LISTING OF CLAIMS

1. A compound comprising a 5-position modified pyrimidine nucleoside, or a salt thereof,

wherein the 5-position modified pyrimidine is substituted with a moiety comprising two phenyl groups covalently attached to one another by a first linker, and wherein the moiety is covalently linked to the 5-position of the pyrimidine by a second linker.

2. The compound of claim 1, wherein the first linker comprises at least one atom selected from a carbon and oxygen or is a bond.

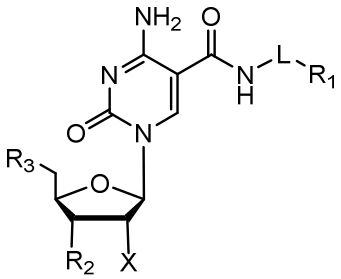
3. The compound of any one of claims 1-2, wherein the 5-position modified pyrimidine comprises a moiety at the 5-position selected from a phenylbenzyl moiety, a phenoxybenzyl moiety, and a diphenylmethyl moiety.

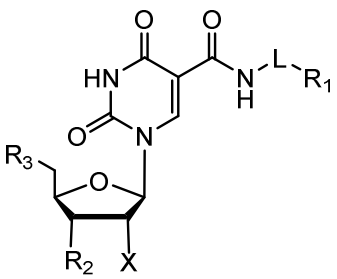
4. The compound of any one of claims 1-3, wherein the second linker comprises a group selected from an amide linker, a carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

5. The compound of any one of claims 1-3, wherein the second linker comprises an amide linker, optionally wherein the amide linker further comprises one or more carbon atoms or 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms.

6. The compound of any one of claims 1-5, comprising a 5-position modified uridine or a 5-position modified cytidine.

7. A compound comprising the structure of Formula IA or Formula IB:



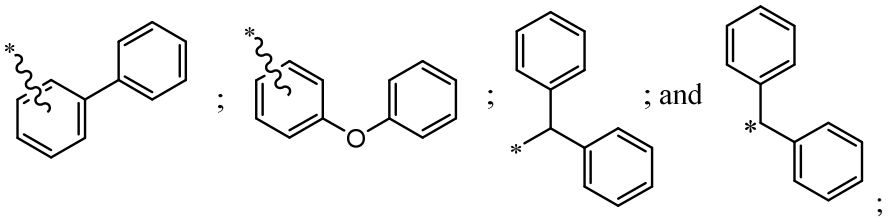


Formula IA Formula IB, or a salt of either one of these,

wherein

each L is independently a $-(CH_2)_n-$, wherein n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each R_1 is independently selected from



wherein * denotes the point of attachment of the R_1 group to the L group;

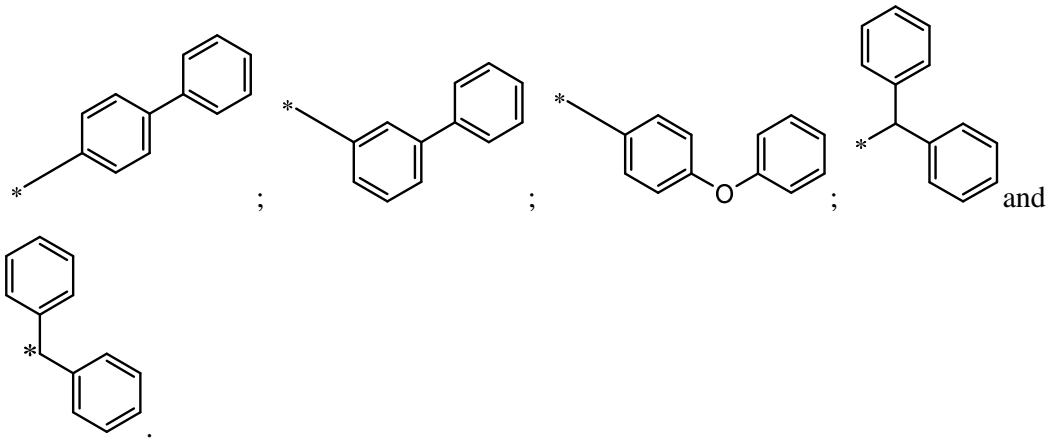
each X is independently selected from -H, -OH, -OMe, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido;

each R_2 is independently selected from -OH; -acetyl; -OBz; $-OP(N(CH_2CH_3)_2)(OCH_2CH_2CN)$, $-OP(N(R_x)_2)(OCH_2CH_2CN)$, wherein each R_x is independently $(C_{1\text{-}6})$alkyl; *tert*-butyldimethylsilyloxy; -O-ss; -OR; -SR; -ZP(Z')(Z")-O-R; wherein ss is a solid support, Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide;

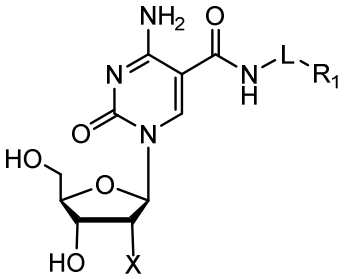
each R_3 is independently selected from -OH, -O-trityl, -O-4,4'-dimethoxytrityl, -O-triphosphate, –OR, -SR, $-NH_2$, -NHR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide.

8 The compound of claim 7, wherein n is 1, 2, or 3; and X is -H or -OMe.

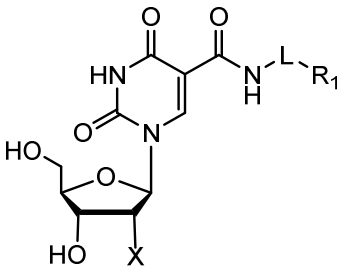
9. The compound of claim 7 or claim 8, wherein each R_1 is independently selected from



10. A compound comprising the structure of Formula IIA or Formula IIB:



Formula IIA

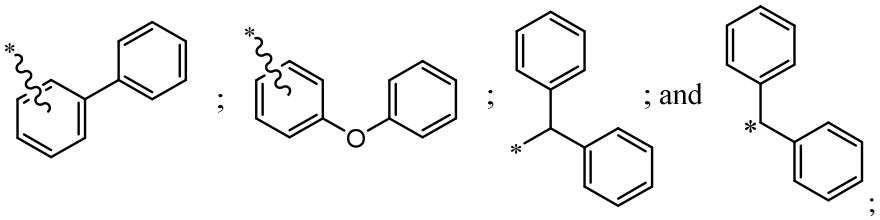


Formula IIB, or a salt of either one of these,

wherein

each L is independently a $-(CH_2)_n-$, wherein n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each R_1 is independently selected from



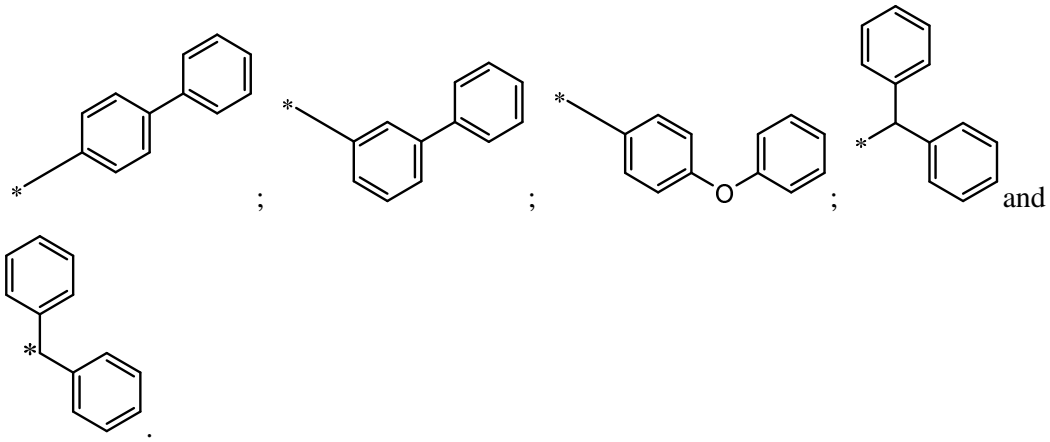
; and ;

wherein * denotes the point of attachment of the R_1 group to the L group;

each X is independently selected from -H, -OH, -OMe, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido.

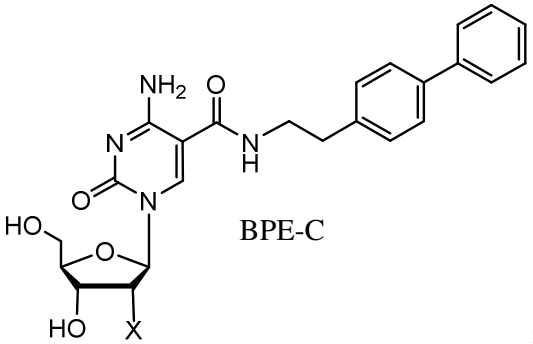
11. The compound of claim 10, wherein n is 1, 2, or 3; and X is -H or -OMe.

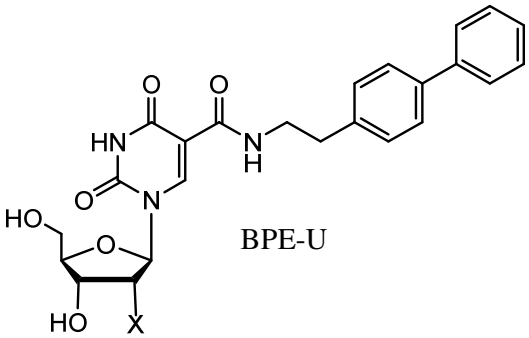
12. The compound of claim 10 or claim 11, wherein each R_1 is independently selected from:

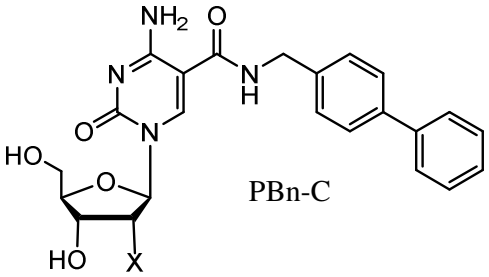


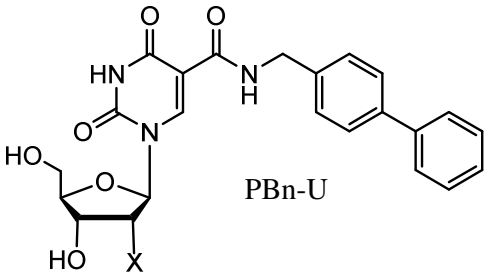
and .

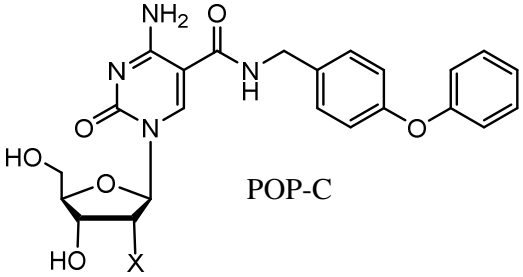
13. A compound comprising the following structure:

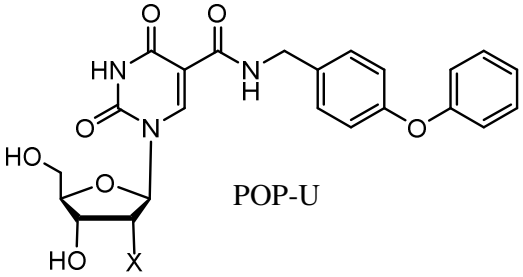

BPE-C ;

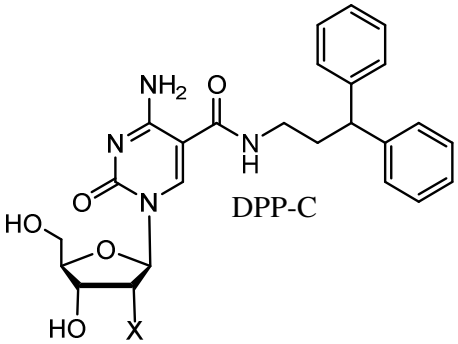

BPE-U ;

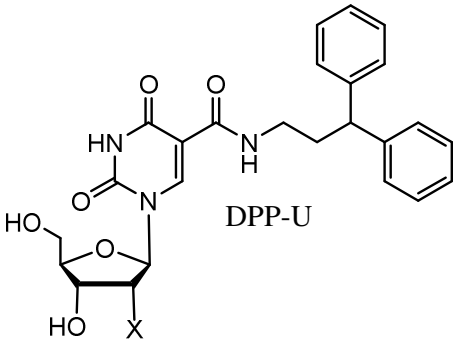

PBn-C ;

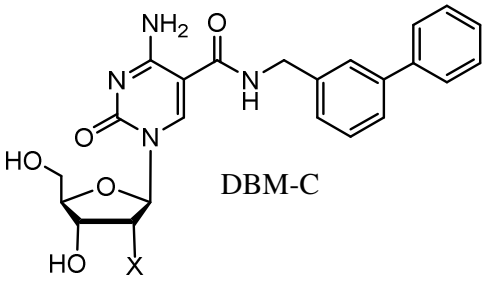

PBn-U ;


POP-C ;


POP-U ;


DPP-C ;


DPP-U ;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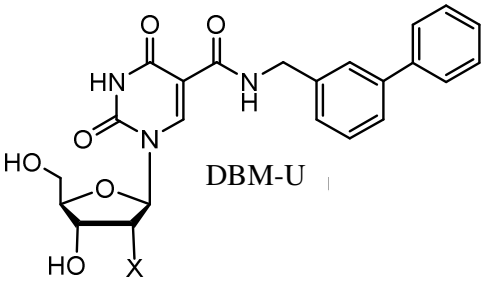
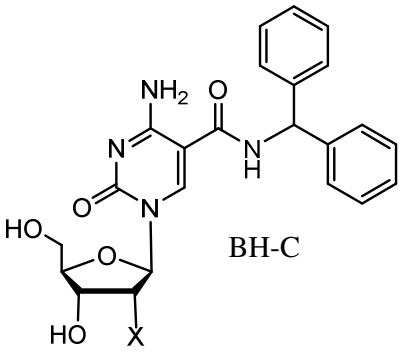
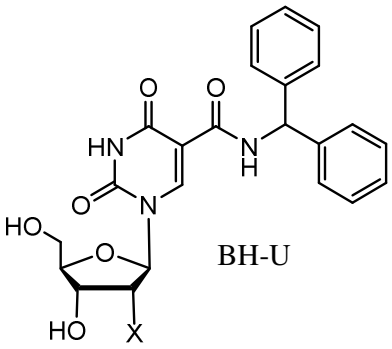

DBM-U ;



BH-C ;



BH-U ;

or a salt of any one of these;

wherein each X is independently selected from -H, -OH, -O-methyl, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido.

14. The compound of claim 13, wherein X is -H or -OMe.

15. An oligonucleotide comprising the compound of any one of claims 1-9.

16. The oligonucleotide of claim 15, which comprises RNA, DNA, or a combination thereof.

17. The oligonucleotide of claim 15 or claim 16, which is 15 to 100, or 15 to 90, or 15 to 80, or 15 to 70, or 15 to 60, or 15 to 50, or 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 30 to 100, or 30 to 90, or 30 to 80, or 30 to 70, or 30 to 60, or 30 to 50, or 40 to 100, or 40 to 90, or 40 to 80, or 40 to 70, or 40 to 60, or 40 to 50 nucleotides in length.

18. The oligonucleotide of any one of claims 15-17, which is an aptamer that binds a target.

19. An aptamer comprising the compound of any one of claims 1-9.

20. The aptamer of claim 19, wherein the aptamer is 15 to 100, or 15 to 90, or 15 to 80, or 15 to 70, or 15 to 60, or 15 to 50, or 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 30 to 100, or 30 to 90, or 30 to 80, or 30 to 70, or 30 to 60, or 30 to 50, or 40 to 100, or 40 to 90, or 40 to 80, or 40 to 70, or 40 to 60, or 40 to 50 nucleotides in length.

21. The aptamer of claim 19 or claim 20, comprising at least one 5-position modified uridine and at least one 5-position modified cytidine.

22. The aptamer of any one of claims 19-21, wherein the aptamer comprises a region at the 5’ end of the aptamer that is at least 10, at least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length, wherein the region at the 5’ end of the aptamer lacks 5-position modified pyrimidines; and/or wherein the aptamer comprises a region at the 3’ end of the aptamer that is at least 10, at

least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length, wherein the region at the 3' end of the aptamer lacks 5-position modified pyrimidines.

23. An aptamer comprising at least one first 5-position modified pyrimidine and at least one second 5-position modified pyrimidine, wherein the first 5-position modified pyrimidine and the second 5-position modified pyrimidine are different 5-position modified pyrimidines, and wherein the at least one first 5-position modified pyrimidine is a compound according to any one of claims 1-9.

24. The aptamer of claim 23, wherein the at least one second 5-position modified pyrimidine is selected from BndC, 2'-OMe-Bn-C, PEdC, 2'-OMe-PE-C, PPdC, 2'-OMe-PP-C, NapdC, 2'-OMe-Nap-C, 2NapdC, 2'-OMe-2Nap-C, NEdC, 2'-OMe-NE-C, 2NEdC, 2'-OMe-2NE-C, TyrdC, 2'-OMe-Tyr-C, BndU, 2'-OMe-Bn-U, NapdU, 2'-OMe-Nap-U, PEdU, 2'-OMe-PE-U, IbdU, 2'-OMe-Ib-U, FBndU, 2'-OMe-FBn-U, 2NapdU, 2'-OMe-2Nap-U, NEdU, 2'-OMe-NE-U, MBndU, 2'-OMe-MBn-U, BFdU, 2'-OMe-BF-U, BTdU, 2'-OMe-BT-U, PPdU, 2'-OMe-PP-U, MOEdU, 2'-OMe-MOE-U, TyrdU, 2'-OMe-Tyr-U, TrpdU, 2'-OMe-Trp-U, ThrdU, and 2'-OMe-Thr-U.

25. The aptamer of any one of claims 19-24, wherein the aptamer has improved nuclease stability and/or a longer half-life in human serum and/or improved affinity and/or improved off-rate compared to an aptamer of the same length and nucleobase sequence that comprises an unmodified pyrimidine in place of the 5-position modified pyrimidine.

26. A composition comprising a plurality of aptamers of any one of claims 19-25.

27. A composition comprising an aptamer and a target, wherein the aptamer and the target are capable of forming a complex, and wherein the aptamer is an aptamer of any one of claims 19-25.

28. A composition comprising a first aptamer, a second aptamer, and a target,

wherein the first aptamer, the second aptamer, and the target are capable of forming a trimer complex; and

wherein the first aptamer is an aptamer comprising a compound of any one of claims 1-9; and

wherein the second aptamer comprises at least one second 5-position modified pyrimidine.

29. The composition of claim 28, wherein the target is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

30. A pharmaceutical composition comprising at least one aptamer of any one of claims 19-25, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

31. A method comprising:

(a) contacting an aptamer capable of binding to a target molecule with a sample;

(b) incubating the aptamer with the sample to allow an aptamer-target complex to form;

(c) enriching for the aptamer-target complex in the sample; and

(d) detecting for the presence of the aptamer, the aptamer-target complex, or the target molecule, wherein the detection of the aptamer, the aptamer-target complex, or the target molecule indicates that the target molecule is present in the sample, and wherein the lack of detection of the aptamer, the aptamer-target complex, or the target molecule indicates that the target molecule is not present in the sample;

wherein the aptamer comprises a compound of any one of claims 1-9 or is an aptamer of any one of claims 19-25.

32. The method of claim 31, wherein the method comprises at least one additional step selected from: adding a competitor molecule to the sample; capturing the aptamer-target complex on a solid support; and adding a competitor molecule and diluting the sample; wherein the at least one additional step occurs after step (a) or step (b), optionally wherein the competitor molecule is selected from a polyanionic competitor, further optionally wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin, and dNTPs.

33. The method of claim 31 or claim 32, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue, optionally wherein the sample is selected from whole blood, leukocytes, peripheral blood mononuclear cells, plasma, serum, sputum, breath, urine, semen, saliva, meningial fluid, amniotic fluid, glandular fluid, lymph fluid, nipple aspirate, bronchial aspirate, synovial fluid, joint aspirate, cells, a cellular extract, stool, tissue, a tissue biopsy, and cerebrospinal fluid.

34. A method for detecting a target in a sample comprising:

(a) contacting the sample with a first aptamer to form a mixture, wherein the first aptamer is capable of binding to the target to form a first complex;

(b) incubating the mixture under conditions that allow for the first complex to form;

(c) contacting the mixture with a second aptamer, wherein the second aptamer is capable of binding the first complex to form a second complex;

(d) incubating the mixture under conditions that allow for the second complex to form;

(e) detecting for the presence or absence of the first aptamer, the second aptamer, the target, the first complex or the second complex in the mixture, wherein the presence of the first aptamer, the second aptamer, the target, the first complex or the second complex indicates that the target is present in the sample;

wherein the first aptamer comprises a compound of any one of claims 1-9; and

wherein the second aptamer comprises at least one second 5-position modified pyrimidine;

wherein the first aptamer, the second aptamer and the target are capable of forming a trimer complex.

35. The method of claim 34, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

36. The method of claim 34 or claim 35, wherein the first aptamer, the second aptamer and the target are capable of forming a trimer complex.

37. The method of any one of claims 34-36, wherein the second aptamer comprises at least one second 5-position modified pyrimidine selected from BndC, 2'-OMe-Bn-C, PEdC, 2'-OMe-PE-C, PPdC, 2'-OMe-PP-C, NapdC, 2'-OMe-Nap-C, 2NapdC, 2'-OMe-2Nap-C, NEdC, 2'-OMe-NE-C, 2NEdC, 2'-OMe-2NE-C, TyrdC, 2'-OMe-Tyr-C, BndU, 2'-OMe-Bn-U, NapdU, 2'-OMe-Nap-U, PEdU, 2'-OMe-PE-U, IbdU, 2'-OMe-Ib-U, FBndU, 2'-OMe-FBn-U, 2NapdU, 2'-OMe-2Nap-U, NEdU, 2'-OMe-NE-U, MBndU, 2'-OMe-MBn-U, BFdU, 2'-OMe-BF-U, BTdU, 2'-OMe-BT-U, PPdU, 2'-OMe-PP-U, MOEdU, 2'-OMe-MOE-U, TyrdU, 2'-OMe-Tyr-U, TrpdU, 2'-OMe-Trp-U, ThrdU, and 2'-OMe-Thr-U.

38. A method for identifying one or more aptamers capable of binding to a target molecule comprising:

(a) contacting a library of aptamers with the target molecule to form a mixture, and allowing for the formation of an aptamer-target complex, wherein the aptamer-target complex forms when an aptamer has affinity for the target molecule;

(b) partitioning the aptamer-target complex from the remainder of the mixture (or enriching for the aptamer-target complex);

(c) dissociating the aptamer-target complex; and

(d) identifying the one or more aptamers capable of binding to the target molecule;

wherein the library of aptamers comprises a plurality of polynucleotides, and which is the composition of any one of claims 26-29.

39. The method of claim 38, wherein each polynucleotide comprises a fixed region at the 5' end of the polynucleotide and/or a fixed region at the 3' end of the polynucleotide, and wherein each polynucleotide comprises a random region.

40. The method of claim 39, wherein the fixed region at the 5' end and/or the 3' end of each polynucleotide is at least 10, at least 15, at least 20, at least 25 or at least 30 nucleotides in length, or 5 to 30, 10 to 30, 15 to 30, 5 to 20, or 10 to 20 nucleotides in length, and wherein the random region is 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 20 to 40, or 30 to 100, or 30 to 90, or 30 to 70, or 30 to 60, or 30 to 50, or 30 to 40 nucleotides in length, and wherein each polynucleotide is 15 to 100, or 15 to 90, or 15 to 80, or 15 to 70, or 15 to 60, or 15 to 50, or 20 to 100, or 20 to 90, or 20 to 80, or 20 to 70, or 20 to 60, or 20 to 50, or 30 to 100, or 30 to 90, or 30 to 80, or 30 to 70, or 30 to 60, or 30 to 50, or 40 to 100, or 40 to 90, or 40 to 80, or 40 to 70, or 40 to 60, or 40 to 50 nucleotides in length.

41. The method of any one of claims 38-40, wherein each polynucleotide is an aptamer that binds a target, and wherein the library comprises at least 1000 aptamers, wherein each aptamer comprises a different nucleotide sequence.

42. The method of any one of claims 38-41, wherein steps (a), (b), and/or (c) are repeated at least one time, two times, three times, four times, five times, six times, seven times, eight times, nine times, or ten times.

43. The method of any one of claims 38-42, wherein the one or more aptamers capable of binding to the target molecule is amplified.

44. The method of any one of claims 38-43, wherein the mixture comprises a polyanionic competitor molecule, optionally wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin and dNTPs.

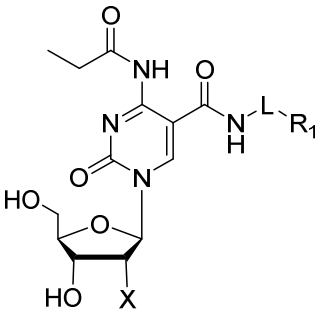
45. The method of any one of claims 38-44, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

46. The compound of any one of claims 1-9, the aptamer of any one of claims 19-25, the composition of any one of claims 26-29, or the method of any one of claims 31-45, wherein the 5-position modified pyrimidine is capable of being incorporated by a polymerase enzyme.

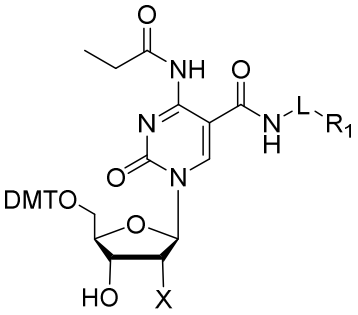
47. A kit comprising the compound of any one of claims 1-9, the compound of any one of claims 10-14, the oligonucleotide of any one of claims 15-18, the aptamer of any one of claims 19-25, the composition of any one of claims 26-29, and optionally one or more of (a) a pharmaceutically acceptable carrier, such as a solvent or solution; (b) a pharmaceutically acceptable excipient, such as a stabilizer or buffer; (c) at least one container, vial, or apparatus

for holding and/or mixing the kit components; and (d) a delivery apparatus; and optionally further comprising one or more of (e) labeling agents useful to detect a target molecule that is bound to an aptamer; (f) a solid support, such as a microarray or bead; and (g) reagents related to quantitation of polymerase chain reaction products, such as intercalating fluorescent dyes or fluorescent DNA probes.

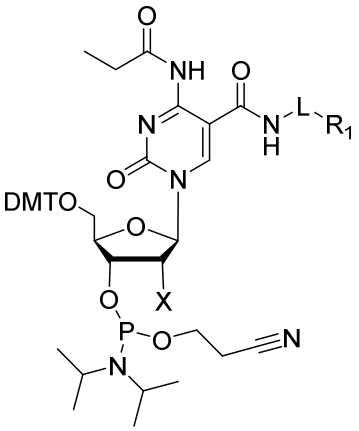
48. A compound comprising the structure of Formula III, Formula IV, or Formula V:



Formula III



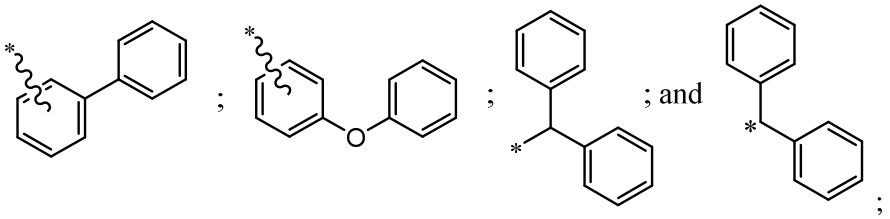
Formula IV



Formula V, or a salt of any one of these, wherein:

each L is independently a $-(CH_2)_n-$, wherein n is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

each R_1 is independently selected from

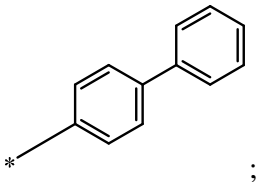
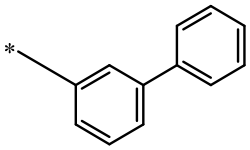
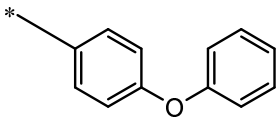
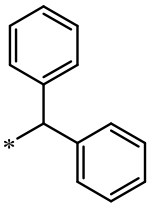
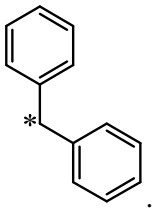


wherein * denotes the point of attachment of the R_1 group to the L group; and

each X is independently selected from -H, -OH, -OMe, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido.

49. The compound of claim 48, wherein n is 1, 2, or 3; and X is -H or -OMe.

50. The compound of claim 48 or claim 49, wherein R_1 is selected from

 ;  ;  ;  and  .