solution to form an oligonucleotide fragment of formula (F3') or (F3'-1),



(F3')



(F3'-1),

or a salt thereof; and

b) sulfurizing or oxidizing the oligonucleotide fragment of formula (F3') or (F3'-1), or a salt thereof, to form the oligonucleotide of formula (VI') or (VI'-1) or a salt thereof,

wherein:

Q is a hydroxyl protecting group;

Base is a nucleobase comprising a NH_2 group which is modified by Z;

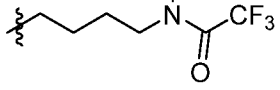
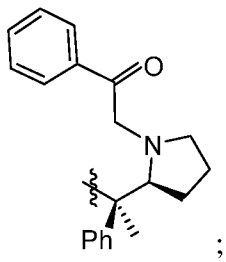
R^{31}, for each occurrence, is independently a nucleobase, wherein the NH_2 of the nucleobase, if present, is protected by an amine protecting group;

R^{32}, for each occurrence, is independently selected from the group consisting of H, halo, OH, and C_{1-6}alkoxy optionally substituted with C_{1-6}alkoxy; wherein the OH group is optionally protected by a hydroxyl protecting group;

R^{34}, for each occurrence, is independently H or forms a ring with the alkoxy group of R^{32};

R^{35} is a hydroxyl protecting group;

R^{36}, for each occurrence, is independently C_{1-6}alkyl group, C_{2-6}alkenyl group, phenyl or benzyl group, each of which is optionally substituted with –CN, -NO_2 or halogen; or

R^{36} is  , or  ;

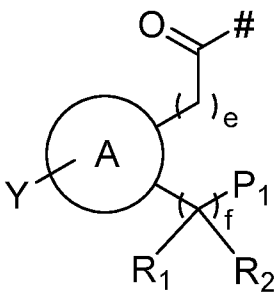
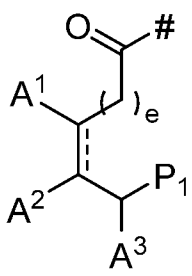
R^{37a} and R^{37b} are independently C_{1-6}alkyl;

p is an integer from 2 to 20;

o is an integer from 1 to 200;

X, for each occurrence, is independently O or S;

Z is a group represented by Formula I^* or B^*,

 (I^*) or  (B^*);

wherein

—# represents the point of attachment for Z;

one of A^1, A^2 and A^3 is Y^A and the others are H;

≡ is a single bond or a double bond;

Y^A is Y-$(CH_2)_{a1}CH_2O(CH_2)_{a2}$-, wherein a1 and a2 are each independently 0 or an integer from 1 to 10;

ring A is phenyl, 8- to 10-membered bicyclic aryl, 5- to 6-membered heteroaryl having 1 to 3 heteroatoms independently selected from oxygen, nitrogen, and sulfur, or 7- to 10-membered bicyclic heteroaryl having 1-4 heteroatoms independently selected from oxygen, nitrogen, and sulfur;

Y is H, halogen, OR^{1A}, $NR^{2A}R^{3A}$, SR^{4A}, $CR^{5A}R^{6A}R^{7A}$, or a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms; wherein R^{1A}, R^{2A}, R^{3A}, R^{4A}, R^{5A}, R^{6A}, and R^{7A} are each independently C_{1-6}alkyl, C_{1-6}alkenyl, C_{1-6}alkynyl, phenyl, OR^{8A}, -$OC(O)R^{8A}$, -$C(O)OR^{8A}$, $NR^{8A}R^{9A}$, -$NR^{8A}COR^{9A}$, -$CONR^{8A}R^{9A}$, 3- to 7-membered saturated or partially unsaturated monocyclic carbocyclyl, or 3- to 7-membered saturated or partially unsaturated monocyclic heterocyclyl having 1-2 heteroatoms independently selected from oxygen, nitrogen, and sulfur; wherein R^{8A} and R^{9A}, for each occurrence, is independently H or C_{1-6}alkyl;

P_1 is NO_2 or a silyl hydroxyl protecting group;

R_1 and R_2 are independently H, C_{1-6}alkyl, or phenyl; wherein C_{1-6}alkyl and phenyl are optionally substituted by 1-3 R_3;

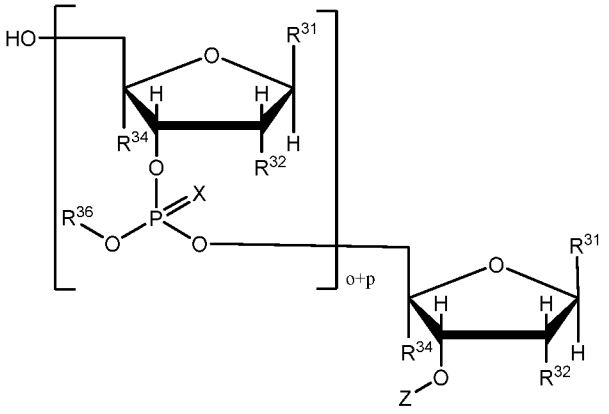
R_3 is C_{1-30}alkoxy;

e is an integer from 0 to 6; and

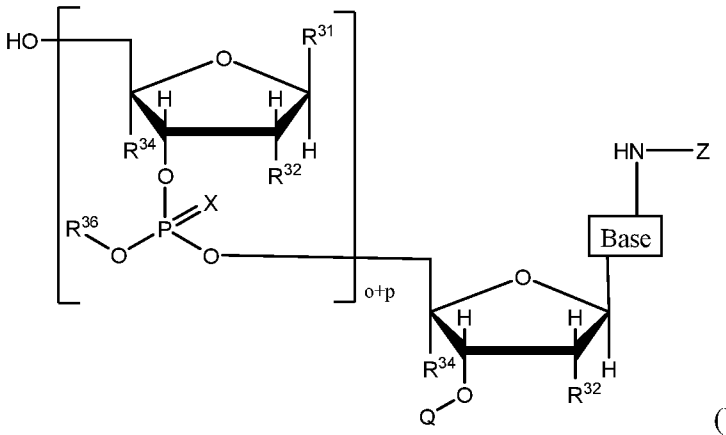
f is an integer from 0 to 6.

86. The process of claim 84 or claim 85, wherein Y is a hydrophobic group comprising one or more aliphatic hydrocarbon group having 10 or more carbon atoms.

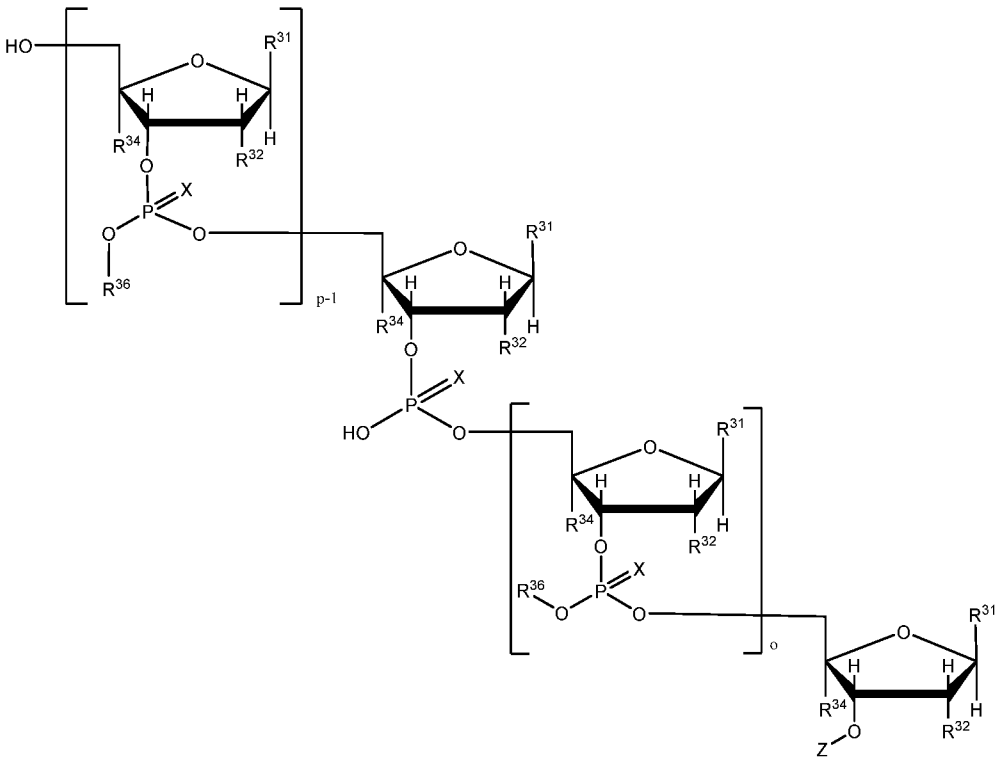
87. The process of claim 84 or claim 85, further comprising step c) deprotecting the oligonucleotide of formula (VI), (VI'), (VI-1), or (VI'-1) to form an oligonucleotide of formula (VII), (VII-1), (VII'), or (VII'-1):



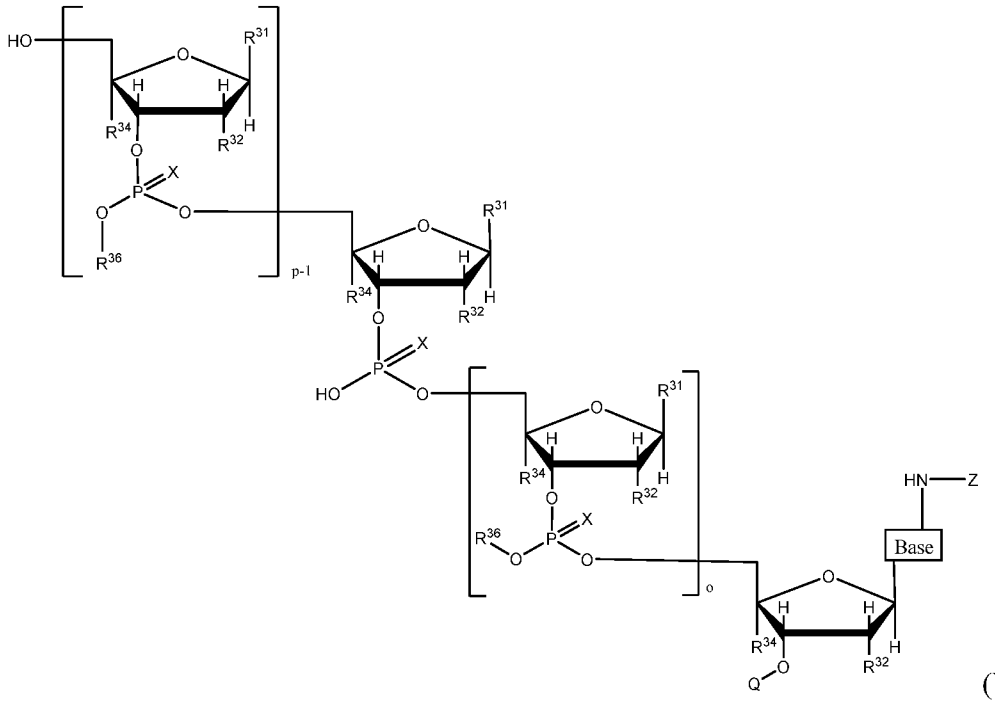
(VII),



(VII-1),



(VII'),



(VII'-1)

or a salt thereof.

88. The process of claim 87, wherein starting from oligonucleotide of formula (VII), (VII-1), (VII'), or (VII'-1), the process further comprises repeating steps a), b) and c) for 1 to 10 times, followed by steps a) and b).

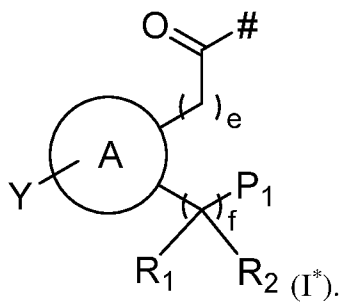
89. The process of claim 88, wherein the process further comprises repeating steps a), b) and c) for 1 to 3 times followed by steps a) and b).

90. The process of any one of claims 84-89, wherein o is an integer from 2 to 20.

91. The process of claim 90, wherein o is 2 to 5.

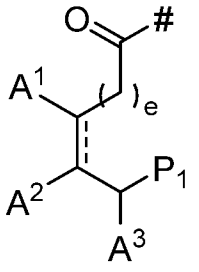
92. The process of claim 91, wherein o is 4.

93. The process of claim of any one of claims 54-92, wherein Z is a group represented by Formula I*,



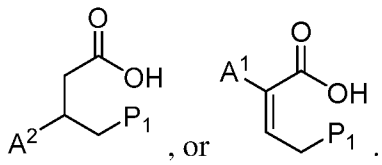
(I*).

94. The process of claim of any one of claims 54-92, wherein Z is a group represented by Formula B*,



(B*).

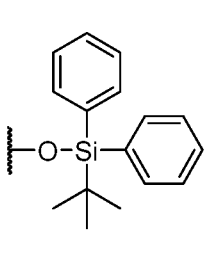
95. The process of claim of any one of claims 54-92, wherein Z is a group represented by Formula B-1* or B-2*:

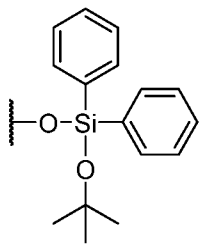


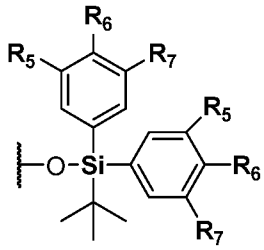
B-1* B-2*.

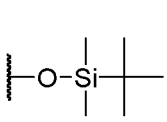
96. The process of any one of claims 54-92, wherein ring A is phenyl or naphthalenyl.

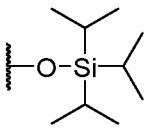
97. The process of any one of claims 54-96, wherein P_1 is a silyl hydroxyl protecting group selected from the following:

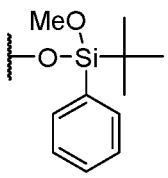

-O-TBDPS ,

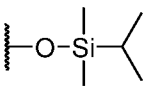

-O-TBoDPS ,

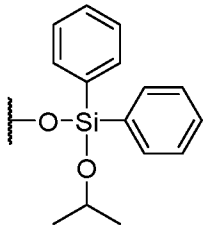

-O-TBDAS ,

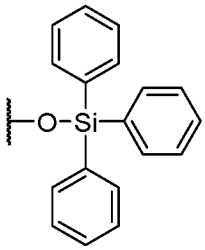

-O-TBDMS ,

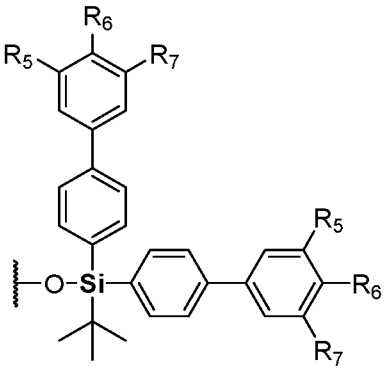

-O-TIPS ,

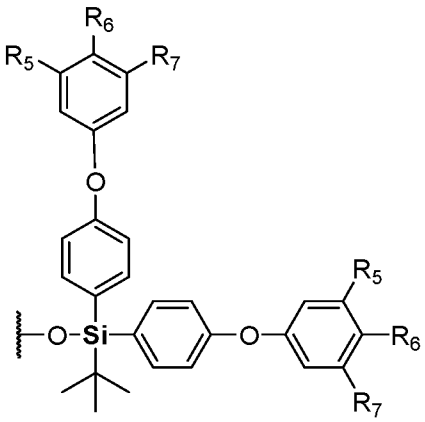

-O-TBMPS ,


-O-DMIPS ,

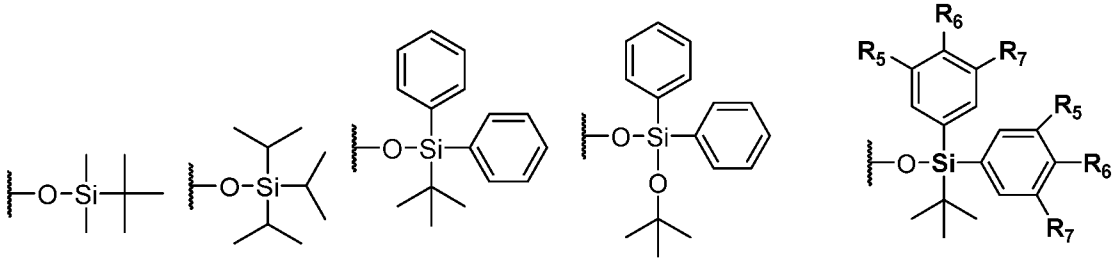

-O-IPoDPS ,


-O-TPS ,

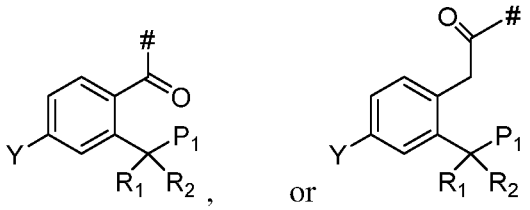

-O-TBDAS-1 ,


and -O-TBDAS-2 ; wherein ⊢ represents the point of attachment for P_1 and . R_5, R_6 and R_7 are each independently H, $C_{1\text{-}30}$alkyl, or $C_{1\text{-}30}$alkoxy.

98. The process of claim 97, wherein P_1 is selected from the group consisting of –O-TBDMS, -O-TIPS, -O-TBDPS, -O-TBoDPS, and –O-TBDAS:


-O-TBDMS , -O-TIPS , -O-TBDPS , -O-TBoDPS , and -O-TBDAS .

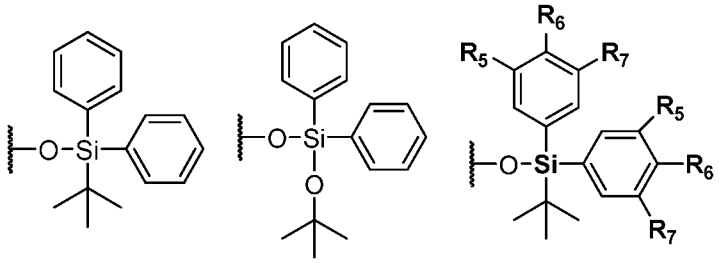
99. The process of claim 93, wherein Z is a group represented by Formula I** or Ia**:



I** or Ia**

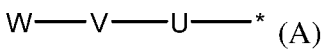
or a salt thereof;

wherein P_1 is selected from the group consisting of -O-TBDPS, -O-TBoDPS, and –O-TBDAS:



-O-TBDPS, -O-TBoDPS, -O-TBDAS.

100. The process of any one of claims 54-99 or a salt thereof, wherein Y is represented by Formula A:

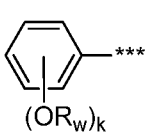
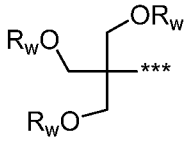
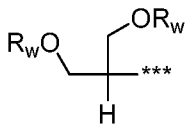
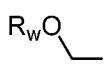


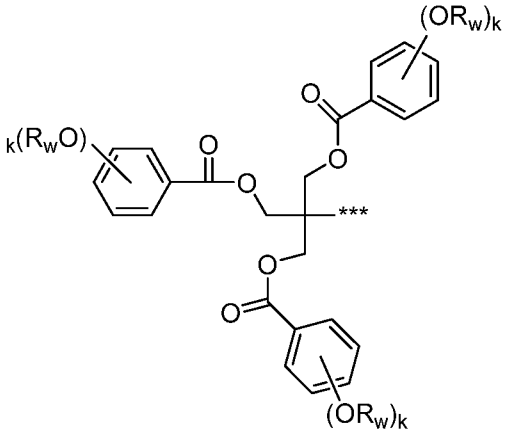
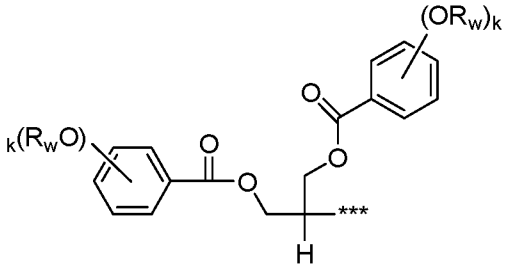
(A)

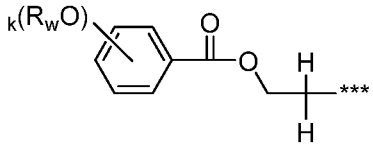
wherein:

—* represents the point of attachment for Y;

W is represented by Formula A1, A2, A2-1, A2-2, A3, A3-1, or A3-2:


(A1),

(A2),

(A2-1),

(A2-2),


(A3),

(A3-1),

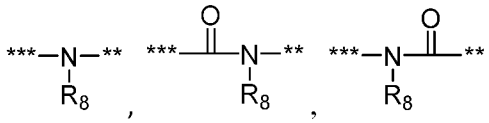
or

(A3-2),

wherein

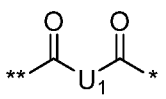
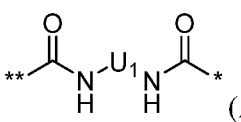
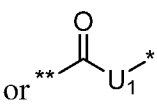
—*** represents the point where W and V connect;

each R_w is independently an aliphatic hydrocarbon group having 10 or more carbon atoms;

k is an integer from 1 to 5;

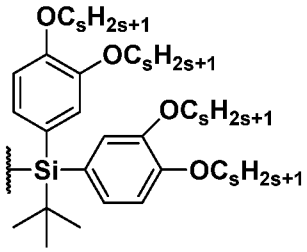
V is a bond, oxygen, C_{1-20}alkylene, C_{1-6}alkynylene, -C(=O)-, ***-C(=O)-O-**, ***-O-C(=O)-**,  , or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; wherein —** represents the point where V and U connect; and R_8 is H or C_{1-30}alkyl; and

U is a bond, oxygen, C_{1-20}alkylene, carbonyl, ***-O-C(=O)-**, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur; 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, wherein the heteroaryl is optionally substituted by 1-3 R_8; or a group represented by formula A4, A5, or A6:


(A4),

(A5), or

(A6),

wherein U_1 is $C_{1\text{-}6}$alkylene, $C_{1\text{-}6}$alkyleneoxy, 5 to 7 member heterocyclyl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur, or 5 to 7 member heteroaryl having 1 to 3 heteroatoms selected from oxygen, nitrogen, and sulfur.

101. The process of any one of claims 97-100, wherein the TBDAS group is:

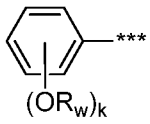


,

wherein s is an integer from 1 to 30.

102. The process of any one of claims 54-100, wherein P_1 is TBDPS.

103. The process of any one of claims 100-102, wherein W is represented by Formula A1:

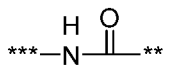


A1,

wherein R_w is C_nH_{2n+1};

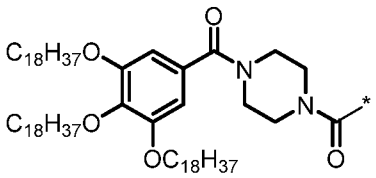
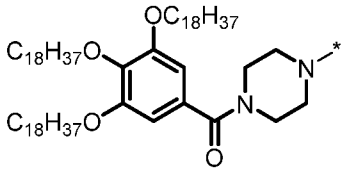
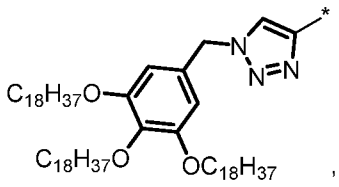
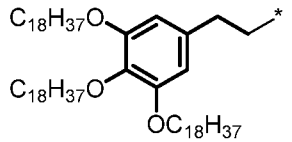
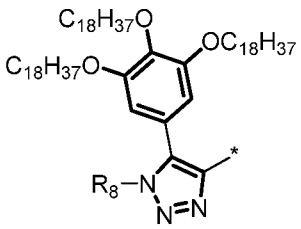
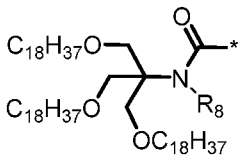
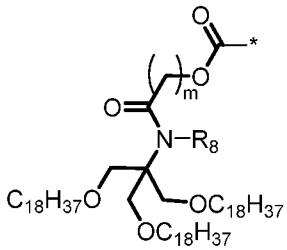
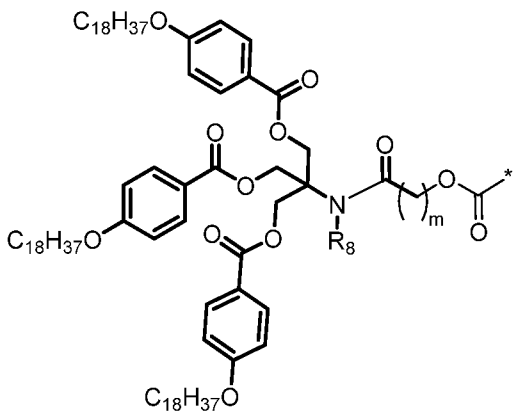
n is an integer from 1 to 30.

104. The process of any one of claims 100-103, wherein R_w is selected from a group consisting of $C_{12}H_{25}$, $C_{18}H_{37}$, $C_{20}H_{41}$, $C_{22}H_{45}$, $C_{24}H_{49}$, $C_{26}H_{53}$, and $C_{28}H_{57}$.

105. The process of any one of claims 100-104, wherein V is a bond, CH_2, CH_2CH_2, C(=O)-, ***-C(=O)-O-**, or 

.

106. The process of any one of claims 54-100, wherein Y is selected from the groups consisting of


,

,

,

,

,


, and

;

wherein

R_8 is H or $C_{1\text{-}6}$alkyl; and

m is an integer from 1 to 5.

107. The process of any one of claims 54-106, wherein R_1 and R_2 are independently H or CH_3.

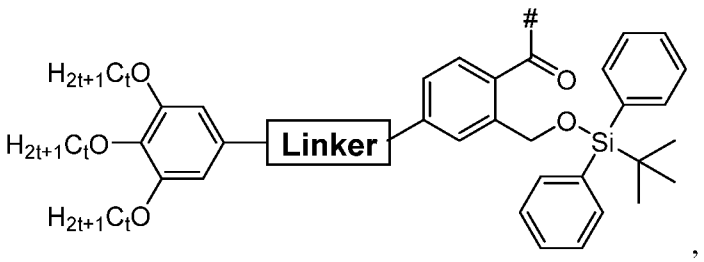
108. The process of any one of claims 54-107, wherein e is 0, 1, or 2; and f is 0, 1, or 2.

109. The process of any one of claims 54-108, wherein e is 1; and f is 1.

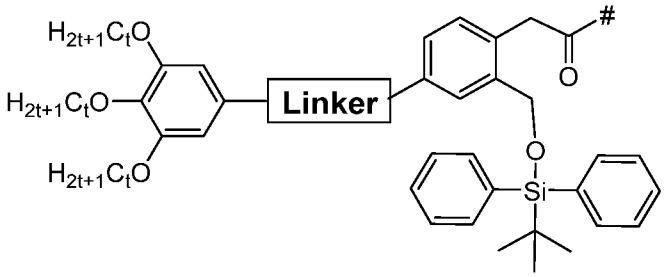
110. The process of any one of claims 54-108, wherein e is 0; and f is 1 or e is 1; and f is 0.

111. The process of any one of claims 54-110, wherein R_8 is H or $C_{1\text{-}4}$alkyl.

112. The process of any one of claims 54-111, wherein Z is represented by Formula II* or IIa*,



II*

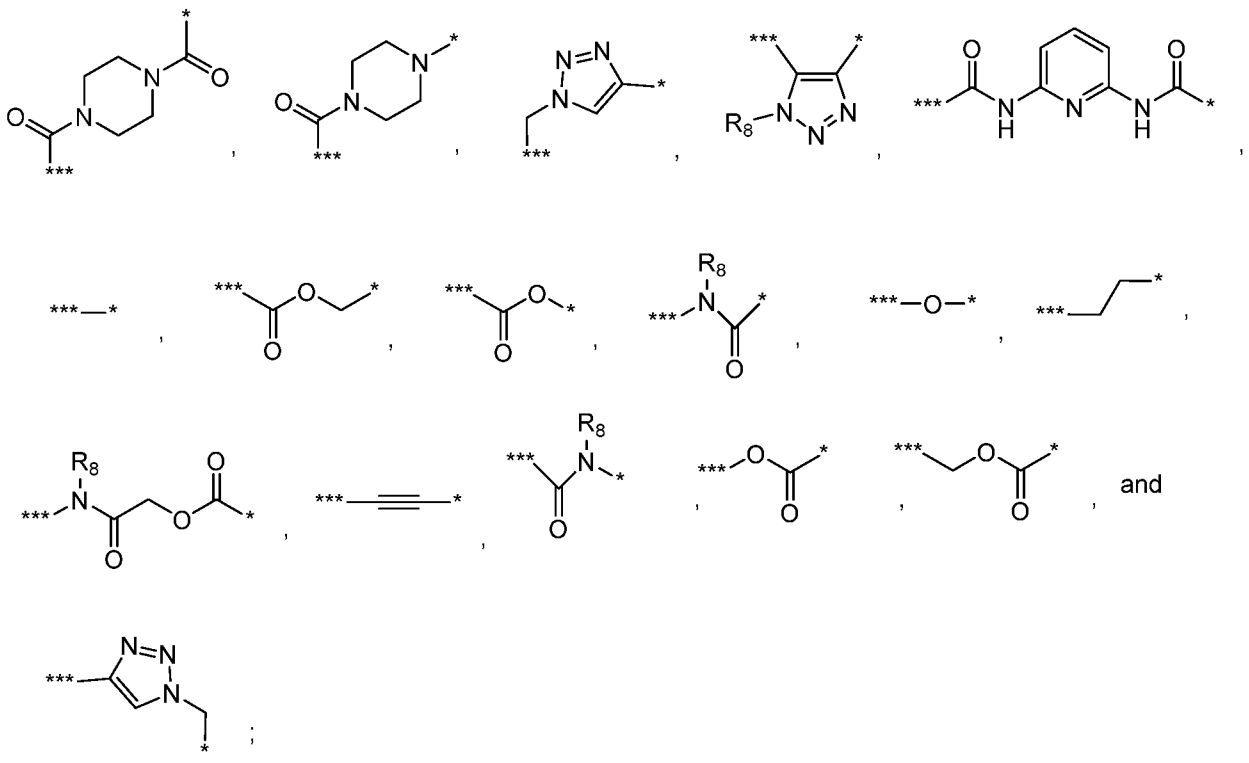


IIa*

wherein

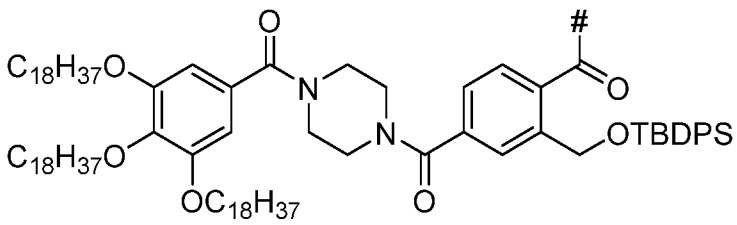
t is an integer from 10 to 30;

Linker is selected from the group consisting of

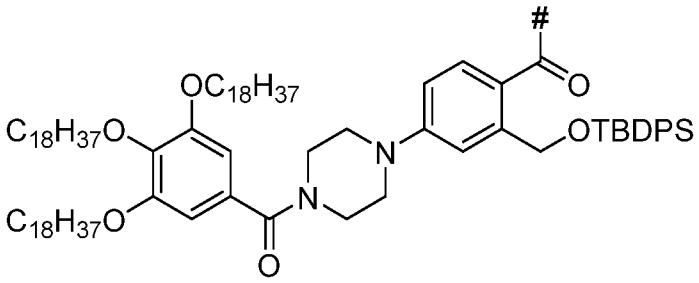


wherein R_8 is H or C_{1-6}alkyl.

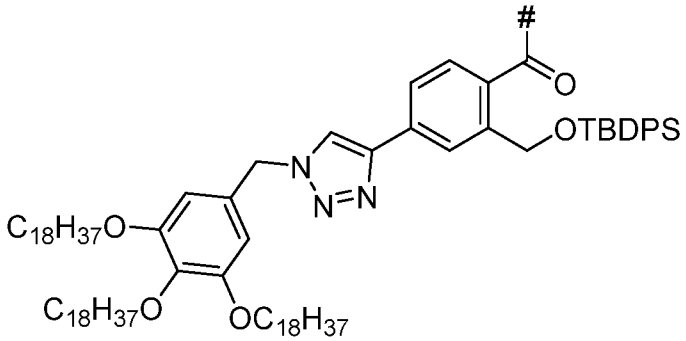
113. The process of any one of claims 54-112, wherein Z is:



,

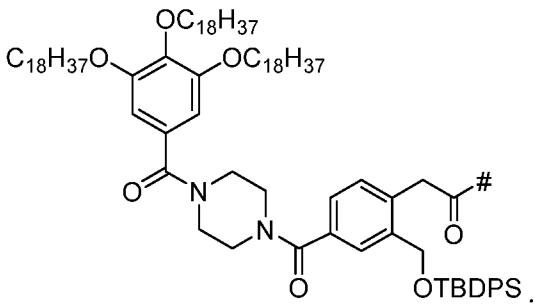


, or



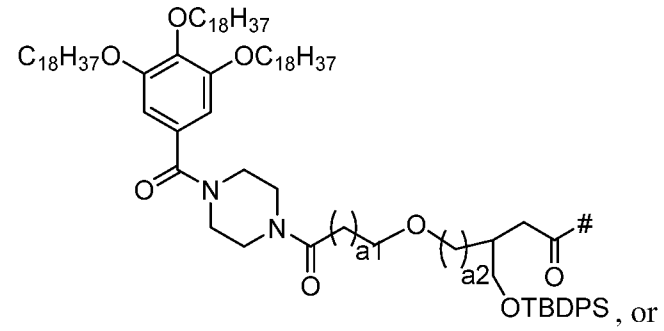
.

114. The process of any one of claims 54-93, or a salt thereof, wherein Z is

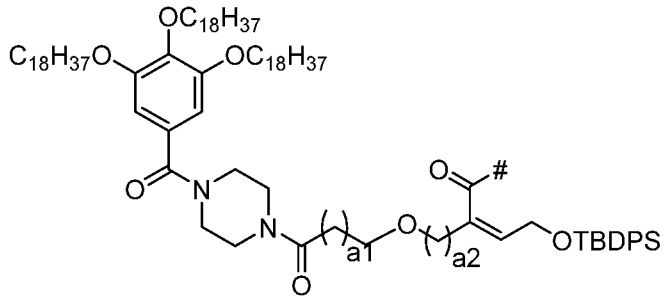


.

115. The process of any one of claims 54-93, or a salt thereof, wherein Z is



, or



.

116. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein all of the P=X groups in the nucleotide or oligonucleotide are P=S.

117. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein all of the P=X groups in the nucleotide or oligonucleotide are P=O.

118. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% or 90% of the P=X groups in the compound or oligonucleotide are P=S.

119. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein 10-90%, 20-80%, 30-70% or 40-60% of the P=X groups in the compound or oligonucleotide are P=S.

120. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein the nucleobase is selected from the group consisting of cytosine, guanine, adenine, thymine, uracil, hypoxanthine, xanthine, 7-methylguanine, 5,6-dihydrouracil, 5-methylcytosine, and 5-hydroxymethylcytosine, wherein the NH_2 group of the nucleobase, if present, is protected by PhCO-, CH_3CO-, *i*PrCO-, Me_2N-CH=, or Me_2N-CMe=.

121. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-115, wherein the nucleobase is selected from the group consisting of cytosine, guanine, adenine, thymine, uracil, and 5-methylcytosine, wherein the NH_2 group of the nucleobase, if present, is protected by PhCO-, CH_3CO-, *i*PrCO-, Me_2N-CH=, or Me_2N-CMe=.

122. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-121, wherein

each R^{32} is independently selected from the group consisting of H, F, and C_{1-4}alkoxy optionally substituted with C_{1-4}alkoxy;

each R^{34} is independently H or forms a ring with the alkoxy group of R^2, wherein the ring is a 5 or 6-membered ring optionally substituted with 1 to 3 C_{1-4} alkyl groups;

each R^{35} is a 4,4'-dimethoxytirtyl group;

R^{36} is $–CH_2CH_2CN$; and

R^{37a} and R^{37b} are independently C_{1-4}alkyl.

123. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-121, wherein

each R^{32} is independently selected from the group consisting of H, F, $-OCH_3$, $–OCH_2CH_2OCH_3$,and -OTBDMS; and

each R^{34} is independently H or forms a ring with the alkoxy group of R^{32}, wherein the ring is a 5-membered ring.

124. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-121, wherein each R^{34} is independently H or together with the alkoxy group of R^{32} form $–CH_2$-O-.

125. The nucleotide or oligonucleotide of any one of claims 27-53 or the process of any one of claims 54-121, wherein

each R^{32} is independently selected from H or $–OCH_2CH_2OMe$;

each R^{34} is H;

each R^{35} is a 4,4'-dimethoxytirtyl group;

R^{36} is $–CH_2CH_2CN$; and

R^{37a} and R^{37b} are both $-CH(CH_3)_2$.

126. The process of any one of claims 55, 64, and 85, wherein the salt of the compound of formula (VD'), (V-2'), or (F2') is selected from trimethyl amine salt, triethyl amine salt, and triisopropyl amine salt.

127. The process of claim 126, wherein the salt of the compound of formula (VD'), (V-2'), or (F2') is triethyl amine salt.

128. The nucleotide or oligonucleotide of claim 28, or the process of any one of claims 58, 59, 69, and 71-92, wherein the Base is adenine, cytosine, or guanine.

129. The nucleotide or oligonucleotide of claim 28, or the process of any one of claims 58, 59, 69, and 71-92, wherein the Q is a silyl protecting group.

130. The nucleotide or oligonucleotide of claim 28, or the process of any one of claims 58, 59, 69, and 71-92, wherein the Q is selected from the group consisting of trimethylsilyl, triethylsilyl, triisopropylsilyl, dimethylisopropylsilyl, diethylisopropylsilyl, dimethylthexylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl, di-t-butylmethylsilyl tri(trimethylsilyl)silyl, t-butylmethoxyphenylsilyl, and t-butoxydiphenylsilyl.

131. The nucleotide or oligonucleotide of claim 28, or the process of any one of claims 58, 59, 69, and 71-92, wherein the Q is t-butyldiphenylsilyl.

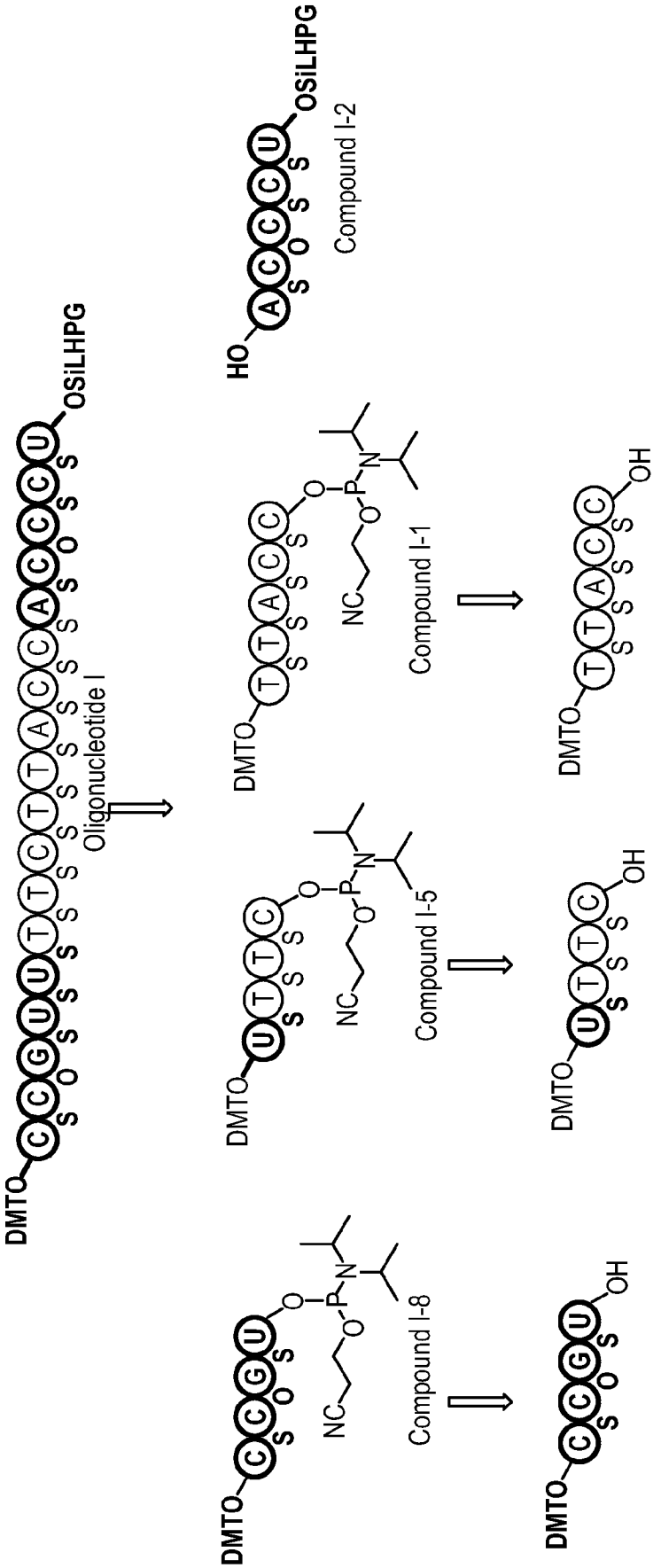

DMTO
OSiLHPG
Oligonucleotide I
HO
OSiLHPG
Compound I-2
DMTO
NC
Compound I-1
OH
DMTO
NC
Compound I-5
OH
DMTO
NC
Compound I-8
OH

FIG. 1

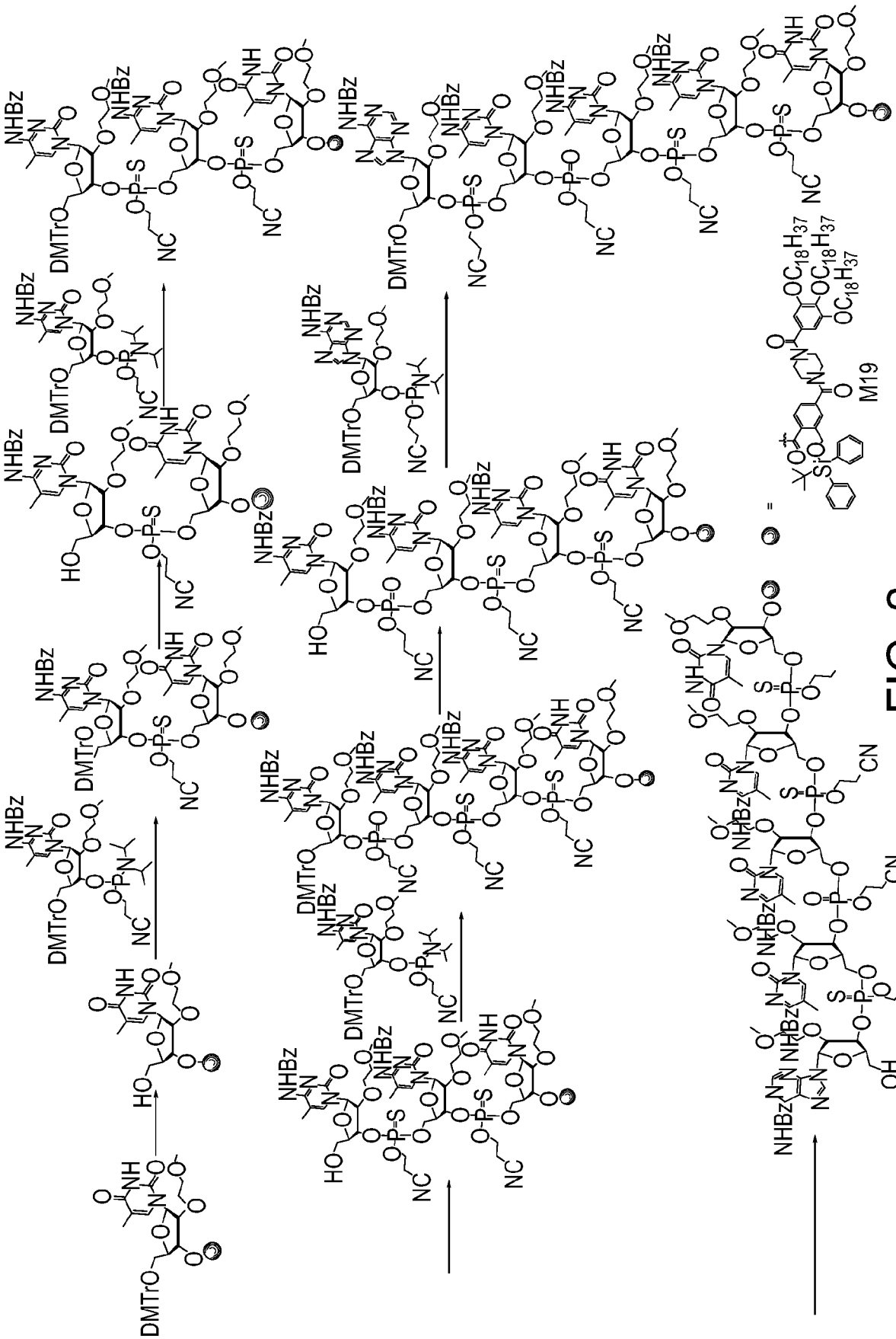


FIG. 2

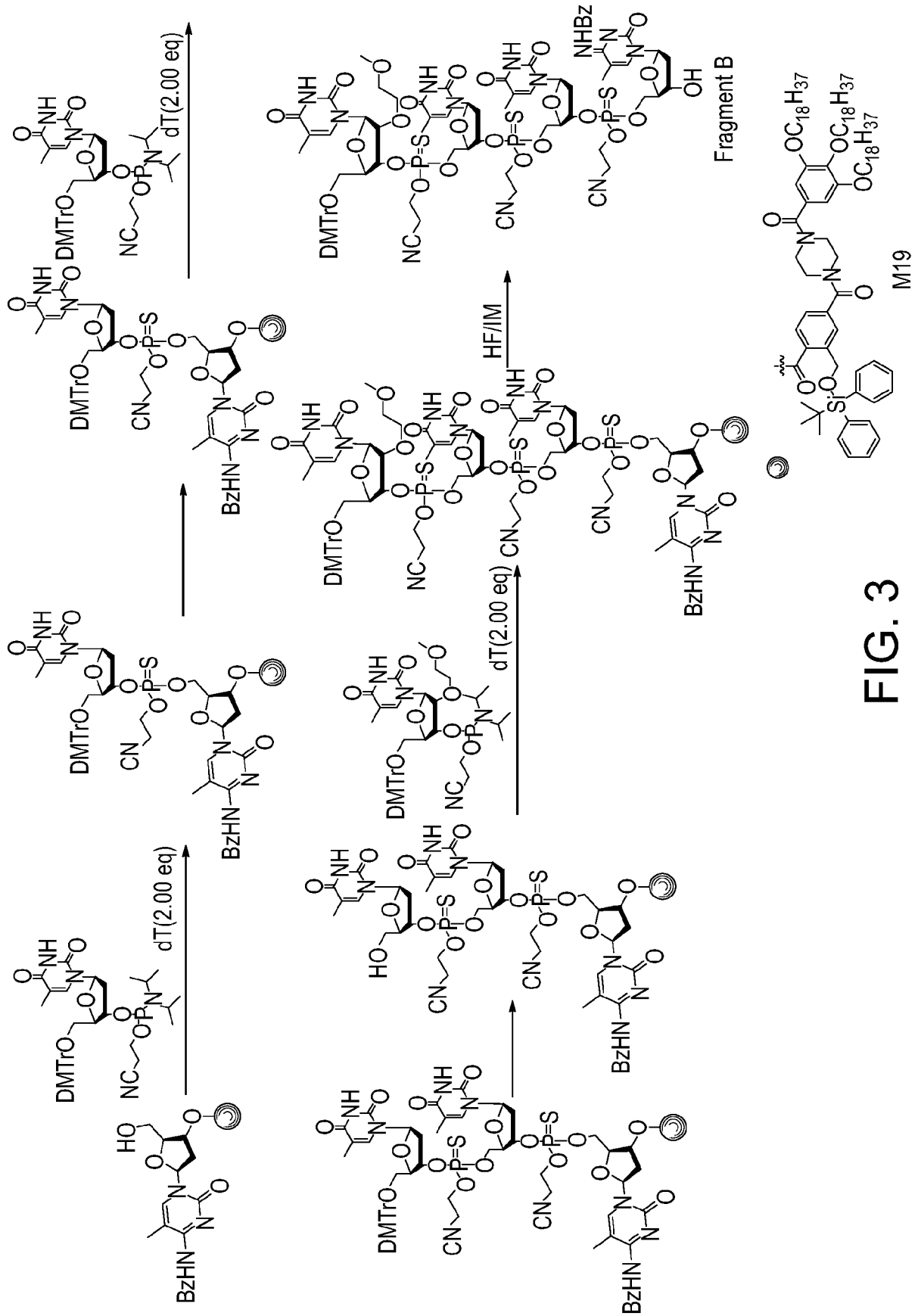


FIG. 3

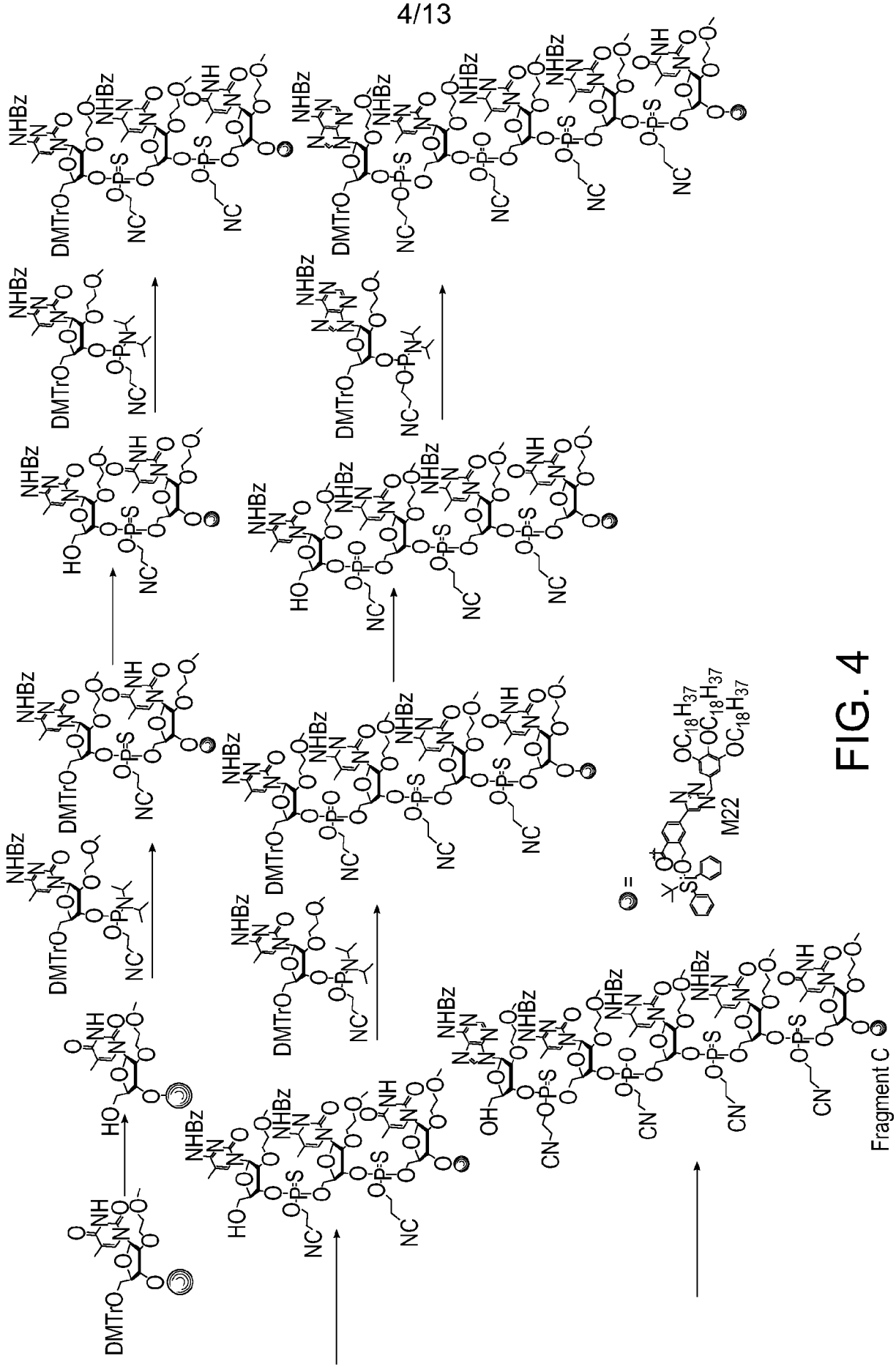


FIG. 4

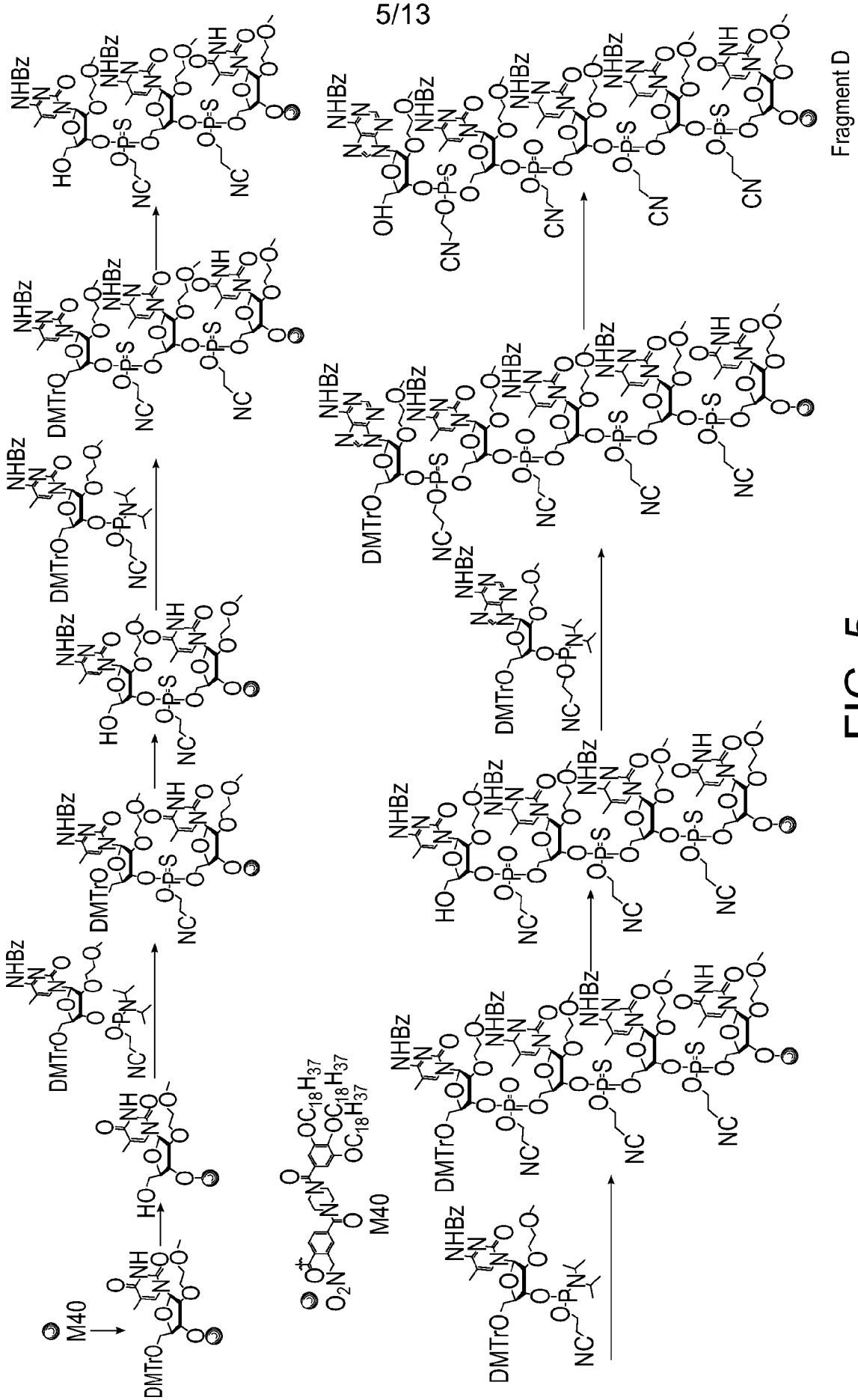


FIG. 5

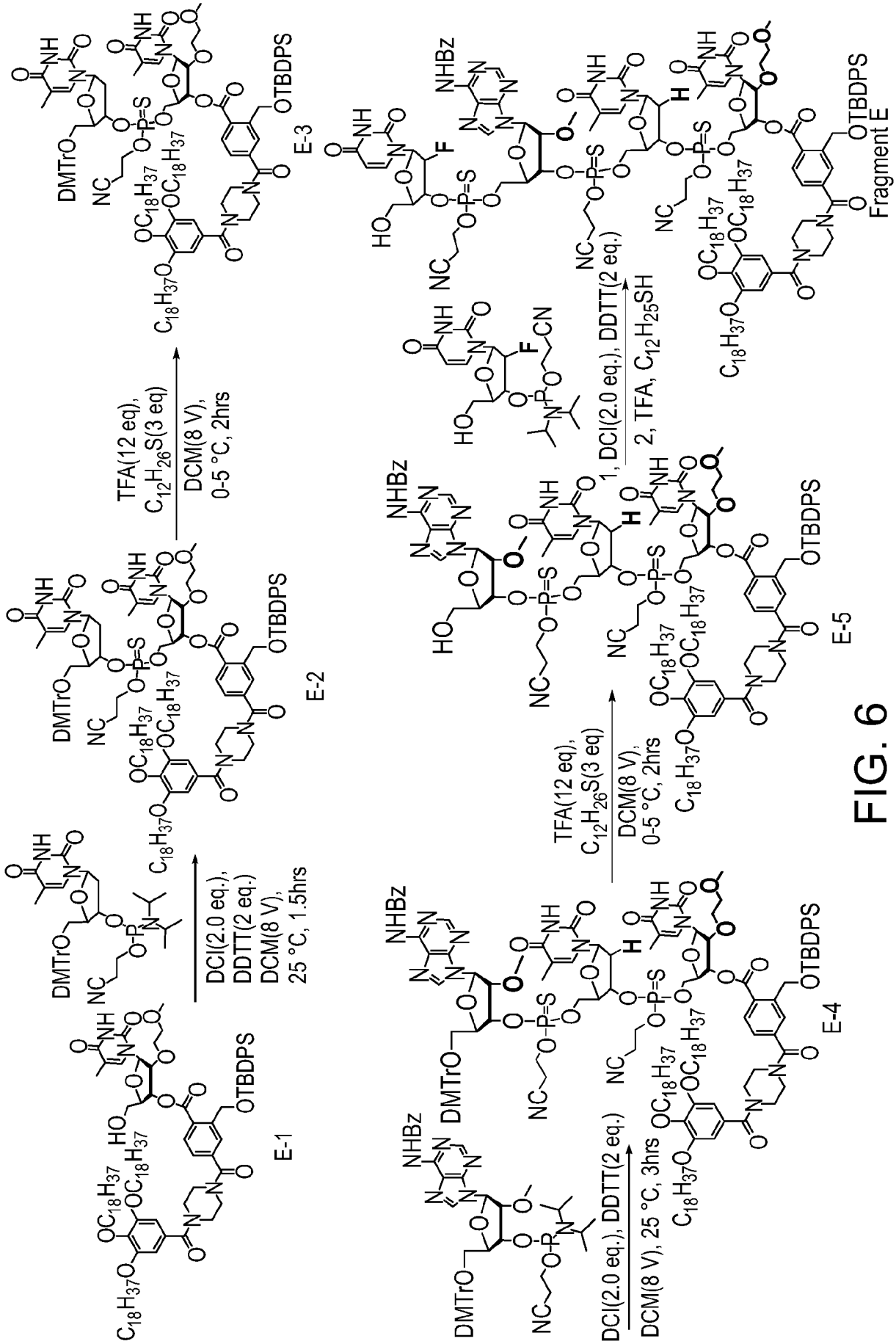

DCI(2.0 eq.), DDTT(2 eq.) DCM(8 V), 25 °C, 1.5hrs
TFA(12 eq), C12H26S(3 eq) DCM(8 V), 0-5 °C, 2hrs
DCI(2.0 eq.), DDTT(2 eq.) DCM(8 V), 25 °C, 3hrs
1, DCI(2.0 eq.), DDTT(2 eq.) 2, TFA, C12H25SH
E-1
E-2
E-3
E-4
E-5
Fragment E

FIG. 6

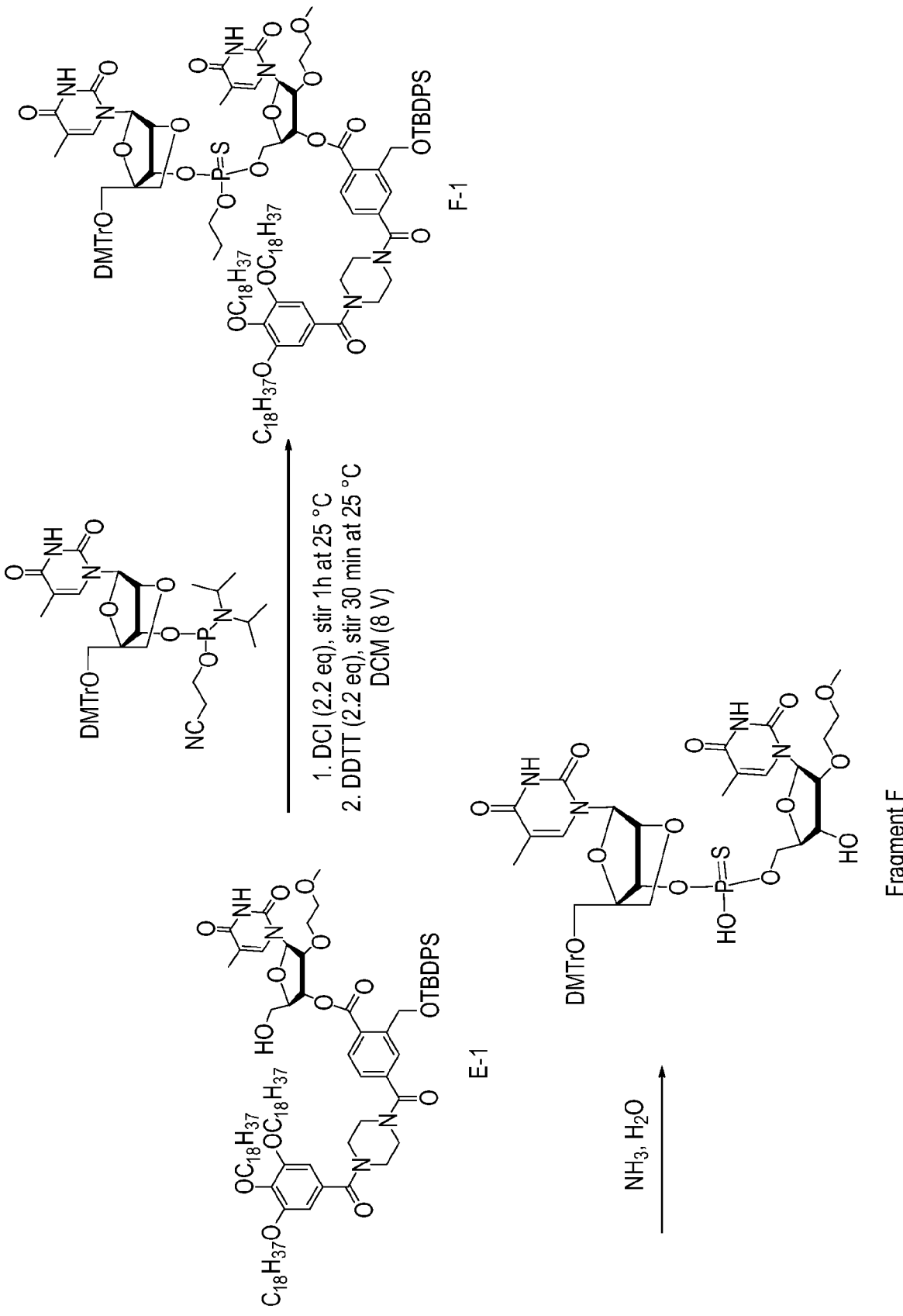

1. DCI (2.2 eq), stir 1h at 25 °C
2. DDTT (2.2 eq), stir 30 min at 25 °C
DCM (8 V)
F-1
E-1
NH_3, H_2O
Fragment F

FIG. 7

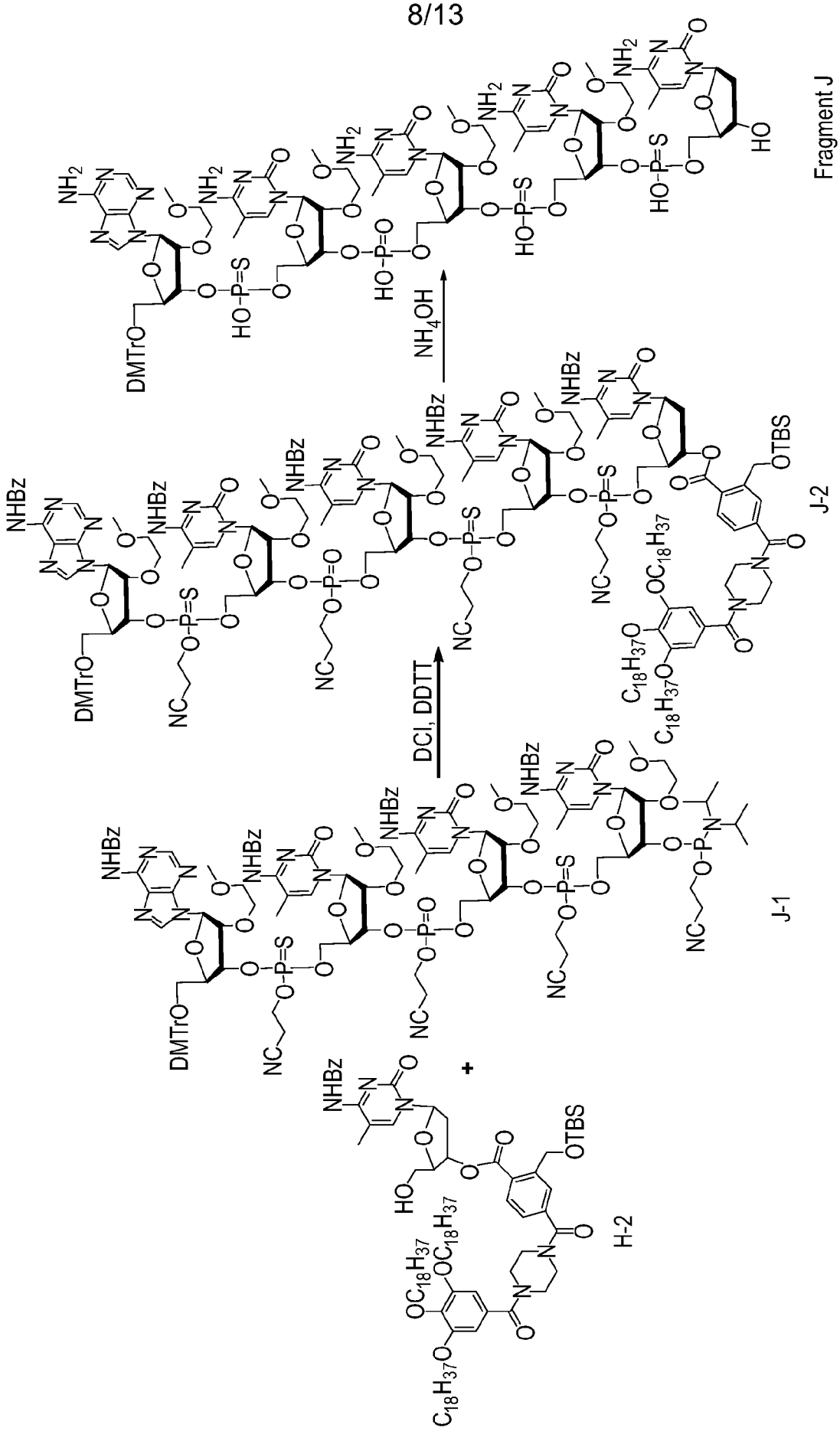

DMTrO
NHBz
NC
O-P=S
O-P=O
OTBS
HO
$OC_{18}H_{37}$
$C_{18}H_{37}O$
DCI, DDTT
NH_4OH
HO-P=S
HO-P=O
Fragment J
J-1
J-2
H-2

FIG. 8

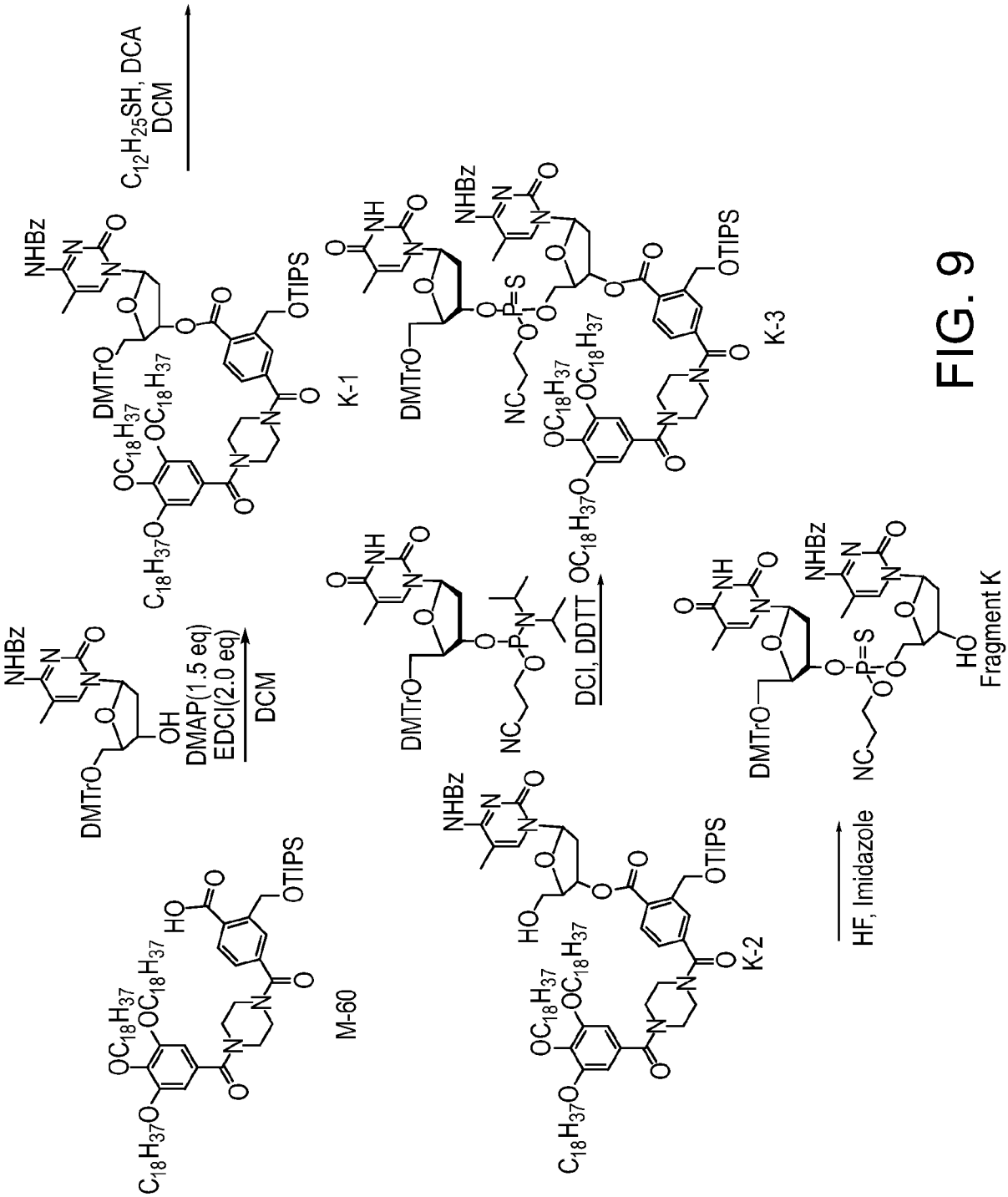

C12H25SH, DCA
DCM
DMAP(1.5 eq)
EDCI(2.0 eq)
DCM
DCI, DDTT
HF, Imidazole
M-60
K-1
K-2
K-3
Fragment K

FIG. 9

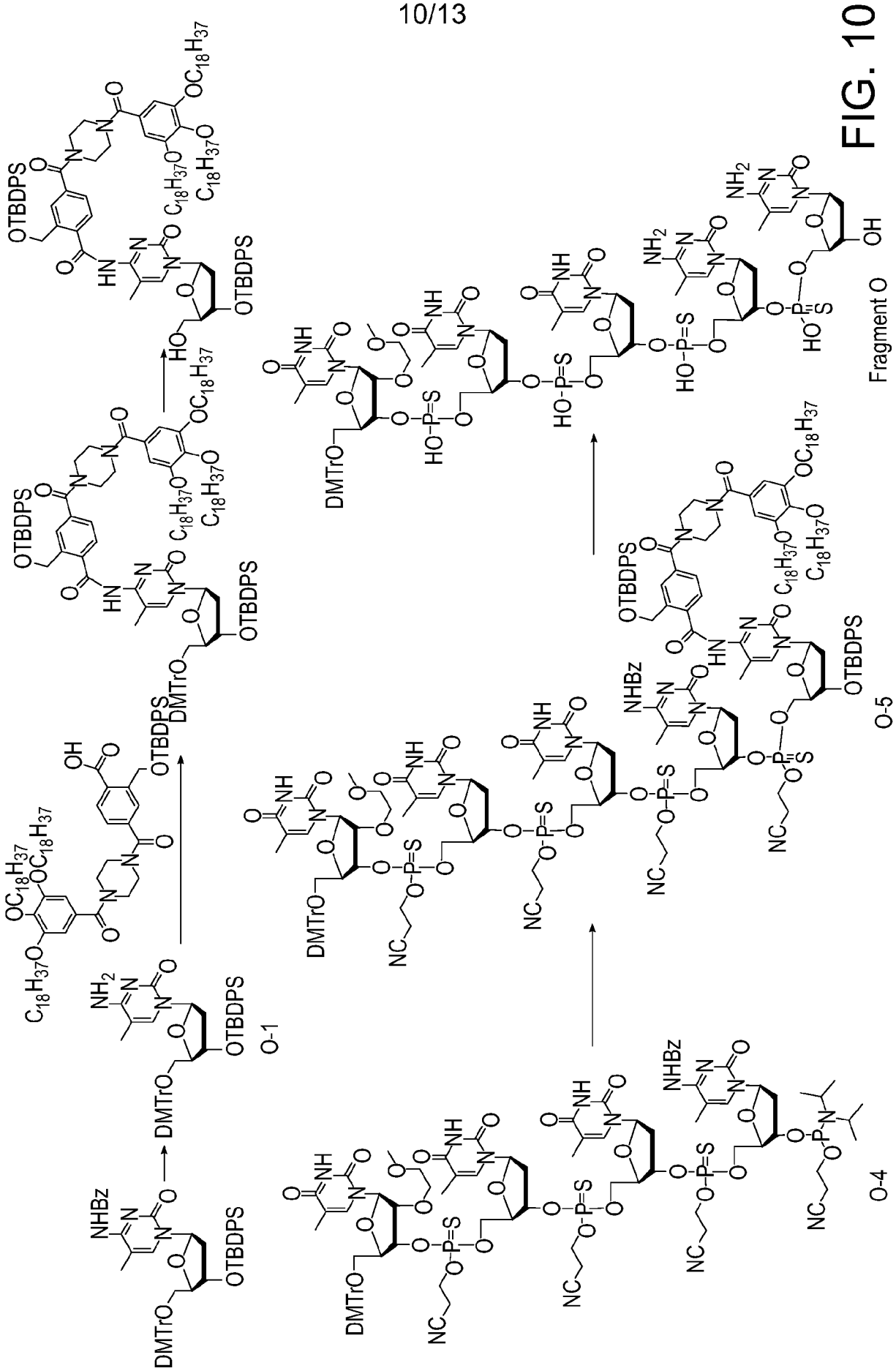


FIG. 10

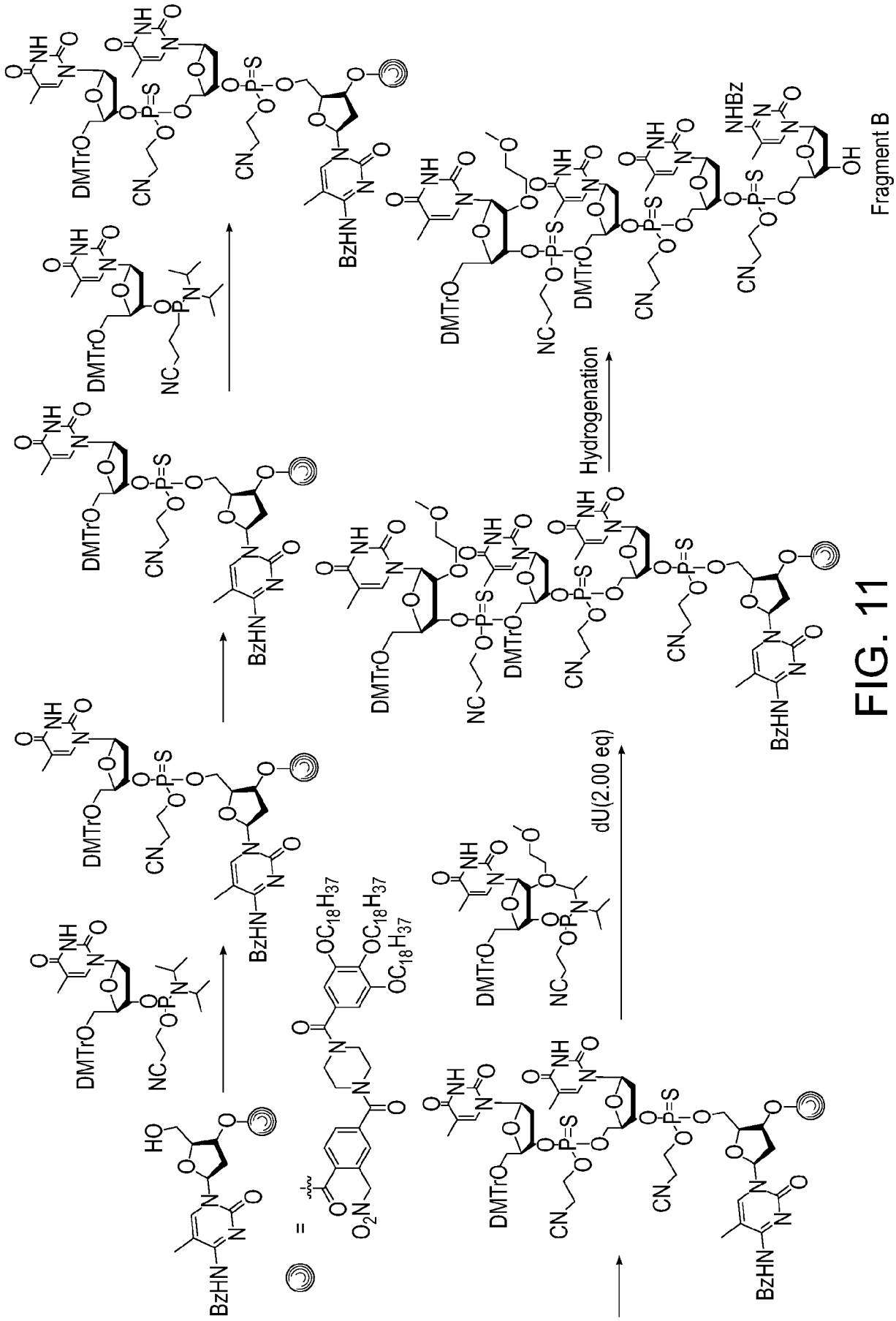


FIG. 11

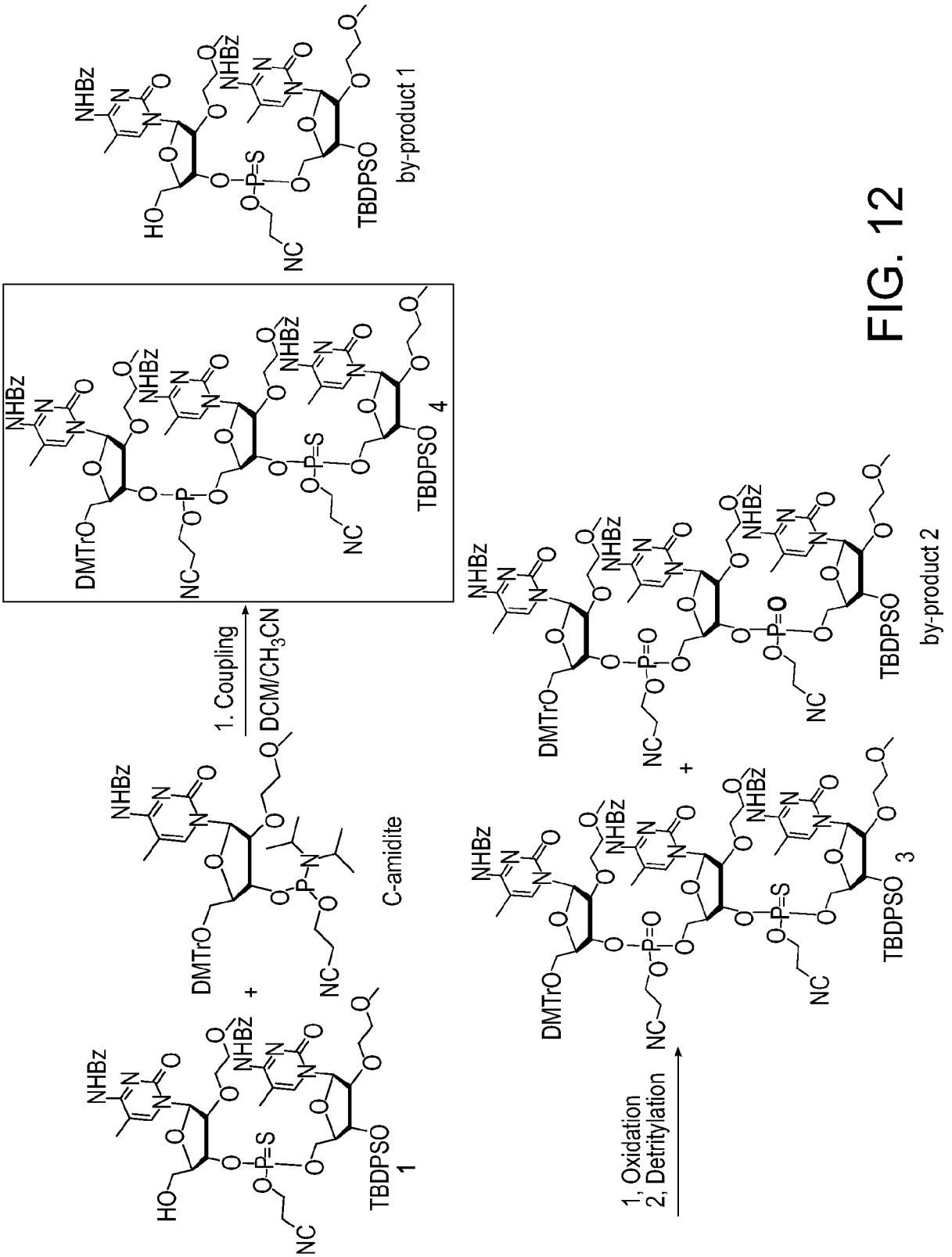

1
C-amidite
1. Coupling
DCM/CH3CN
4
by-product 1
1, Oxidation
2, Detritylation
3
by-product 2

FIG. 12

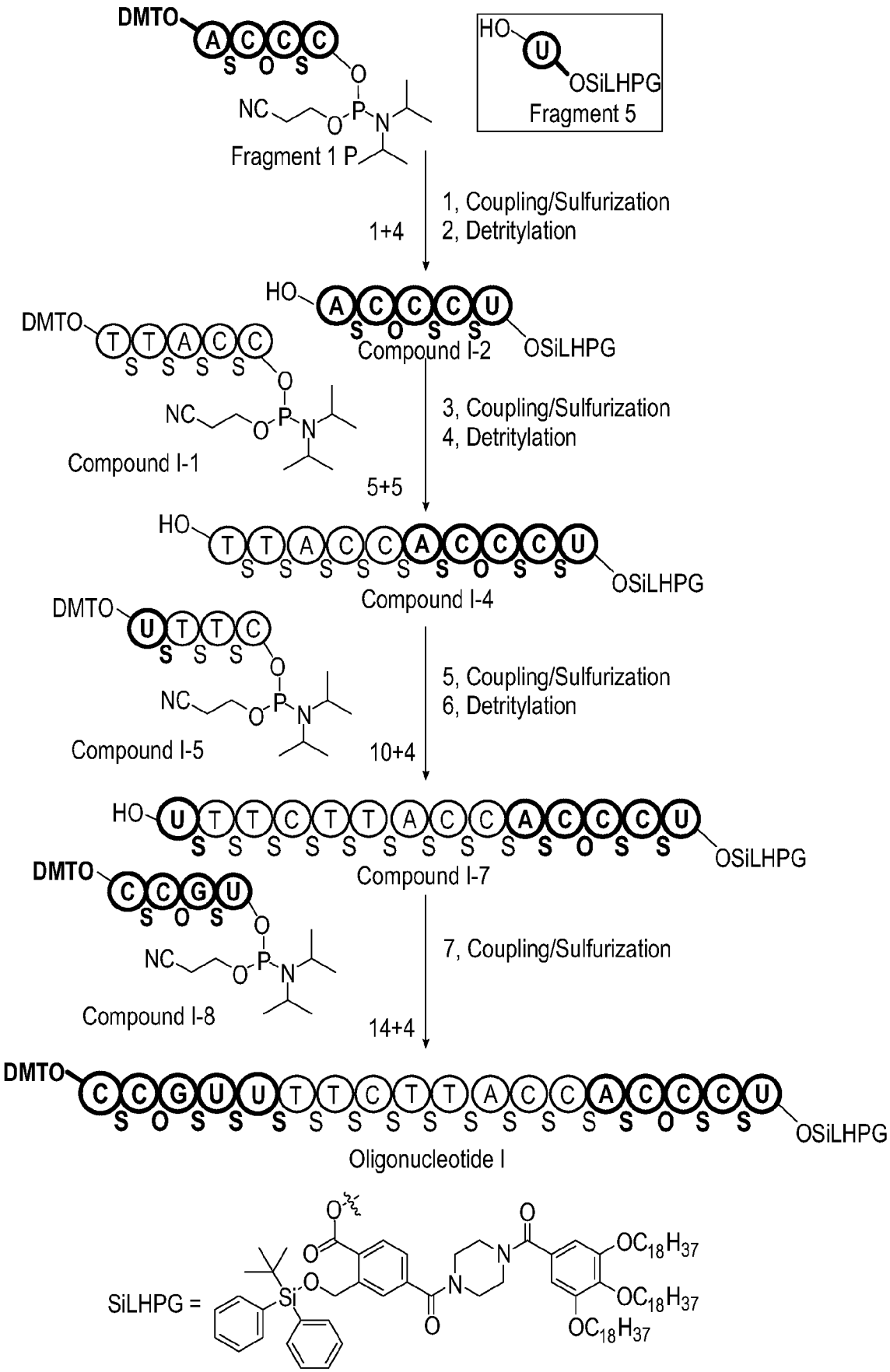

DMTO
A C C C
S O S
O
NC
O P N
Fragment 1 P
HO
U
OSiLHPG
Fragment 5
1, Coupling/Sulfurization
2, Detritylation
1+4
HO
A C C C C U
S O S S
Compound I-2
OSiLHPG
DMTO
T T A C C
S S S S
O
NC
O P N
Compound I-1
3, Coupling/Sulfurization
4, Detritylation
5+5
HO
T T A C C A C C C U
S S S S S S O S S
OSiLHPG
Compound I-4
DMTO
U T T C
S S S
O
NC
O P N
Compound I-5
5, Coupling/Sulfurization
6, Detritylation
10+4
HO
U T T C T T A C C A C C C U
S S S S S S S S S S S O S S
OSiLHPG
Compound I-7
DMTO
C C G U
S O S
O
NC
O P N
Compound I-8
7, Coupling/Sulfurization
14+4
DMTO
C C G U U T T C T T A C C A C C C U
S O S S S S S S S S S S S S S O S S
OSiLHPG
Oligonucleotide I
SiLHPG =
O
O
O
Si
O
N
N
O
O
OC18H37
OC18H37
OC18H37

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/058786

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07H21/00 C07H23/00 C07H1/00 C07D249/06 C07D241/38
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07H C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TYPHAINE GUERLAVAIS-DAGLAND ET AL: "Fluoride-Labile Protecting Groups for the Synthesis of Base-Sensitive Methyl-SATE Oligonucleotide Prodrugs", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, vol. 2003, no. 12, 1 June 2003 (2003-06-01), pages 2327-2335, XP055046163, ISSN: 1434-193X, DOI: 10.1002/ejoc.200300069 cited in the application figure 1 -----	1,2,6-9, 12,19-21
X	CN 110 128 299 B (UNIV ZHEJIANG) 10 November 2020 (2020-11-10) compound 11 -----	1,2,6-9, 19-21

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 April 2022	13/04/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Klein, Didier

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEÓN THIERRY ET AL: "Ni-Catalyzed Direct Carboxylation of Benzyl Halides with CO 2", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 135, no. 4, 30 January 2013 (2013-01-30), pages 1221-1224, XP055906024, ISSN: 0002-7863, DOI: 10.1021/ja311045f Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/ja311045f> compound 2I	1,2,6-8, 19-21
X	CN 107 513 020 B (UNIV NANCHANG) 29 October 2019 (2019-10-29) paragraph [0108] - paragraph [0110]; compounds 1-3,5-7,14	1,2,6, 19-21
X	LEYI GONG ET AL: "Design and synthesis of novel ccr3 antagonists", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 13, no. 20, 20 October 2003 (2003-10-20), pages 3597-3600, XP002562676, ISSN: 0960-894X, DOI: 10.1016/S0960-894X(03)00748-0 [retrieved on 2003-08-27] Scheme 3 first compound	1,2,6, 19-21
X	WO 2018/138359 A1 (GENFIT [FR]) 2 August 2018 (2018-08-02) Figures 1AC, 1AK,1AL	1,2,6, 19-21
X	EP 1 479 666 A1 (JAPAN TOBACCO INC [JP]) 24 November 2004 (2004-11-24) paragraph [0494]	1,2,6, 19-21
X	BURGESS K ET AL: "Rapid and Efficient Solid Phase Syntheses of Cyclic Peptides with Endocyclic Biaryl Ether Bonds", TETRAHEDRON LETTERS, ELSEVIER, AMSTERDAM , NL, vol. 38, no. 19, 12 May 1997 (1997-05-12), pages 3345-3348, XP004061422, ISSN: 0040-4039, DOI: 10.1016/S0040-4039(97)00654-0 compound 4	1,2,6, 19-21

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2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Laïb Taoues ET AL: "Synthesis of Model of Phomopsin-Ustiloxin-Type Antimitotic Agents", Synlett, 1 January 2000 (2000-01-01), pages 1363-1365, XP055905495, DOI: 10.1055/s-2000-7137 Retrieved from the Internet: URL:https://www.thieme-connect.de/products/ejournals/pdf/10.1055/s-2000-7137.pdf [retrieved on 2022-03-25] compound 3	1,2,6, 19-21
X	HU ET AL: "Carboxylic acid based quinolines as liver X receptor modulators that have LXR@b receptor binding selectivity", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 18, no. 1, 9 November 2007 (2007-11-09), pages 54-59, XP022410856, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2007.11.013 compounds 13,14	1,2,6, 19-21
X	WO 00/49020 A2 (SCHERING AG [DE]; KLAR ULRICH [DE] ET AL.) 24 August 2000 (2000-08-24) example 1f	1,2,6-8, 12,19-21
X	EVANO GWILHERM ET AL: "A Convergent Synthesis of the Macrocyclic Core of Cytotrienins:? Application of RCM for Macrocyclization", ORGANIC LETTERS, vol. 6, no. 4, 1 February 2004 (2004-02-01), pages 525-528, XP055906637, US ISSN: 1523-7060, DOI: 10.1021/ol036284k Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/ol036284k> compound 20 -->10 step d)	1,3,4,12

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MORI SHUNJI ET AL: "Synthesis of a Glandular Secretion of the Civet Cat, (2 S ,6 S)-(6-Methyltetrahydropyran-2-yl)acetic Acid and Its Enantiomer, by Using the Yeast-Reduction Product and Recovered Substrate from Yeast Reduction", BIOSCIENCE, BIOTECHNOLOGY, AND BIOCHEMISTRY, vol. 70, no. 3, 1 January 2006 (2006-01-01), pages 712-717, XP055906398, JP ISSN: 0916-8451, DOI: 10.1271/bbb.70.712 Retrieved from the Internet: URL:https://watermark.silverchair.com/bbb0712.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAtMwggLPBgkqhkiG9w0BBwagggLAMIICvAIBADCCArUGCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMyoQ2kj41xY8-w83rAgEQgIIChgmcM7x3hu7VkzrTi8q8-vnjvCaeyEkO3vhAHn65XNNM4akafj0whC8HbPQa2zIC5sPZp9fQm43MX7JCWjX7bljXXar2> compound 6	1,3,12

X	DUTHALER RUDOLF O.: "Construction of Highly Substituted Nitroaromatic Systems by Cyclocondensation. Part I. Synthesis of 4-nitro-3-oxobutyrate", HELVETICA CHIMICA ACTA, vol. 66, no. 5, 27 July 1983 (1983-07-27), pages 1475-1492, XP055906571, Hoboken, USA ISSN: 0018-019X, DOI: 10.1002/hlca.19830660516 compound 23	1,3

X	US 2 694 635 A (ILMARI SALMINEN ET AL) 16 November 1954 (1954-11-16) compound IV	1,2,5,6, 19-21

X	US 5 969 148 A (VALENTE RONALD R [US] ET AL) 19 October 1999 (1999-10-19) column 14 - column 15; compounds NAS-2,9	1,2,5,6, 19-21

X	CONNOLLY S ET AL: "Design and Synthesis of a Novel and Potent Series of Inhibitors of Cytosolic Phospholipase A2 Based on a 1,3-Disubstituted Propan -2-one Skeleton", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 45, 2 August 2002 (2002-08-02), pages 1348-1362, XP002287473, ISSN: 0022-2623, DOI: 10.1021/JM011050X table 4; compound 28	1,2,5,6, 19-21

	-/--	

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TOBLER ERNST ET AL: "On-column deracemization of an atropisomeric biphenyl by quinine-based stationary phase and determination of rotational energy barrier by enantioselective stopped-flow HPLC and CEC : On-Column Deracemization of a Chiral Biphenyl", CHIRALITY., vol. 13, no. 10, 1 January 2001 (2001-01-01), pages 641-647, XP055907787, US ISSN: 0899-0042, DOI: 10.1002/chir.10015 compound 1	1,2,5,6, 10, 13-16, 19-21
X	GB 1 335 603 A (AGFA GEVAERT AG) 31 October 1973 (1973-10-31) coupler 3	1,2,5,6, 19-21
X	CN 101 875 617 B (INST MATERIA MEDICA CAMS) 20 May 2015 (2015-05-20) 8th -10th compound; page 5	1,2,5,6, 10, 13-17, 19-21
X	WO 2019/180683 A1 (TAKEDA PHARMACEUTICALS CO [JP]) 26 September 2019 (2019-09-26) Example 26, PNB and PNB protected compound	1,2,6, 27,29, 33, 46-48, 120-125
X	QUINN JORDAN R. ET AL: "Structure-Function Studies on a Synthetic Guanosine Receptor That Simultaneously Binds Watson-Crick and Hoogsteen Sites", THE JOURNAL OF ORGANIC CHEMISTRY, vol. 70, no. 19, 1 September 2005 (2005-09-01), pages 7459-7467, XP055906176, ISSN: 0022-3263, DOI: 10.1021/jo0501689 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/jo0501689> compounds 18,19,21,22	1,2,6, 19-21, 28,29, 33,123, 124,128, 130,131

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JAVAID SUMAIRA ET AL: "Thymidine esters as substrate analogue inhibitors of angiogenic enzyme thymidine phosphorylasein vitro", BIOORGANIC CHEMISTRY, ACADEMIC PRESS INC., NEW YORK, NY, US, vol. 70, 23 November 2016 (2016-11-23), pages 44-56, XP029957265, ISSN: 0045-2068, DOI: 10.1016/J.BIOORG.2016.11.007 compound 16	27,29, 33,120, 121,123, 124
X	WO 2019/147050 A1 (ST PHARM CO LTD) 1 August 2019 (2019-08-01) example 30 & EP 3 744 726 A1 (ST PHARM CO LTD [KR]) 2 December 2020 (2020-12-02) example 30	1,2,28, 29
X	EP 3 398 955 A1 (AJINOMOTO KK [JP]) 7 November 2018 (2018-11-07) the whole document	27-131
X	EP 2 816 053 A1 (AJINOMOTO KK [JP]) 24 December 2014 (2014-12-24) the whole document	27-131
X	SHOKAKU KIM ET AL: "Liquid-Phase RNA Synthesis by Using Alkyl-Chain-Soluble Support", CHEMISTRY - A EUROPEAN JOURNAL, vol. 19, no. 26, 24 June 2013 (2013-06-24) , pages 8615-8620, XP055211240, ISSN: 0947-6539, DOI: 10.1002/chem.201300655 the whole document	27-131
X	EP 3 378 869 A1 (NISSAN CHEMICAL CORP [JP]) 26 September 2018 (2018-09-26) the whole document	27-131
X	WO 2020/017085 A1 (FUJIMOTO CHEMICALS CO LTD [JP]) 23 January 2020 (2020-01-23) the whole document & EP 3 825 300 A1 (FUJIMOTO CHEMICALS CO LTD [JP]) 26 May 2021 (2021-05-26) the whole document	27-131
1 X	JP 2012 111728 A (NOKODAI TLO KK; HOKKAIDO SYSTEM SCIENCE CO LTD) 14 June 2012 (2012-06-14) the whole document	27-131
2		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/058786

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-17, 19-21(all partially)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1-17, 19-21(all partially)

So many documents were retrieved that it is impossible to determine which parts of the claims 1-17,19-21 may be said to define subject-matter for which protection might legitimately be sought (Article 6 PCT). For these reasons, the search was performed taking into consideration the non-compliance in determining the extent of the search of claim 1-17,19-21.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/058786

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 110128299 B	10-11-2020	NONE	
CN 107513020 B	29-10-2019	NONE	
WO 2018138359 A1	02-08-2018	CA 3050056 A1	02-08-2018
		CN 110234643 A	13-09-2019
		EP 3573972 A1	04-12-2019
		JP 2020505410 A	20-02-2020
		US 2020000815 A1	02-01-2020
		WO 2018138359 A1	02-08-2018
EP 1479666 A1	24-11-2004	AU 2003211617 A1	09-09-2003
		BR 0306292 A	24-08-2004
		CA 2460682 A1	04-09-2003
		CN 1630629 A	22-06-2005
		CN 1943786 A	11-04-2007
		CN 101838218 A	22-09-2010
		CO 5570695 A2	31-10-2005
		EP 1479666 A1	24-11-2004
		JP 2010043098 A	25-02-2010
		KR 20040077852 A	07-09-2004
		KR 20060053023 A	19-05-2006
		MX PA04002602 A	11-08-2004
		NO 329178 B1	06-09-2010
		NZ 531890 A	24-02-2006
		RU 2293721 C2	20-02-2007
		SG 165154 A1	28-10-2010
		US 2005075367 A1	07-04-2005
		US 2010158996 A1	24-06-2010
		WO 03072532 A1	04-09-2003
		ZA 200402275 B	23-04-2005
		ZA 200502495 B	20-09-2005
		ZA 200502496 B	12-10-2005
WO 0049020 A2	24-08-2000	AU 3156600 A	04-09-2000
		DE 19908763 A1	24-08-2000
		WO 0049020 A2	24-08-2000
US 2694635 A	16-11-1954	NONE	
US 5969148 A	19-10-1999	JP 2000171953 A	23-06-2000
		US 5969148 A	19-10-1999
		US 6008369 A	28-12-1999
GB 1335603 A	31-10-1973	BE 769116 A	28-12-1971
		DE 2032171 A1	13-01-1972
		FR 2100089 A5	17-03-1972
		GB 1335603 A	31-10-1973
CN 101875617 B	20-05-2015	NONE	
WO 2019180683 A1	26-09-2019	EP 3768685 A1	27-01-2021
		JP 2021518400 A	02-08-2021
		US 2021015915 A1	21-01-2021
		WO 2019180683 A1	26-09-2019
WO 2019147050 A1	01-08-2019	CN 111902419 A	06-11-2020
		EP 3744726 A1	02-12-2020

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/058786

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		JP 2021511354 A	06-05-2021
		KR 20190090301 A	01-08-2019
		US 2021040135 A1	11-02-2021
		WO 2019147050 A1	01-08-2019
EP 3398955 A1	07-11-2018	CN 108473526 A	31-08-2018
		EP 3398955 A1	07-11-2018
		JP 2022009923 A	14-01-2022
		JP WO2017104836 A1	11-10-2018
		US 2018282365 A1	04-10-2018
		WO 2017104836 A1	22-06-2017
EP 2816053 A1	24-12-2014	CN 104114569 A	22-10-2014
		CN 106977567 A	25-07-2017
		EP 2816053 A1	24-12-2014
		EP 3296306 A1	21-03-2018
		JP 6237237 B2	29-11-2017
		JP WO2013122236 A1	18-05-2015
		WO 2013122236 A1	22-08-2013
EP 3378869 A1	26-09-2018	CN 108350018 A	31-07-2018
		EP 3378869 A1	26-09-2018
		JP 6950529 B2	13-10-2021
		JP 2022000444 A	04-01-2022
		JP WO2017086397 A1	06-09-2018
		TW 201731861 A	16-09-2017
		US 2019169223 A1	06-06-2019
		WO 2017086397 A1	26-05-2017
WO 2020017085 A1	23-01-2020	CN 112533892 A	19-03-2021
		EP 3825300 A1	26-05-2021
		JP 6770553 B2	14-10-2020
		JP 2020011932 A	23-01-2020
		KR 20210032478 A	24-03-2021
		US 2021317159 A1	14-10-2021
		WO 2020017085 A1	23-01-2020
JP 2012111728 A	14-06-2012	JP 5705512 B2	22-04-2015
		JP 2012111728 A	14-06-2012