WHAT IS CLAIMED IS:CLAIMS

1. A method of linearizing a plurality of immobilized double-stranded polynucleotides, comprising:

providing a solid support comprising double-stranded polynucleotides, each double-stranded polynucleotide comprises a first strand and a second strand, wherein the first strand and the second strand are immobilized to the solid support at their 5′ ends, and wherein each first strand comprises a first cleavage site capable of undergoing chemical cleavage by a transition metal complex, wherein the transition metal complex is a palladium complex or a nickel complex;

contacting the double-stranded polynucleotides with an aqueous solution of the transition metal complex, thereby cleaving one or more first strands at the first cleavage site, and generating one or more cleaved first nucleic acids and cleaved immobilized first strands; and

removing the cleaved first nucleic acids from the solid support.

2. The method of claim 1, wherein each first strand is extended from a first extension primer immobilized to the solid support.

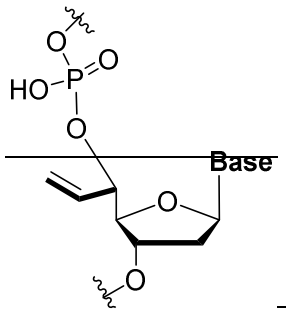
3. The method of claim 2, wherein the first extension primer comprises the first cleavage site.

4. The method of claim 2 or 3, wherein the first extension primer comprises a P5 sequence or a modified P5 sequence.

5. The method of any one of claims 1 to 4, wherein the first cleavage site comprises a modified nucleoside or nucleotide moiety that is capable of undergoing chemical cleavage by the transition metal complex.

6. The method of claim 5, wherein the modified nucleoside or nucleotide moiety comprises an allyl group.

7. The method of claim 5 or 6, wherein the first cleavage site comprises the modified nucleoside or nucleotide moiety having a structure of Formula (II´):



(II´)

wherein Base is adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof.

8. The method of any one of claims 5 to 7, wherein the modified nucleoside or nucleotide moiety has a thymine base.

9. The method of any one of claims 1 to 8, wherein the transition metal complex is a palladium (0) complex.

10. The method of claim 9, wherein the palladium (0) complex is $Pd(THP)_2$, $Pd(THP)_4$, $Pd(THM)_4$, or combinations thereof.

11. The method of claim 9, wherein the palladium (0) complex is generated *in situ*.

12. The method of claim 11, wherein the palladium (0) complex is generated by mixing one or more palladium (II) compounds with tris(hydroxypropyl)phosphine (THP), and wherein the palladium (II) compound is selected from the group consisting of Na_2PdCl_4, $(PdAllylCl)_2$, [PdAllyl(THP)]Cl, and $[PdAllyl(THP)_2]Cl$.

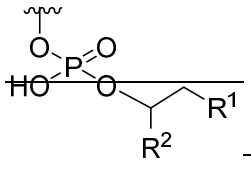
13. The method of claim 11, wherein the palladium (0) complex is generated by mixing $Pd(PPh_3)_4$ with tris(hydroxypropyl)phosphine.

14. The method of any one of claims 1 to 8, wherein the transition metal complex is a nickel (0) complex.

15. The method of claim 14, wherein the nickel (0) complex is generated *in situ* from a nickel (II) compound.

16. The method of any one of claims 1 to 15, wherein the 3′ end of each cleaved immobilized first strands comprises a protecting group.

17. The method of claim 16, wherein the protecting group is a 3′ end phosphate moiety having a structure of Formula (I):



(I)

wherein:

R^1 is NH_2, OH, $NHC(O)OR^a$ or $OCH_2OSi(R^b)_3$;

R^a is C_{1-4} alkyl, tert-butyl, allyl, benzyl, or 9-fluorenylmethyl;

each R^b is independently selected from the group consisting of C_{1-4}alkyl and phenyl; and

R^2 is H, C_{1-4} alkyl, an optionally substituted tetrahydrofuran, or a nucleotide.

18. The method of any one of claims 1 to 17, wherein each second strand is extended from a second extension primer immobilized to the solid support, and each second strand comprises a second cleavage site.

19. The method of claim 18, wherein the second extension primer comprises the second cleavage site.

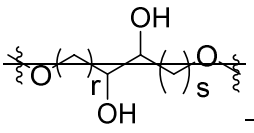
20. The method of claim 18 or 19, wherein the second cleavage site is not capable of undergoing chemical cleavage by the palladium complex or the nickel complex.

21. The method of claim 18 to 20, wherein the second extension primer comprises a P7 nucleotide sequence or a modified P7 nucleotide sequence or a P17 nucleotide sequence.

22. The method of any one of claims 18 to 21, wherein the second cleavage site is cleaved by a method selected from the group consisting of chemical cleavage, photo cleavage, enzymatic cleavage, and a combination thereof.

23. The method of claim 22, wherein the second cleavage site is cleaved by a chemical cleavage and wherein the second cleavage site comprises a diol linker or an azobenzene linker.

24. The method of claim 23, wherein the diol linker comprises a structure of Formula (VIII):.



(VIII)

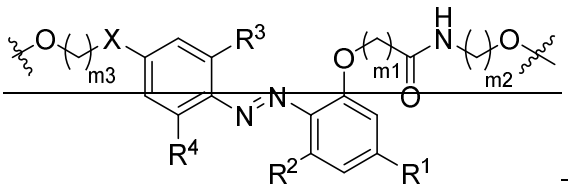
wherein

r is 2, 3, 4, 5, or 6; and

s is 2, 3, 4, 5, or 6.

25. The method of claim 24, wherein the diol linker may be cleaved by a periodate salt.

26. The method of claim 23, wherein the azobenzene linker comprises a structure of Formula (X):



(X)

wherein

R^1 is H, hydroxyl, or a protected hydroxyl;

R^2 is H, C_{1-6} alkyl, or C_{1-6} alkoxy;

each R^3 and R^4 is independently H, halo, $-C(O)OR^5$, or $-C(O)NHR^6$;

each R^5 and R^6 is independently H, C_{1-6} alkyl, or C_{6-10} aryl;

X is $-C(O)-$, $-CH_2-$, or $-C(O)NH-$; and

each m1, m2 and m3 is independently 1, 2, 3, 4, 5, or 6.

27. The method of claim 26, wherein the azobenzene linker may be cleaved by $Na_2S_2O_4$.

28. The method of claim 22, wherein the second cleavage site is cleaved by an enzymatic cleavage.

29. The method of any of claims 1 to 28, wherein the double-stranded polynucleotides are immobilized to the solid support through covalent bonding.

30. A method of linearizing a plurality of immobilized double-stranded polynucleotides, comprising:

providing a solid support comprising double-stranded polynucleotides, each double-stranded polynucleotide comprises a first strand and a second strand, wherein the first strand and the second strand are immobilized to the solid support at their 5′ ends, wherein each first strand comprises a first cleavage site, and wherein each second strand comprises a second cleavage site comprising an azobenzene linker;

cleaving one or more first strands at the first cleavage site, and generating one or more cleaved first nucleic acids and cleaved immobilized first strands;

removing the cleaved first nucleic acids from the solid support;

sequencing the immobilized second strands;

resynthesizing derivative first strands that are complementary to the second strands; and

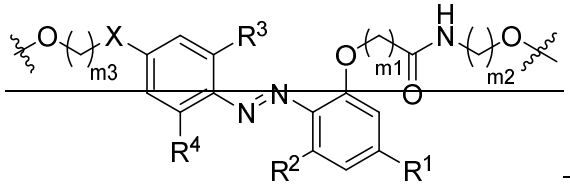
cleaving one or more second strands at the second cleavage site, and generating one or more cleaved second nucleic acids and cleaved immobilized second strands.

31. The method of claim 30, wherein each first strand is extended from a first extension primer immobilized to the solid support, and wherein the first extension primer comprises the first cleavage site.

32. The method of claim 30 or 31, wherein the first cleavage site is cleaved by a method selected from the group consisting of chemical cleavage, photo cleavage, enzymatic cleavage, and a combination thereof.

33. The method of any one of claims 30 to 32, wherein each second strand is extended from a second extension primer immobilized to the solid support, and wherein the second extension primer comprises the second cleavage site.

34. The method of any one of claims 30 to 33, wherein the azobenzene linker comprises a structure of Formula (X):



(X)

wherein

R^1 is H, hydroxyl, or a protected hydroxyl;

R^2 is H, C_{1-6} alkyl, or C_{1-6} alkoxy;

each R^3 and R^4 is independently H, halo, $-C(O)OR^5$, or $-C(O)NHR^6$;

each R^5 and R^6 is independently H, C_{1-6} alkyl, or C_{6-10} aryl;

X is $-C(O)-$, $-CH_2-$, or $-C(O)NH-$; and

each m1, m2 and m3 is independently 1, 2, 3, 4, 5, or 6.

35. The method of any one of claims 30 to 34, wherein the azobenzene linker is cleaved by $Na_2S_2O_4$.

36. The method of any one of claims 30 to 35, wherein the sequencing of the immobilized second strands comprises successively incorporating labeled nucleotides complementary to the immobilized second strands and detecting the labeled nucleotides.

37. The method of any one of claims 30 to 36, wherein the 3′ end of each cleaved immobilized first strands comprises a protecting group.

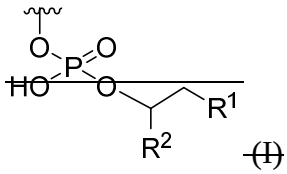
38. The method of claim 37, wherein the protecting group of the 3′ end cleaved immobilized first strands is deprotected prior to resynthesizing the derivative first strands.

39. The method of any one of claims 30 to 38, further comprising removing the cleaved second nucleic acids from the solid support, and sequencing the derivative first strands.

40. A method of removing a 3′end protecting group from an oligonucleotide, comprising:

providing a solid support comprising a plurality of oligonucleotides immobilized thereon at their 5′ ends, wherein the oligonucleotides each comprises a 3′ end protecting group having a structure of Formula (I); and

contacting the oligonucleotides with a deprotecting reagent, thereby cleaving the 3′ end protecting group to produce oligonucleotides each with a free 3′ end hydroxyl group;



(I)

wherein

R^1 is $-NH_2$, $-OH$, $-NHC(O)OR^a$ or $-OCH_2OSi(R^b)_3$;

R^a is C_{1-4}alkyl, tert-butyl, allyl, benzyl, or 9-fluorenylmethyl;

each R^b is independently selected from the group consisting of C_{1-4} alkyl and phenyl; and

R^2 is H, C_{1-4} alkyl, an optionally substituted tetrahydrofuran, or a nucleotide.

41. The method of claim 40, wherein R^1 is $-OCH_2OSi(R^b)_3$.

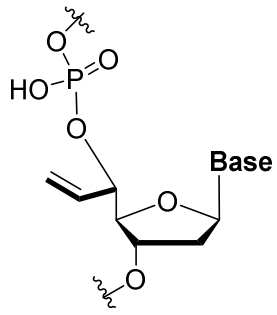
42. The method of claim 41, wherein each R^b is isopropyl.

43. The method of any one of claims 40 to 42, wherein R^2 is a nucleotide.

~~44. The method of any one of claims 40 to 43, wherein the deprotecting reagent comprises a fluoride ion or a base.~~

~~45. The method of claim 44, wherein the deprotecting reagent is selected from the group consisting of tetra-n-butylammonium fluoride (TBAF), HF, NH_4F, CsF, NaOH, and KOH, and combination thereof.~~

~~46.~~1. A solid support comprising a plurality of ~~first strand~~ double-stranded polynucleotides, each double-stranded polynucleotide comprises a first strand and a second strand immobilized ~~there~~on the solid support, each first strand ~~polynucleotide~~ comprises a first cleavage site capable of undergoing chemical cleavage by a transition metal complex; wherein the transition metal complex is a palladium complex or a nickel complex, ~~and~~ wherein the ~~plurality of~~ first strand ~~polynucleotides~~ and second strand are immobilized to the solid support at their 5′ ends~~.~~, and wherein the first cleavage site comprises the modified nucleoside or nucleotide having a structure of Formula (II´):



(II′)

wherein Base is adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof.

~~47.~~2. The solid support of claim ~~46~~ 1, wherein each first strand ~~polynucleotide~~ comprises a first extension primer immobilized to the solid support.

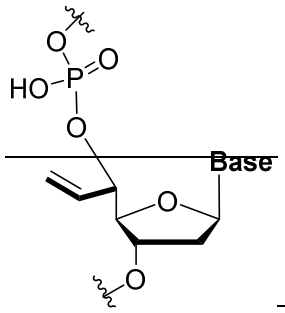
~~48.~~3. The solid support of claim ~~47~~ 2, wherein the first extension primer comprises the first cleavage site.

~~49.~~4. The solid support of claim ~~47 or 48~~ 2 or 3, wherein the first extension primer comprises a P5 sequence ~~or a modified P5 sequence~~ having a sequence of SEQ ID NO. 1 or 3.

~~50.~~5. The solid support of any one of claims ~~46 to 49~~ 1 to 4, wherein the first cleavage site comprises a modified nucleoside or nucleotide that is capable of undergoing chemical cleavage by the palladium complex or the nickel complex.

~~51.~~6. The solid support of claim ~~50~~ 5, wherein the modified nucleoside or nucleotide comprises an allyl group.

~~52. The solid support of claim 51, wherein the first cleavage site comprises the modified nucleoside or nucleotide having a structure of Formula (II´):~~



~~(II')~~

~~wherein Base is adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof.~~

~~53. The solid support of any one of claim 50 to 52, wherein the modified nucleoside or nucleotide comprises a thymine base.~~

~~54. The solid support of any one of claims 50 to 53, wherein the first extension primer comprises a P15 sequence.~~

~~55. The solid support of any one of claims 46 to 54, wherein the palladium complex is a palladium (0) complex.~~

~~56. The solid support of claim 55, wherein the palladium complex is generated *in situ* by mixing one or more palladium (II) compounds with tris(hydroxypropyl)phosphine (THP), and wherein the palladium (II) compound is selected from the group consisting of Na_2PdCl_4, $(PdAllylCl)_2$, [PdAllyl(THP)]Cl, and $[PdAllyl(THP)_2]Cl$.~~

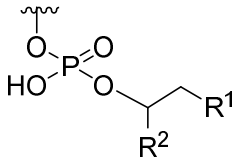
~~57. The solid support of claim 55, wherein, the palladium (0) complex is generated by mixing $Pd(PPh_3)_4$ with tris(hydroxypropyl)phosphine.~~

~~58. The solid support of any one of claims 46 to 54, wherein the transition metal complex is a nickel (0) complex.~~

~~59. The solid support of claim 58, wherein the nickel (0) complex is generated *in situ* from a nickel (II) compound.~~

~~60. The solid support of any one of claims 46 to 59, wherein the first cleavage site generates a 3′ blocking moiety after chemical cleavage.~~

~~61.~~7. ~~The~~ A solid support ~~of claim 60, wherein the~~ comprising a 3′ blocking moiety comprises a phosphate moiety having a structure of Formula (I):



(I)

wherein

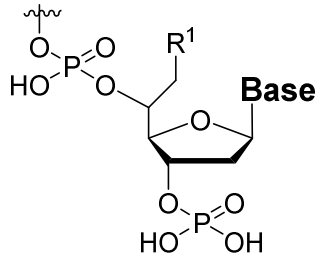
R^1 is $–NH_2$, –OH, $–NHC(O)OR^a$ or $–OCH_2OSi(R^b)_3$;

R^a is $C_{1\text{-}4}$alkyl, tert-butyl, allyl, benzyl group, or 9-fluorenylmethyl;

each R^b is independently selected from the group consisting of C_{1-4} alkyl and phenyl; and

R^2 is H, C_{1-4} alkyl, ~~an optionally substituted~~ tetrahydrofuran, or a nucleotide~~.~~;

~~62. The solid support of claim 61, wherein the structure of Formula (I) is also represented by~~ or of Formula (V):



(V)

wherein R^1 is $–NH_2$, –OH, $–NHC(O)OR^a$ or $–OCH_2OSi(R^b)_3$;

R^a is C_{1-4}alkyl, tert-butyl, allyl, benzyl group, or 9-fluorenylmethyl;

each R^b is independently selected from the group consisting of C_{1-4} alkyl and phenyl; and

~~wherein~~ Base is ~~an optionally protected~~ adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof, wherein the solid support is generated by cleaving the first cleavage site of the solid support of any one of claims 1 to 6.

8. The solid support of claim 7, wherein tetrahydrofuran in R^2 in Formula (I) substituted.

9. The solid support of claim 7, wherein Base in Formula (V) is protected.

~~63. The solid support of claim 61 or 62, wherein R^1 is $–OCH_2OSi(R^b)_3$.~~

~~64. The solid support of claim 63, wherein R^b is isopropyl.~~

~~65. The solid support of any one of claims 60 to 64, wherein the 3′ blocking moiety may be removed in an enzymatic reaction or a chemical reaction.~~

~~66.~~10. The solid support of any one of claims ~~46 to 65~~ 7 to 9, ~~further~~ comprising a ~~plurality of~~ second strand according to any one of claims 1 to 9 ~~polynucleotides~~ immobilized thereon~~,~~ wherein each second strand ~~polynucleotide~~ comprises a second cleavage site~~, wherein the plurality of second strand polynucleotides are immobilized to the solid support at their 5′ ends~~.

~~67.~~11. The solid support of claim ~~66~~ 10, wherein ~~each~~ the second strand ~~polynucleotide~~ comprises a second extension primer immobilized to the solid support.

~~68.~~12. The solid support of claim ~~67~~ 11, wherein the second extension primer comprises the second cleavage site.

~~69.~~13. The solid support of any one of claims ~~66 to 68~~ 10 to 12, wherein the second cleavage site is not capable of undergoing chemical cleavage by the palladium complex or the nickel complex.

~~70.~~14. The solid support of any one of claims ~~66 to 69~~ 10 to 12, wherein the second cleavage site ~~may be~~ had been cleaved by a ~~method selected from the group consisting of~~ chemical cleavage~~, photo cleavage, enzymatic cleavage, and a combination thereof~~.

~~71.~~15. The solid support of ~~claim 70~~any one of claims 10 to 12, ~~wherein the second cleavage site is cleaved by a chemical cleavage and wherein~~ the second cleavage site comprises a diol linker or an azobenzene linker.

16. A method of linearizing a plurality of double-stranded polynucleotides which are immobilized on a solid support, comprising:

providing the solid support of any one of claims 1-6

contacting the double-stranded polynucleotides with an aqueous solution of the transition metal complex, thereby cleaving one or more first strands at the first cleavage site, and generating one or more cleaved first nucleic acids and cleaved immobilized first strands; and removing the cleaved first nucleic acids from the solid support.

17. The method of claim 16, wherein the transition metal complex is a palladium (0) or a nickel (0) complex.

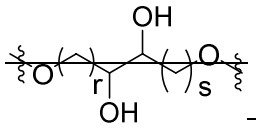
18. The method of claim 17, wherein the palladium (0) complex is $Pd(THP)_2$, $Pd(THP)_4$, $Pd(THM)_4$, or combinations thereof.

19. The method of claim 17 or 18, wherein the palladium (0) complex is generated in situ.

20. The method of claim 17, wherein the nickel (0) complex is generated in situ from a nickel (II) compound.

21. The solid support of any one of claims 10 to 13 and 15, wherein the second cleavage site is cleaved by a method selected from the group consisting of chemical cleavage, photo cleavage, enzymatic cleavage, and a combination thereof.

~~72. The solid support of claim 71, wherein the diol linker comprises a structure of Formula (VIII):~~



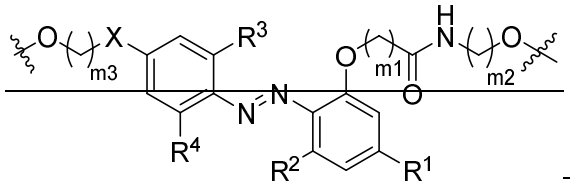
~~(VIII)~~

~~wherein~~

~~r is 2, 3, 4, 5, or 6; and~~

~~s is 2, 3, 4, 5, or 6.~~

~~73. The solid support of claim 71, wherein the azobenzene linker comprises a structure of Formula (X):~~



~~(X)~~

~~wherein~~

~~R^1 is H, hydroxyl, or a protected hydroxyl;~~

~~R^2 is H, C_{1-6} alkyl, or C_{1-6} alkoxy;~~

~~each R^3 and R^4 is independently H, halo, $-C(O)OR^5$, or $-C(O)NHR^6$;~~

~~each R^5 and R^6 is independently H, C_{1-6} alkyl, or C_{6-10} aryl;~~

~~X is $-C(O)-$, $-CH_2-$, or $-C(O)NH-$; and~~

~~each m1, m2 and m3 is independently 1, 2, 3, 4, 5, or 6.~~

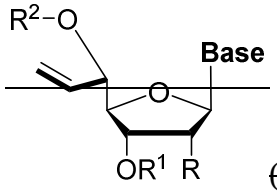
~~74. The solid support of any one of claims 67 to 70, wherein the second extension primer comprises a P7 nucleotide sequence, a modified P7 nucleotide sequence, or a P17 nucleotide sequence.~~

~~75. The solid support of any one of claims 66 to 74, wherein the first and the second strand polynucleotides are immobilized on the solid support through covalent bonding with a polymer or hydrogel coating on a surface of the solid support.~~

~~76. The solid support of claim 75, wherein the polymer or hydrogel coating comprises PAZAM.~~

~~77. The solid support of any one of claims 46 to 76, wherein the solid support comprises a flow cell.~~

~~78. A modified nucleoside or nucleotide comprising a structure of Formula (II):~~



~~(II)~~

~~wherein R is H, OH or OPG;~~

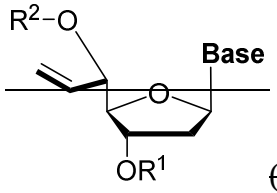
~~R^1 is H or PG;~~

~~R^2 is H, PG, or $-OR^2$ is a phosphate;~~

~~PG is a hydroxyl protecting group; and~~

~~Base is adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof.~~

~~79. The modified nucleoside or nucleotide of claim 78, having the structure of Formula (IIa):~~

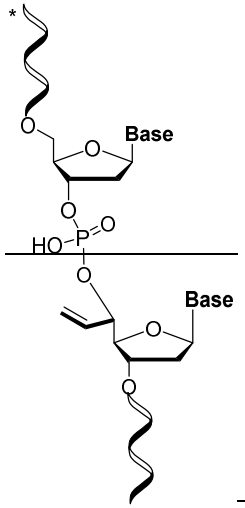


(IIa).

80. The modified nucleoside or nucleotide of claim 78 or 79, wherein the Base is thymine.

81. A oligonucleotide comprising the modified nucleoside or nucleotide of claim 79 or 80.

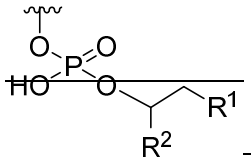
82. The oligonucleotide of claim 81, wherein the oligonucleotide has a structure of Formula (III):



(III).

83. The oligonucleotide of claim 81 or 82, wherein the 5' end of the oligonucleotide is bound to a solid support (the position of the asterisk).

84. A nucleoside or nucleotide comprising a 3´ blocking moiety of a structure of Formula (I):



(I)

wherein

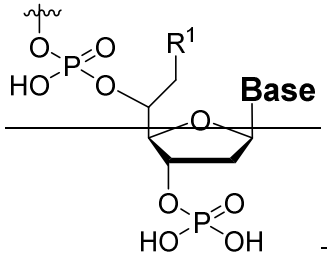
R^1 is NH_2, OH, $NHC(O)OR^a$ or $OCH_2OSi(R^b)_3$;

R^a is C_{1-4} alkyl, tert-butyl, allyl, benzyl, or 9-fluorenylmethyl;

each R^b is independently selected from the group consisting of C_{1-4} alkyl and phenyl; and

R^2 is H, C_{1-4} alkyl, an optionally substituted tetrahydrofuran, or a nucleotide.

85. The nucleoside or nucleotide of claim 84, wherein the structure of Formula (I) is also represented by Formula (V):



(V)

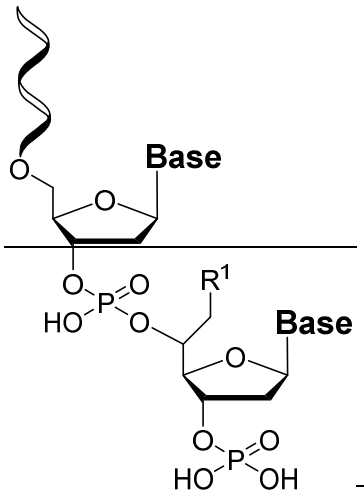
wherein Base is an optionally protected adenine, guanine, cytosine, thymine, or uracil, or a derivative thereof.

86. The nucleoside or nucleotide of claim 84 or 85, wherein R^1 is $-OCH_2OSi(R^b)_3$.

87. The nucleoside or nucleotide of claim 86, wherein R^b is isopropyl.

88. An oligonucleotide comprising a nucleotide of any one of claims 84 to 87.

89. The oligonucleotide of claim 88, having a structure of Formula (VI):



(VI).