Claims

1. An isolated compound according to Structure 1:

X - R1 - R2 - A - R3 - B (Structure 1)

wherein:

X is a nucleic acid bonded to R1 through its 3’ or 5’ terminus;

R1 is a phosphodiester, thiophosphodiester, sulfate, amide, glycol, or is absent;

R2 is a C2-C10 alkyl, alkoxy, or aryl group, or is absent;

A is a covalent linker that comprises a reaction product of a first nucleophile and a first electrophile;

R3 is a covalent linker that comprises a C2-C10 alkyl, alkoxy, aryl, alkyldithio group, ether, thioether, ester, thiopropionate, or disulfide; and

B is a group that comprises a second nucleophile or a second electrophile having a structure that is the same as used in the formation of A.

2. The isolated compound of claim 1, wherein the first nucleophile and first electrophile of A comprise (i) a thiol and a maleimide, (ii) a thiol and a vinylsulfone, (iii) a thiol and a pyridyldisulfide, (iv) a thiol and an iodoacetamide, (v) a thiol and an acrylate, (vi) an azide and an alkyne, or (vii) an amine and a carboxyl group.

3. The isolated compound of claim 1, wherein the second nucleophile or second electrophile of B comprise a thiol, maleimide, vinylsulfone, pyridyldisulfide, iodoacetamide, acrylate, azide, alkyne, amine, or carboxyl group.

4. The isolated compound of claim 1, wherein:

R1 is a phosphodiester or thiophosphodiester;

R2 is a C2-C10 alkyl or alkoxy;

A comprises a reaction product of a first thiol and a first maleimide;

R3 comprises a disulfide; and

B comprises a second thiol or second maleimide having a structure that is the same as used in the formation of A.

5. The isolated compound of claim 1, wherein X is an siRNA.

6. The isolated compound of claim 1, wherein the nucleic acid is RNA, DNA, or comprises an artificial or non-natural nucleic acid analog.

7. The isolated compound of claim 6, wherein the nucleic acid is DNA.

8. The isolated compound of claim 7, wherein the DNA is an antisense DNA (aDNA) or antisense gapmer.

9. The isolated compound of claim 6, wherein the nucleic acid is RNA.

10. The isolated compound of claim 9, wherein the RNA is an antisense RNA (aRNA), CRISPR RNA (crRNA), long noncoding RNA (lncRNA), microRNA (miRNA), piwi-interacting RNA (piRNA), small interfering RNA (siRNA), messenger RNA (mRNA), short hairpin RNA (shRNA), small activating (saRNA), antagomir, or ribozyme.

11. The isolated compound of claim 1, wherein the nucleic acid is an aptamer.

12. The isolated compound of claim 1, wherein the nucleic acid further comprises a chemical modification.

13. The isolated compound of claim 12, wherein the chemical modification comprises a modified nucleoside, modified backbone, modified sugar, or modified terminus.

14. The isolated compound of claim 1, further comprising a targeting ligand.

15. The isolated compound of claim 14, wherein the targeting ligand is bound to the nucleic acid.

16. The isolated compound of claim 15, wherein the targeting ligand is bonded to the nucleic acid through its 3’ or 5’ terminus.

17. The isolated compound of claim 14, wherein the targeting ligand comprises N-Acetylgalactosamine (GalNAc), cholesterol, tocopherol, folate, 2-[3-(1,3-dicarboxypropyl)-ureido]pentanedioic acid (DUPA), or anisamide.

18. The isolated compound of claim 1, wherein the nucleic acid is single stranded.

19. The isolated compound of claim 1, wherein the nucleic acid is double stranded.

20. The isolated compound of claim 1, wherein the nucleic acid is 15-30, 17-27, 19-26, 20-25, 40-50, 40-150, 100-300, 1000-2000, or up to 10000 nucleotides in length.

21. The isolated compound of claim 1, wherein R1 is a phosphodiester or thiophosphodiester.

22. The isolated compound of claim 1, wherein R2 is C2-C10, C3-C6, or C6 alkyl.

23. The isolated compound of claim 1, wherein A comprises the reaction product of a first thiol and a first maleimide .

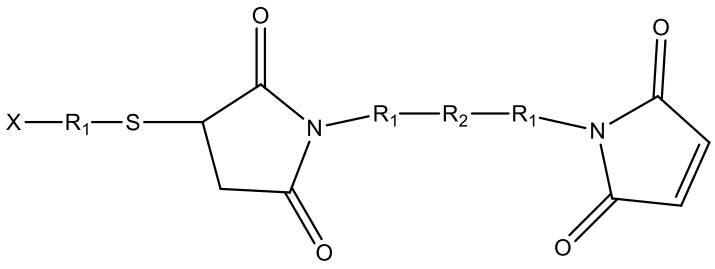
24. The isolated compound of claim 1, wherein R3 comprises a thiopropionate or disulfide.

25. The isolated compound of claim 1, wherein R3 comprises a linker that is cleavable under intracellular conditions.

26. The isolated compound of claim 1, wherein the compound is at least 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100 % pure.

27. The isolated compound of claim 1, wherein the compound is 85-95 % pure.

28. An isolated compound according to Structure 2:



(Structure 2)

wherein:

X is a nucleic acid bonded to R1 via a phosphate or thiophosphate at its 3’ or 5’ terminus;

each R1 is independently a C2-C10 alkyl, alkoxy, or aryl group; and

R2 is a linker that comprises a thiopropionate or disulfide group.

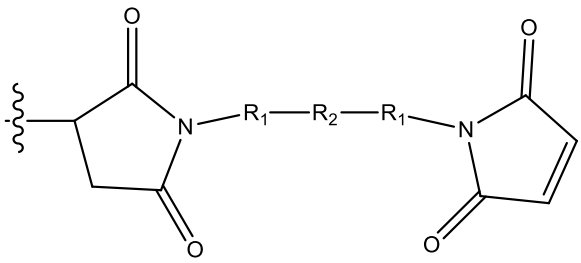
29. The isolated compound of claim 28, wherein R1-R2-R1 is cleavable under intracellular conditions.

30. The isolated compound of claim 28, wherein X comprises an siRNA.

31. The isolated compound of claim 28, wherein the compound is at least 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100 % pure.

32. The isolated compound of claim 28, wherein the compound is 85-95 % pure.

33. The isolated compound of claim 28, wherein the moiety



comprises the reaction product of a DTME (dithiobismaleimidoethane).

34. A method for synthesizing an isolated compound according to claim 1, the method comprising:
reacting a functionalized nucleic acid X - R1 - R2 - A' and a covalent linker A'' - R3 - B, wherein A' and A'' comprise the nucleophile and the electrophile, in a dilute solution of X - R1 - R2 - A' and with a stoichiometric excess of A'' - R3 – B, thereby forming the compound X - R1 - R2 - A - R3 - B (Structure 1).

35. The method of claim 34, further comprising the step of synthesizing the functionalized nucleic acid X - R1 - R2 - A', wherein A' comprises a thiol (-SH) by (i) introducing the thiol during solid phase synthesis of the nucleic acid using phosphoramidite oligomerization chemistry or (ii) reduction of a disulfide introduced during the solid phase synthesis.

36. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A' and the covalent linker A'' - R3 - B is carried out at a X - R1 - R2 - A' concentration of below 1 mM, 500 µM, 250 µM, 100 µM, or 50 µM.

37. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A' and the covalent linker A''- R3 - B is carried out at a X - R1 - R2 - A' concentration of 1 mM, 500 µM, 250 µM, 100 µM, or 50 µM.

38. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A’ and the covalent linker A’’ - R3 - B is carried out with a molar excess of A’’ - R3 – B of at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 100.

39. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A’ and the covalent linker A’’ - R3 - B is carried out with a molar excess of A’’ - R3 - B of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or 100.

40. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A’ and the covalent linker A’’ - R3 - B is carried out at a pH of below 7, 6, 5, or 4.

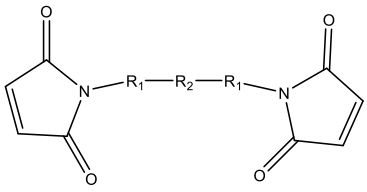
41. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A’ and the covalent linker A’’ - R3 - B is carried out at a pH of 7, 6, 5, or 4.

42. The method of claim 34, wherein reacting the functionalized nucleic acid X - R1 - R2 - A’ and the covalent linker A’’ - R3 - B is carried out in a solution comprising water and a water miscible organic co-solvent.

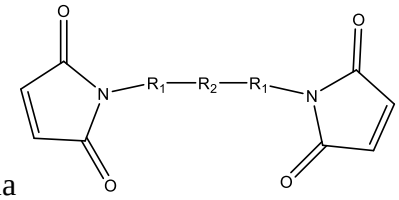
43. The method of claim 42, wherein the water miscible organic co-solvent comprises DMF, NMP, DMSO, or acetonitrile.

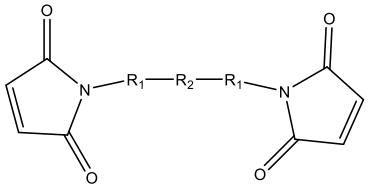
44. The method of claim 42, wherein the water miscible organic co-solvent comprises 10, 15, 20, 25, 30, 40, or 50 % (v/v) of the solution.

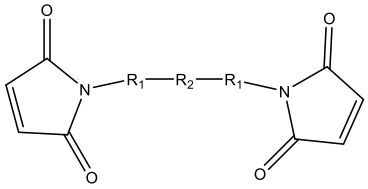
45. A method for synthesizing an isolated compound of claim 28 according to Structure 2, the method comprising:

reacting a functionalized nucleic acid X - R1 - SH and a compound of the formula 

, in a dilute solution of X - R1 - SH and with a

stoichiometric excess of the compound of formula , thereby forming the compound according to Structure 2.

46. The method of claim 45, wherein reacting the functionalized nucleic acid X - R1 - SH and the covalent linker  is carried out at a X - R1 - SH concentration of below 1 mM, 500 µM, 250 µM, 100 µM, or 50 µM.

47. The method of claim 45, wherein reacting the functionalized nucleic acid X - R1 - SH and the covalent linker  is carried out at a X - R1 - SH concentration of 1 mM, 500 µM, 250 µM, 100 µM, or 50 µM.