MODIFIED NUCLEOSIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of US Provisional Application No. 63/174,495, filed April 13, 2021, and US Provisional Application No. 63/174,792, filed April 14, 2021, each of which is incorporated by reference herein in its entirety for any purpose.

FIELD

[0002] The present disclosure relates generally to the field of oligonucleotides comprising modified nucleosides, such as aptamers that are capable of binding to target molecules. In some embodiments, the present disclosure relates to oligonucleotides, such as aptamers, that comprise one or more base-modified nucleoside, and methods of making and using such aptamers.

BACKGROUND

[0003] Modified nucleosides have been used as therapeutic agents, diagnostic agents, and for incorporation into oligonucleotides to improve their properties (e.g., stability).

[0004] SELEX (Systematic Evolution of Ligands for EXponential Enrichment) is a method for identifying oligonucleotides (referred to as "aptamers") that selectively bind target molecules. The SELEX process is described, for example, in U.S. Patent No. 5,270,163. The SELEX method involves the selection and identification of oligonucleotides from a random mixture of oligonucleotides to achieve virtually any desired criterion of binding affinity and selectivity. By introducing specific types of modified nucleosides to the oligonucleotides identified in the course of the SELEX process, the nuclease stability, net charge, hydrophilicity or lipophilicity may be altered to provide differences in the three dimensional structure and target binding capabilities of the oligonucleotides.

[0005] There remains a need in the art for alternative modified nucleosides and modified nucleotides, which can be incorporated into oligonucleotides such as aptamers. The present disclosure aims to meet one or more of these needs or provide other benefits.

SUMMARY

[0006] The foregoing and other objects, features, and advantages of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

[0007] Certain non-limiting exemplary examples are as follows:

Example 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.

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

Example 3. The compound of any one of Examples 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.

Example 4. The compound of any one of Examples 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.

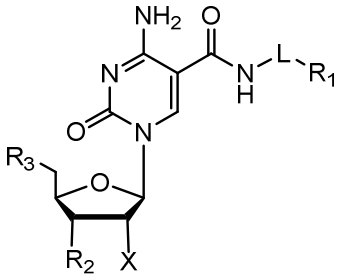
Example 5. The compound of any one of Examples 1-3, wherein the second linker comprises an amide linker.

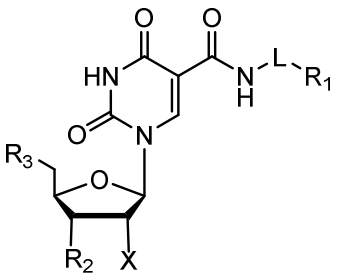
Example 6. The compound of Example 5, wherein the amide linker further comprises one or more carbon atoms or 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms.

Example 7. The compound of any one of Examples 1-6, comprising a 5-position modified uridine.

Example 8. The compound of any one of Examples 1-7, comprising a 5-position modified cytidine.

Example 9. A compound comprising the structure of Formula IA or Formula IB:

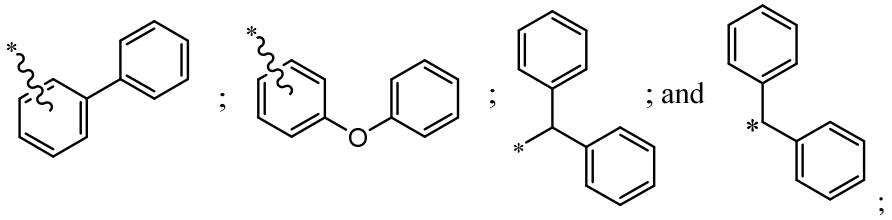




Formula IA Formula IB, or a salt of either one of these, wherein

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 the group consisting of



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

each X is independently selected from the group consisting of -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 the group consisting of -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;

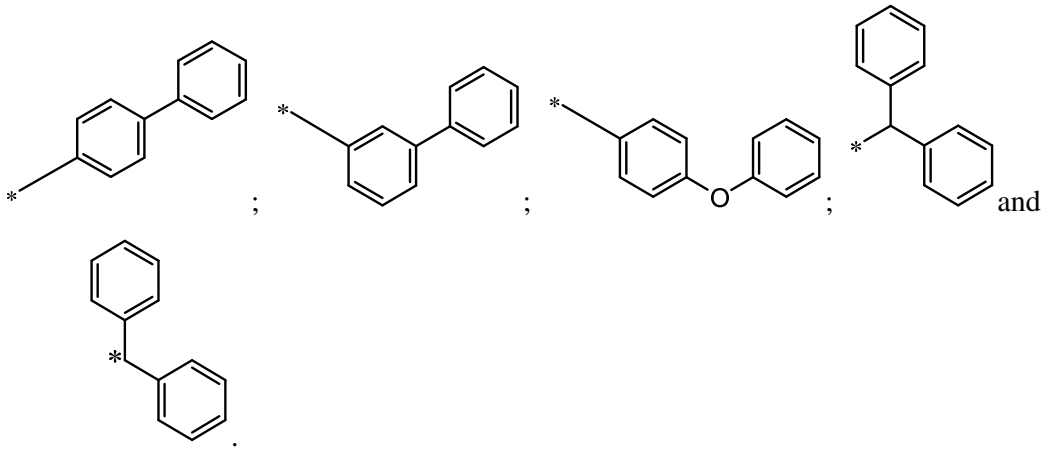
each R_3 is independently selected from the group consisting of -OH, -O-trityl, -O-4,4'-dimethoxytrityl, -O-triphosphate, –OR, -SR, $-NH_2$, -NHR, and –Z-P(Z')(Z")O-R, wherein Z, Z', and Z" are each independently selected from O and S, and R is an adjacent nucleotide.

Example 10. The compound of Example 9, wherein n is 1, 2, or 3.

Example 11. The compound of any one of Examples 9-10, wherein X is -H.

Example 12. The compound of any one of Examples 9-10, wherein X is -OMe.

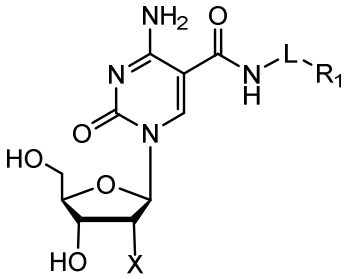
Example 13. The compound of any one of Examples 9-12, wherein each R_1 is independently selected from the group consisting of



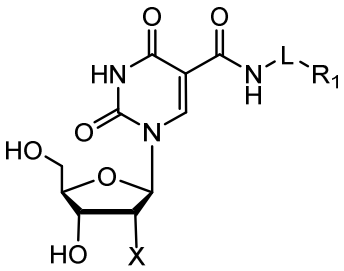
Example 14. The compound of any one of Examples 1-13, wherein the 5-position modified pyrimidine is selected from a BPEdU, a 2'-OMe-BPE-U, a 2'-F-BPE-U, a PBndU, a 2'-OMe-PBn-U, a 2'-F-PBn-U, a POPdU, a 2'-OMe-POP-U, a 2'-F-POP-U, a

DPPdU, a 2'-OMe-DPP-U, a 2'-F-DPP-U, a DBMdU, a 2'-OMe-DBM-U, a 2'-F-DBM-U, a BHdU, a 2'-OMe-BH-U, a 2'-F-BH-U,a BPEdC, a 2'-OMe-BPE-C, a 2'-F-BPE-C, a PBndC, a 2'-OMe-PBn-C, a 2'-F-PBn-C, a POPdC, a 2'-OMe-POP-C, a 2'-F-POP-C, a DPPdC, a 2'-OMe-DPP-C, a 2'-F-DPP-C, a DBMdC, a 2'-OMe-DBM-C, a 2'-F-DBM-C, a BHdC, a 2'-OMe-BH-C, and a 2'-F-BH-C.

Example 15. A compound comprising the structure of Formula IIA or Formula IIB:



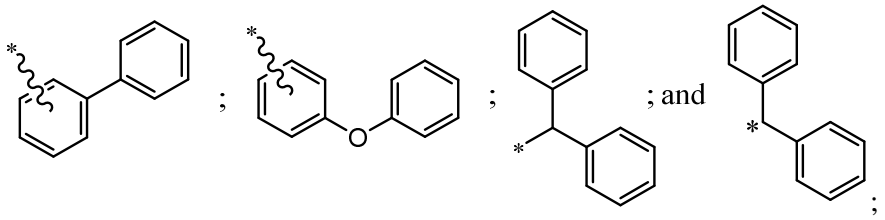
Formula IIA



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 the group consisting of



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

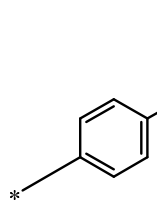
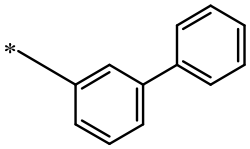
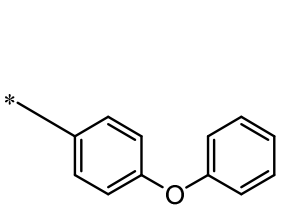
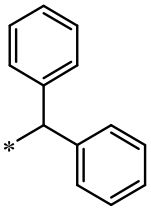
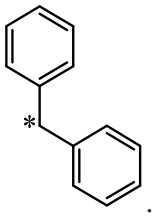
each X is independently selected from the group consisting of -H, -OH, -OMe, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido.

Example 16. The compound of Example 15, wherein n is 1, 2, or 3.

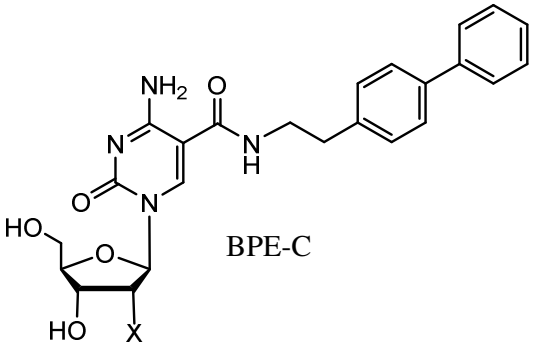
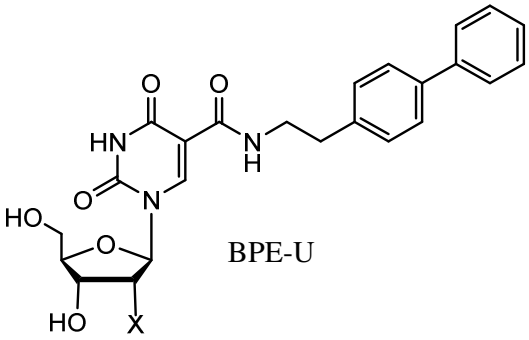
Example 17. The compound of any one of Examples 15-16, wherein X is -H.

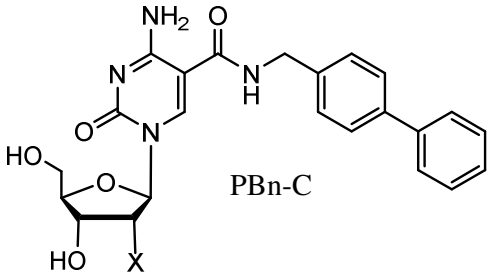
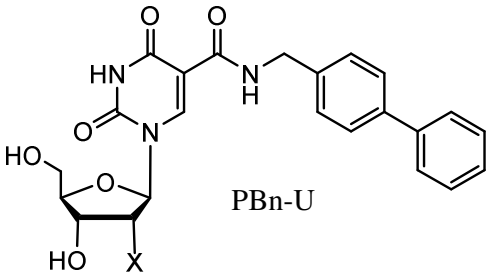
Example 18. The compound of any one of Examples 15-16, wherein X is -OMe.

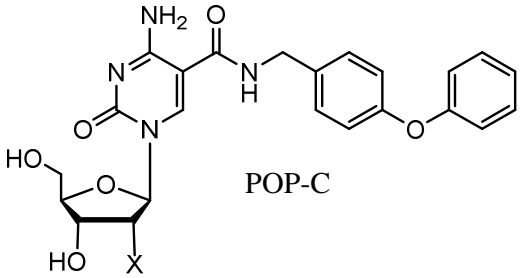
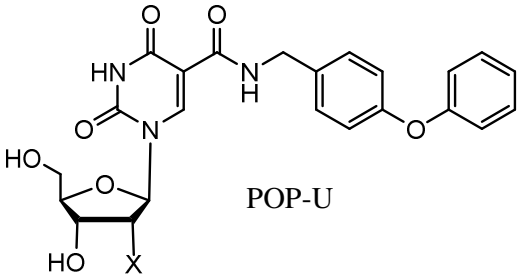
Example 19. The compound of any one of Examples 15-18, wherein each R_1 is independently selected from the group consisting of

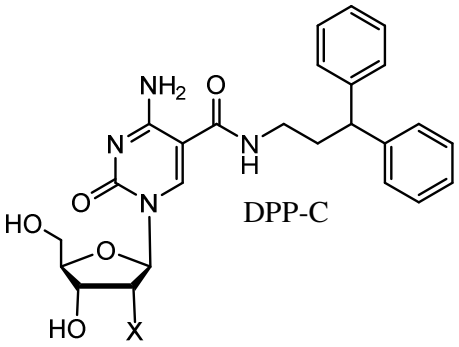

;

;

;

and

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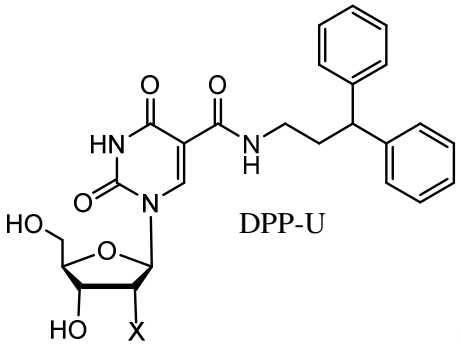
Example 20. A compound comprising the following structure:

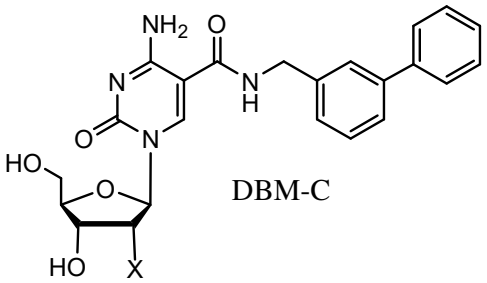

BPE-C
;

BPE-U
;

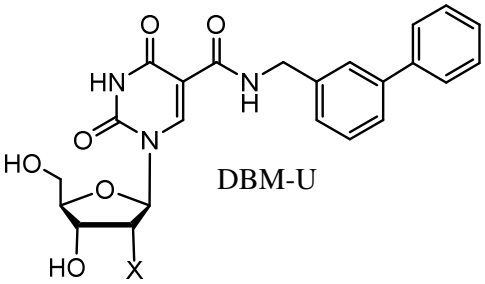

PBn-C
;

PBn-U
;

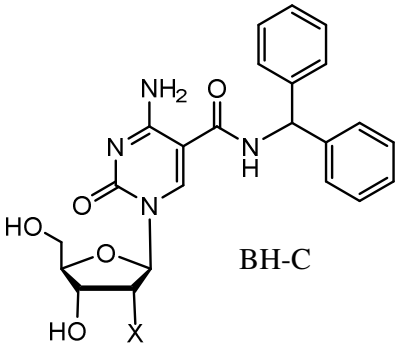

POP-C
;

POP-U
;

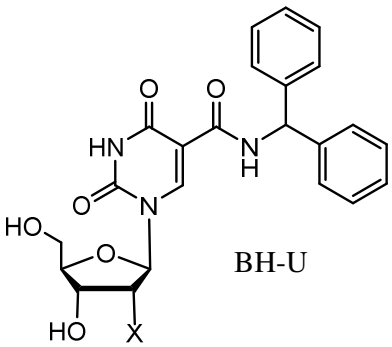

DPP-C ;


DPP-U ;


DBM-C ;


DBM-U ;


BH-C ;


BH-U ;

or a salt of any one of these;

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

Example 21. The compound of Example 20, wherein X is -H.

Example 22. The compound of Example 20, wherein X is -OMe.

Example 23. An oligonucleotide comprising the compound of any one of Examples 1-14.

Example 24. The oligonucleotide of Example 23, which comprises RNA, DNA, or a combination thereof.

Example 25. The oligonucleotide of any one of Examples 23-24, 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.

Example 26. The oligonucleotide of any one of Examples 23-25, which is an aptamer that binds a target.

Example 27. An aptamer comprising the compound of any one of Examples 1-14.

Example 28. The aptamer of any one of Examples 26-27, 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.

Example 29. The aptamer of any one of Examples 26-28, comprising a 5-position modified pyrimidine selected from a BPEdU, a 2'-OMe-BPE-U, a 2'-F-BPE-U, a PBndU, a 2'-OMe-PBn-U, a 2'-F-PBn-U, a POPdU, a 2'-OMe-POP-U, a 2'-F-POP-U, a DPPdU, a 2'-OMe-DPP-U, a 2'-F-DPP-U, a DBMdU, a 2'-OMe-DBM-U, a 2'-F-DBM-U, a BHdU, a 2'-OMe-BH-U, a 2'-F-BH-U, a BPEdC, a 2'-OMe-BPE-C, a 2'-F-BPE-C, a PBndC, a 2'-OMe-PBn-C, a 2'-F-PBn-C, a POPdC, a 2'-OMe-POP-C, a 2'-F-POP-C, a DPPdC, a 2'-OMe-DPP-C, a 2'-F-DPP-C, a DBMdC, a 2'-OMe-DBM-C, a 2'-F-DBM-C, a BHdC, a 2'-OMe-BH-C, and a 2'-F-BH-C.

Example 30. The aptamer of any one of Examples 26-29, comprising at least one 5-position modified uridine selected from a BPEdU, a 2'-OMe-BPE-U, a 2'-F-BPE-U, a PBndU, a 2'-OMe-PBn-U, a 2'-F-PBn-U, a POPdU, a 2'-OMe-POP-U, a 2'-F-POP-U, a DPPdU, a 2'-OMe-DPP-U, a 2'-F-DPP-U, a DBMdU, a 2'-OMe-DBM-U, a 2'-F-DBM-U, a BHdU, a 2'-OMe-BH-U, a 2'-F-BH-U,, and at least one 5-position modified cytidine selected from a BPEdC, a 2'-OMe-BPE-C, a 2'-F-BPE-C, a PBndC, a 2'-OMe-PBn-C, a 2'-F-PBn-C, a POPdC, a 2'-OMe-POP-C, a 2'-F-POP-C, a DPPdC, a 2'-OMe-DPP-C, a 2'-F-DPP-C, a DBMdC, a 2'-OMe-DBM-C, a 2'-F-DBM-C, a BHdC, a 2'-OMe-BH-C, and a 2'-F-BH-C.

Example 31. The aptamer of any one of Examples 26-30, 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.

Example 32. The aptamer of any one of Examples 26-31, 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.

Example 33. 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 Examples 1-14.

Example 34. The aptamer of Example 33, wherein the at least one second 5-position modified pyrimidine is selected from the group consisting of one or more of BndC, 2'-OMe-Bn-C, 2'-F-Bn-C, PEdC, 2'-OMe-PE-C, 2'-F-PE-C, PPdC, 2'-OMe-PP-C, 2'-F-PP-C, NapdC, 2'-OMe-Nap-C, 2'-F-Nap-C, 2NapdC, 2'-OMe-2Nap-C, 2'-F-2Nap-C, NEdC, 2'-OMe-NE-C, 2'-F-NE-C, 2NEdC, 2'-OMe-2NE-C, 2'-F-2NE-C, TyrdC, 2'-OMe-Tyr-C, 2'-F-Tyr-C, BndU, 2'-OMe-Bn-U, 2'-F-Bn-U, NapdU, 2'-OMe-Nap-U, 2'-F-Nap-U, PEdU, 2'-OMe-PE-U, 2'-F-PE-U, IbdU, 2'-OMe-Ib-U, 2'-F-Ib-U, FBndU, 2'-OMe-FBn-U, 2'-F-FBn-U, 2NapdU, 2'-OMe-2Nap-U, 2'-F-2Nap-U, NEdU, 2'-OMe-NE-U, 2'-F-NE-U, MBndU, 2'-OMe-MBn-U, 2'-F-MBn-U, BFdU, 2'-OMe-BF-U, 2'-F-BF-U, BTdU, 2'-OMe-BT-U, 2'-F-BT-U, PPdU, 2'-OMe-PP-U, 2'-F-PP-U, MOEdU, 2'-OMe-MOE-U, 2'-F-MOE-U, TyrdU, 2'-OMe-Tyr-U, 2'-F-Tyr-U, TrpdU, 2'-OMe-Trp-U, 2'-F-Trp-U, ThrdU, 2'-OMe-Thr-U, and 2'-F-Thr-U.

Example 35. The aptamer of any one of Examples 33-34, wherein the at least one second 5-position modified pyrimidine is selected from the group consisting of one or more of NapdC, 2'-OMe-Nap-C, 2'-F-Nap-C, 2NapdC, 2'-OMe-2Nap-C, 2'-F-2Nap-C, TyrdC, 2'-OMe-Tyr-C, 2'-F-Tyr-C, PPdC, 2'-OMe-PP-C, 2'-F-PP-C, NapdU, 2'-OMe-Nap-U, 2'-F-Nap-U, PPdU, 2'-OMe-PP-U, 2'-F-PP-U, MOEdU, 2'-OMe-MOE-U, 2'-F-MOE-U, TyrdU, 2'-OMe-Tyr-U, 2'-F-Tyr-U, TrpdU, 2'-OMe-Trp-U, 2'-F-Trp-U, ThrdU, 2'-OMe-Thr-U, and 2'-F-Thr-U.

Example 36. The aptamer of any one of Examples 26-35, 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.

Example 37. A composition comprising a plurality of aptamers of any one of Examples 26-36.

Example 38. The composition of Example 37, wherein each aptamer comprises a random region.

Example 39. The composition of Example 38, 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.

Example 40. 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 Examples 26-35.

Example 41. 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 Examples 1-14; and wherein the second aptamer comprises at least one second 5-position modified pyrimidine.

Example 42. The composition of Example 41, wherein the target is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

Example 43. The composition of any one of Examples 41-42, wherein the target is a target protein selected from IL-33, XIAP, K-Ras, and TNF-alpha.

Example 44. A pharmaceutical composition comprising at least one aptamer of any one of Examples 26-35, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Example 45. The pharmaceutical composition of Example 44, for treating or preventing a disease or condition mediated by a protein selected from IL-33, XIAP, K-Ras, and TNF-alpha.

Example 46. A method of treating or preventing a disease or condition in a subject, comprising administering to a subject in need thereof an aptamer of any one of Examples 26-35 or a pharmaceutical composition of any one of Examples 44-45.

Example 47. The method of Example 46, wherein the disease or condition is mediated by a protein selected from IL-33, XIAP, K-Ras, and TNF-alpha.

Example 48. The method of any one of Examples 46-47, wherein the disease or condition is traumatic brain injury (TBI) or rheumatoid arthritis.

Example 49. 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 Examples 1-14 or is an aptamer of any one of Examples 26-35.

Example 50. The method of Example 49, 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).

Example 51. The method of Example 50, wherein the competitor molecule is selected from a polyanionic competitor.

Example 52. The method of Example 51, wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin, and dNTPs.

Example 53. The method of Example 52, wherein polydextran is dextran sulfate; and DNA is herring sperm DNA or salmon sperm DNA.

Example 54. The method of any one of Examples 49-53, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

Example 55. The method of any one of Examples 49-54, 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.

Example 56. 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 Examples 1-14; 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.

Example 57. The method of Example 56, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

Example 58. The method of any one of Examples 56-57, wherein the first aptamer, the second aptamer and the target are capable of forming a trimer complex.

Example 59. The method of any one of Examples 56-58, wherein the second aptamer comprises at least one second 5-position modified pyrimidine selected from the group consisting of one or more of 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.

Example 60. The method of any one of Examples 56-59, wherein the second aptamer comprises at least one second 5-position modified pyrimidine selected from the group consisting of one or more of NapdC, 2'-OMe-Nap-C, 2NapdC, 2'-OMe-2Nap-C, TyrdC, 2'-OMe-Tyr-C, PPdC, 2'-OMe-PP-C, NapdU, 2'-OMe-Nap-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.

Example 61. 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 Examples 37-43.

Example 62. The method of Example 61, wherein each polynucleotide comprises a fixed region at the 5' end of the polynucleotide.

Example 63. The method of Example 62, wherein the fixed region at the 5' 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.

Example 64. The method of any one of Examples 61-63, wherein each polynucleotide comprises a fixed region at the 3' end of the polynucleotide.

Example 65. The method of Example 64, wherein the fixed region at the 3' end of the 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.

Example 66. The method of any one of Examples 61-65, wherein each polynucleotide comprises a random region.

Example 67. The method of Example 66, 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.

Example 68. The method of any one of Examples 61-67, 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.

Example 69. The method of any one of Examples 61-68, 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.

Example 70. The method of any one of Examples 61-69, 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.

Example 71. The method of any one of Examples 61-70, wherein the one or more aptamers capable of binding to the target molecule is amplified.

Example 72. The method of any one of Examples 61-71, wherein the mixture comprises a polyanionic competitor molecule.

Example 73. The method of Example 72, wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin and dNTPs.

Example 74. The method of Example 73, wherein polydextran is dextran sulfate; and DNA is herring sperm DNA or salmon sperm DNA.

Example 75. The method of any one of Examples 61-74, wherein the target molecule is selected from a protein, a peptide, a carbohydrate, a small molecule, a cell and a tissue.

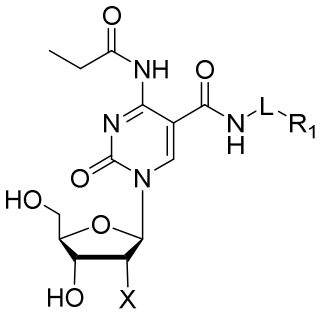
Example 76. The compound of any one of Examples 1-14, the aptamer of any one of Examples 26-35, the composition of any one of Examples 37-45, or the method

of any one of Examples 46-75, wherein the 5-position modified pyrimidine is capable of being incorporated by a polymerase enzyme.

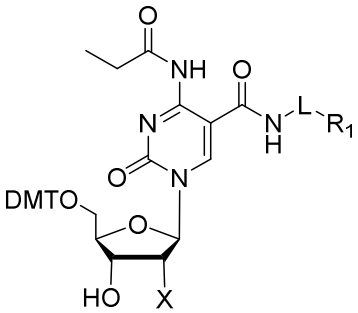
Example 77. A kit comprising the compound of any one of Examples 1-14, the compound of any one of Examples 15-22, the oligonucleotide of any one of Examples 23-25, the aptamer of any one of Examples 26-35, the composition of any one of Examples 37-43, and optionally one or more of (a) a pharmaceutically 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.

Example 78. The kit of Example 77, 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.

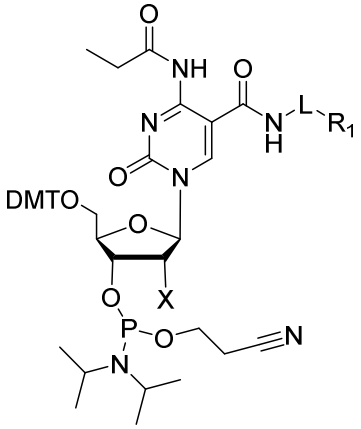
Example 79. A compound comprising the structure of Formula III, Formula IV, or Formula V:



Formula III



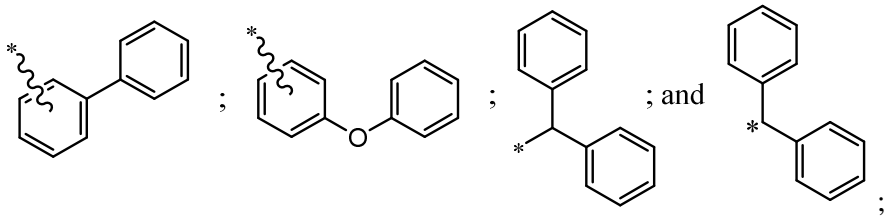
Formula IV



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



; ; ; and ;

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

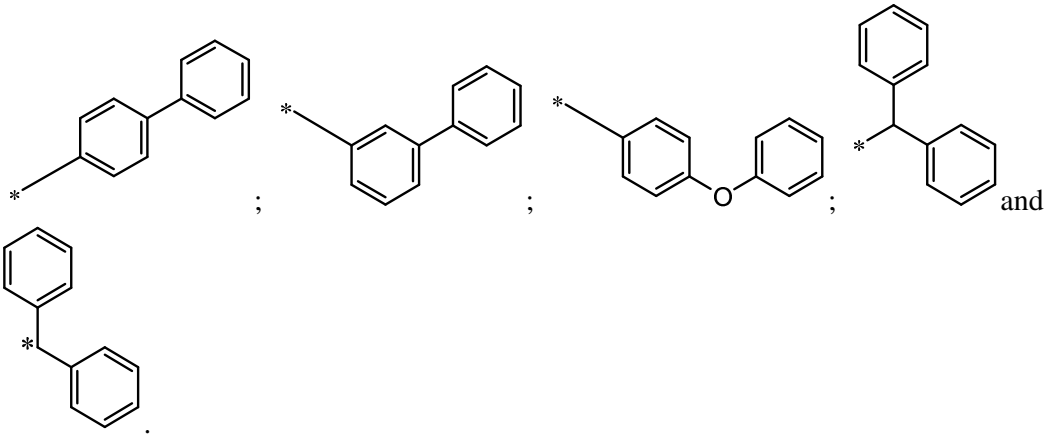
each X is independently selected from -H, -OH, -OMe, -O-allyl, -O-ethyl, -O-propyl, $-OCH_2CH_2OCH_3$, -fluoro, *tert*-butyldimethylsilyloxy, $-NH_2$, and -azido.

Example 80. The compound of claim 79, wherein n is 1, 2, or 3.

Example 81. The compound of any one of claims 79-80, wherein X is -H.

Example 82. The compound of any one of claims 79-80, wherein X is -OMe.

Example 83. The compound of any one of claims 79-82, wherein R_1 is selected from

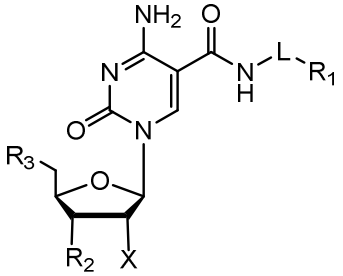


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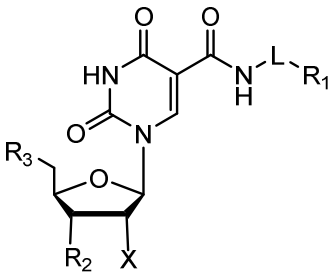
[0007a] In one aspect, the disclosure provides a compound comprising a 5-position modified pyrimidine nucleoside, or a salt thereof,

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.

[0007b] In one aspect, the disclosure provides a compound comprising the structure of Formula IA or Formula IB:



Formula IA

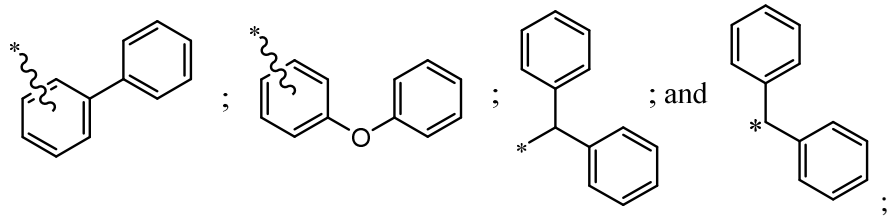


Formula IB, or a salt of either one of these,

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



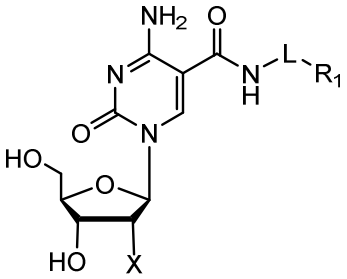
wherein * denotes the point of attachment of the R_1 group to the L group;

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;

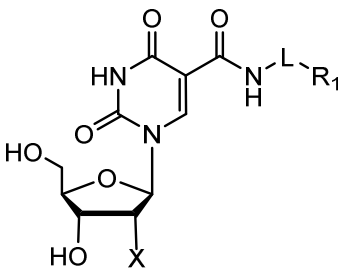
each R_2 is independently selected from -OH; -acetyl; -OBz; $-OP(N(CH_2CH_3)_2)(OCH_2CH_2CN)$, $-OP(N(R_x)_2)(OCH_2CH_2CN)$, wherein each R_x is independently $(C_{1\text{-}6})$alkyl; *tert*-butyldimethylsilyloxy; -O-ss; -OR; -SR; -ZP(Z')(Z")-O-R; wherein ss is a solid support, Z, Z', and Z" are each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

[0007c] In one aspect, the disclosure provides a compound comprising the structure of Formula IIA or Formula IIB:



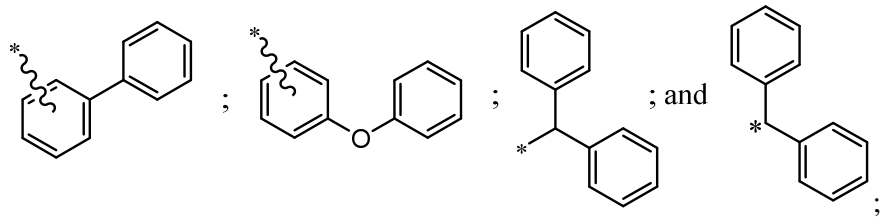
Formula IIA



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;

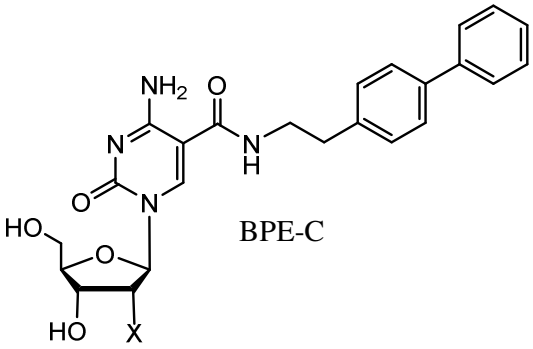
each R_1 is independently selected from



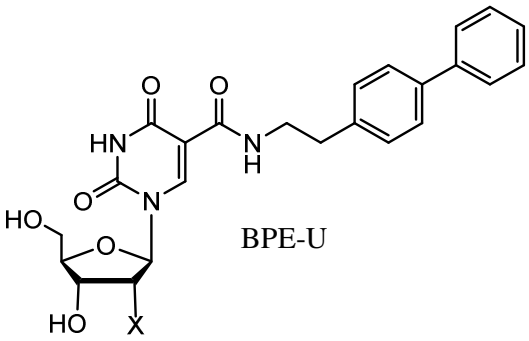
wherein * denotes the point of attachment of the R_1 group to the L group;

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.

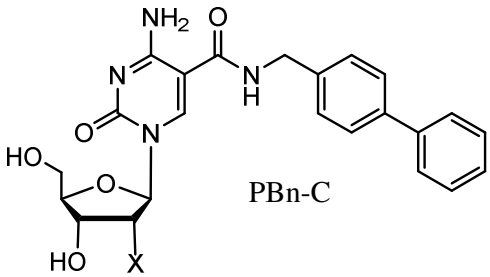
[0007d] In one aspect, the disclosure provides a compound comprising the following structure:



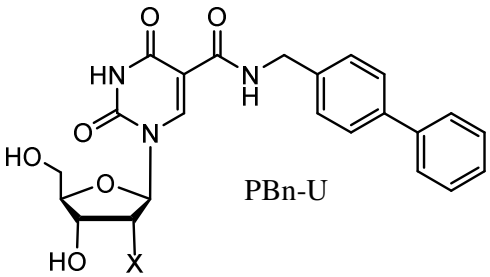
BPE-C ;



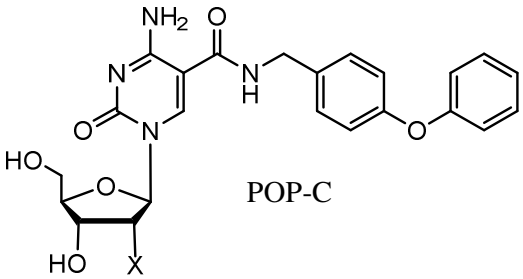
BPE-U ;



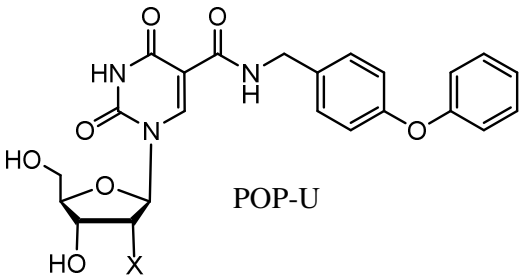
PBn-C ;



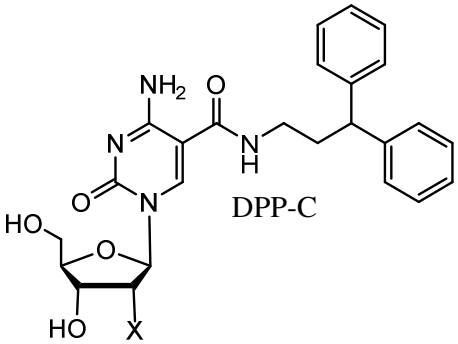
PBn-U ;



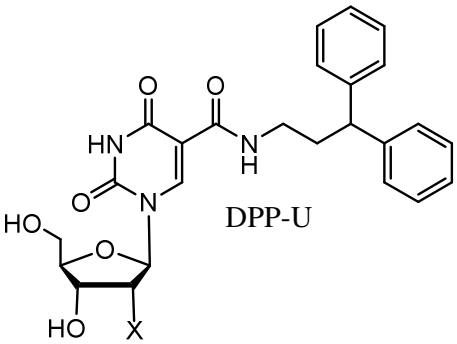
POP-C ;



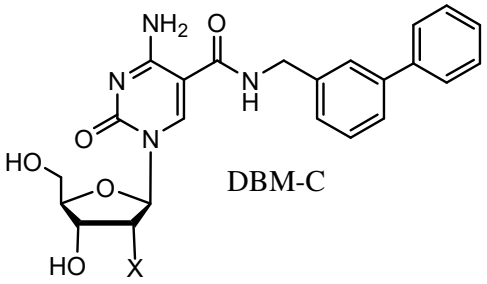
POP-U ;



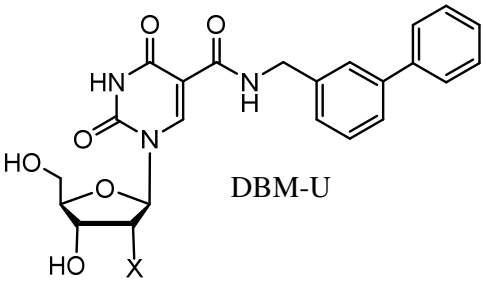
DPP-C ;



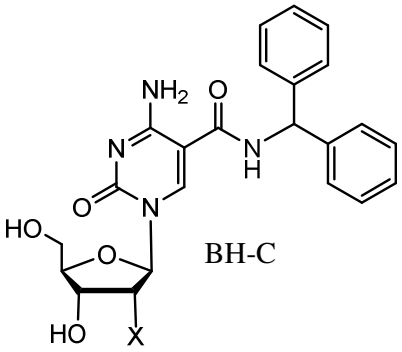
DPP-U ;



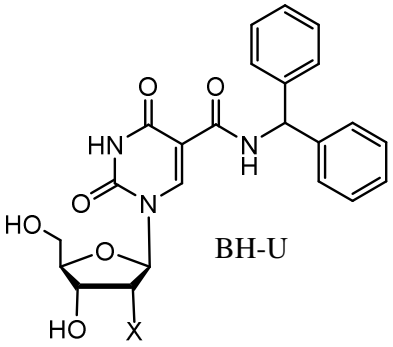
DBM-C ;



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.

[0007e] In one aspect, the disclosure provides an oligonucleotide comprising the compound as disclosed herein.

[0007f] In one aspect, the disclosure provides an aptamer comprising the compound as disclosed herein.

[0007g] In one aspect, the disclosure provides 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 as disclosed herein.

[0007h] In one aspect, the disclosure provides a composition comprising a plurality of aptamers as disclosed herein.

[0007i] In one aspect, the disclosure provides 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 as disclosed herein.

[0007j] In one aspect, the disclosure provides 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 as disclosed herein; and

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

[0007k] In one aspect, the disclosure provides a pharmaceutical composition comprising at least one aptamer as disclosed herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0007l] In one aspect, the disclosure provides 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 as disclosed herein or is an aptamer as disclosed herein.

[0007m] In one aspect, the disclosure provides 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 as disclosed herein; 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.

[0007n] In one aspect, the disclosure provides 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);

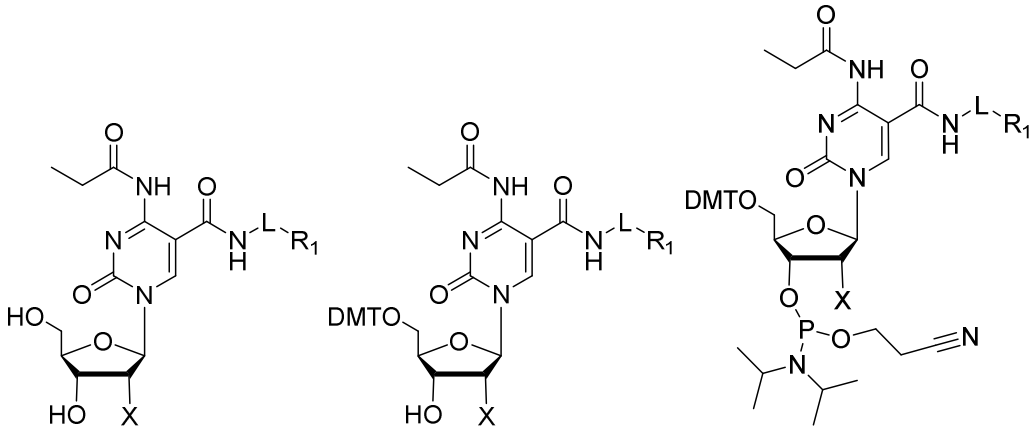
(c) dissociating the aptamer-target complex; and

(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 as disclosed herein.

[0007o] In one aspect, the disclosure provides a kit comprising the compound as disclosed herein, the oligonucleotide as disclosed herein, the aptamer as disclosed herein, the composition as disclosed herein, 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.

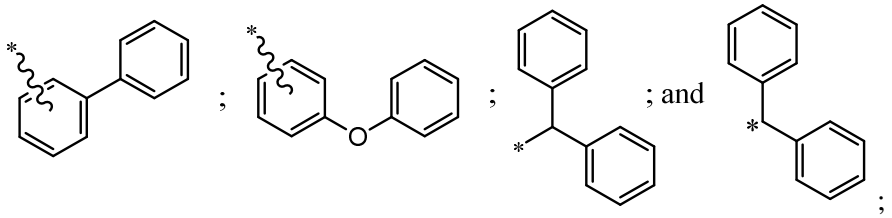
[0007p] In one aspect, the disclosure provides a compound comprising the structure of Formula III, Formula IV, or Formula V:



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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Fig. 1A-1I. Dose-dependent binding of round 7 enriched SELEX pools to various protein targets. For each binding curve, the fraction of library-protein complex was plotted as a function of protein concentration. Equilibrium binding constants (Kd values) were determined by fitting the data to a four-parameter sigmoid dose-response model. (**A**) Comparison of round 7 enriched SELEX pools for PP-dUTP and DPP-dUTP containing libraries for the XIAP target. (**B**) Comparison of round 7 enriched SELEX pools for PP-dUTP and PBn-dUTP containing libraries for the XIAP target. (**C**) Comparison of round 7 enriched SELEX pools for PP-dUTP and DPP-dUTP containing libraries for the IL-33 target. (**D**) Comparison of round 7 enriched SELEX pools for PP-dUTP and PBn-dUTP containing libraries for the IL-33 target. (**E**) Comparison of round 7 enriched SELEX pools for PP-dUTP and POP-dUTP containing libraries for the K-Ras target. (**F**) Comparison of round 7 enriched SELEX pools for PP-dUTP and DPP-dUTP containing libraries for the K-Ras target. (**G**) Comparison of round 7 enriched SELEX pools for PP-dUTP and PBn-dUTP containing libraries for the K-Ras target. (**H**) Comparison of round 7 enriched SELEX pools for PP-dUTP and BPE-dUTP containing libraries for the K-Ras