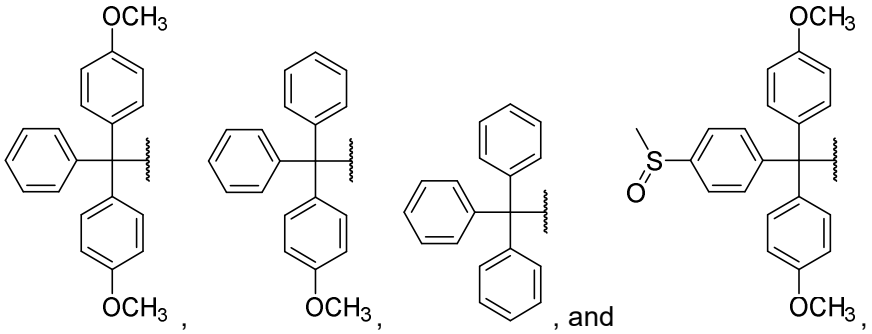
CLAIMS

What is claimed is:

1. A method of deprotecting an oligonucleotide comprising an amine group or a hydroxyl group protected by a trityl-based protecting group, the method comprising heating the oligonucleotide in an acid-free aqueous solution at a temperature of at least 60 °C, thereby cleaving the trityl-based protecting group to provide the oligonucleotide comprising the deprotected amine group or hydroxyl group.

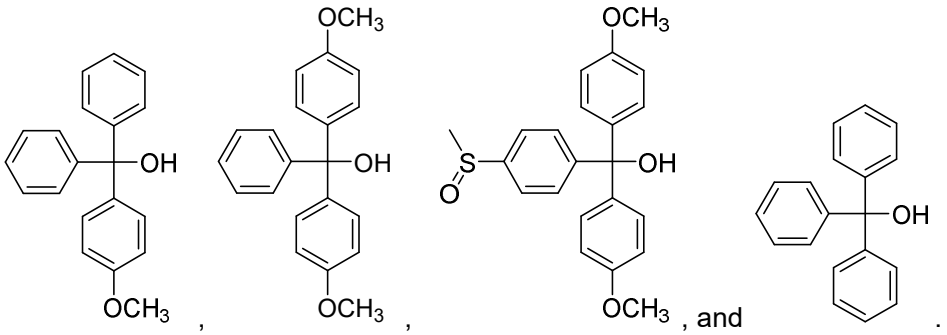
2. The method of claim 1, wherein (i) the trityl-based protecting group is selected from: trityl, monomethoxytrityl (MMTr), dimethoxytritryl (DMTr), and dimethoxymethylsulfonyltrityl (DMS(O)MTr); and/or (ii) the trityl-based protecting group has a structure selected from:



wherein  represents a covalent bond to the nitrogen atom of the amine group, or the oxygen atom of the hydroxyl group.

3. The method of claim 1 or claim 2, wherein deprotection of the amine group or hydroxyl group produces an insoluble trityl byproduct, and the method further comprises separating the insoluble trityl byproduct from the deprotected oligonucleotide; optionally wherein the insoluble trityl byproduct is separated by extraction, desalting, or precipitation and filtration.

4. The method of claim 3, wherein the insoluble trityl byproduct (i) is MMTr-OH, DMTr-OH, Tr-OH, or DMS(O)Tr-OH; and/or (ii) has a structure selected from:



5. The method of any one of claims 1-4, wherein

   (i) the amine group or hydroxyl group is attached to the 5' phosphate group of the oligonucleotide; and/or

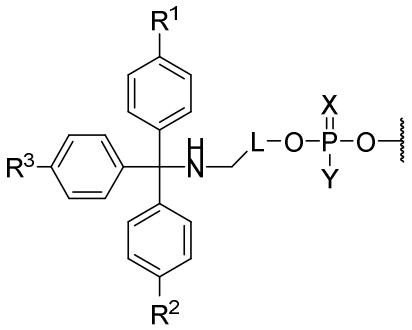
   (ii) the amine group or hydroxyl group is attached to the 5' phosphate group of the oligonucleotide through a linker group; optionally wherein the linker group comprises a 5-carbon to 12-carbon unbranched alkylene chain, optionally, wherein the alkylene chain further comprises at least one ether linkage, or optionally wherein the linker group is selected from: a succinate linker, a PEG3 linker, a PEG4 linker, a sarcosine-glutarate linker, a hydrazone linker, a disulfide linker, a valine-citrulline linker, a valine-alanine linker, a tris-hexylamino linker, a hydroquinone-O,O'-diacetate ("Q") linker, a hexylamine linker, a hexyloxy linker, pentethyleneglycol linker, and a derivative thereof.

6. The method of any one of claims 1-5, wherein the amine group of the oligonucleotide protected by a trityl-based protecting group is a primary amine group.

7. The method of claim 6, further comprising conjugating a ligand to the oligonucleotide by reacting the deprotected primary amine group of the oligonucleotide with a ligand molecule comprising an amine-reactive group, thereby forming a ligand-oligonucleotide conjugate; optionally wherein the amine-reactive group is selected from an ester, an NHS ester, and a carboxylic acid, or optionally wherein the ligand molecule is selected from from N-acetylgalactosamine (GalNAc), a triantennary cluster of N-acetylgalactosamine moieties, a lipid molecule, a small molecule peptide, a protein, and an antibody.

8. The method of any one of claims 1-7, wherein the percentage conversion of the oligonucleotide with trityl-based protecting group to the oligonucleotide with deprotected amine group or hydroxyl group is at least 95%, at least 97%, at least 99%, at least 99.5%, or at least 100%.

9. A method of deprotecting a 5'-amine modified oligonucleotide compound of Formula (**I**), the method comprising heating the 5'-amino modified oligonucleotide compound of Formula (**I**) in an acid-free aqueous solution at a temperature of at least 60 °C to form an oligonucleotide of Formula (**II**) and a byproduct compound of Formula (**III**):



(I)

or a salt thereof or a pharmaceutically acceptable salt thereof,

wherein:

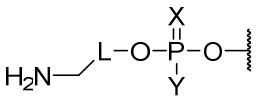
each of R^1, R^2, and R^3 is independently hydrogen, $-OCH_3$, or $-S(O)CH_3$;

L is an optional linker;

X is O or S;

Y is OH or SH; and

 represents a covalent bond to the 5'-end of the oligonucleotide;



(II)

or a salt thereof, or a pharmaceutically acceptable salt thereof,

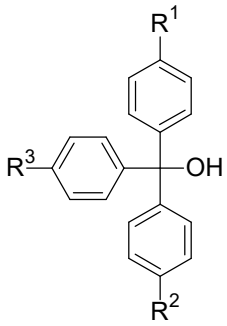
wherein:

X is O or S;

Y is -OH or -SH;

L is an optional linker; and

 represents a covalent bond to the 5'-end of the oligonucleotide;

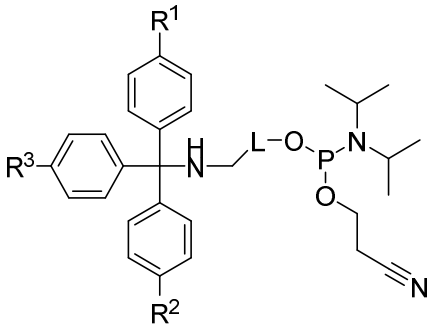


(III)

wherein:

each of R^1, R^2, and R^3 is independently hydrogen, $-OCH_3$, or $-S(O)CH_3$.

10. The method of claim 9, (i) further comprising separating the oligonucleotide compound of Formula (**II**) from the byproduct compound of Formula (**III**) by extraction, desalting, or precipitation and filtration; and/or (ii) wherein the compound of Formula (**I**) is prepared by reacting a phosphoramidite compound of Formula (**A**) with an oligonucleotide compound of Formula (**B**):

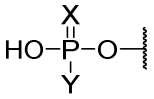


(**A**)

wherein:

each of R^1, R^2, and R^3 is independently hydrogen, $-OCH_3$, or $-S(O)CH_3$; and

L is an optional linker;



(**B**)

or a salt thereof, or a pharmaceutically acceptable salt thereof,

wherein:

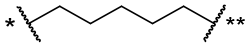
X is O or S;

Y is -OH or -SH; and

 represents a covalent bond to the 5'-end of the oligonucleotide.

11. The method of claim 9 or claim 10, wherein

(i) L is a divalent linker of Formula (**L-i**):



(**L-i**)

wherein:

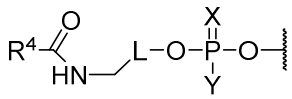
 represents a covalent bond to the nitrogen atom of the primary amine group; and

 represents a covalent bond to the oxygen atom of the phosphate group; or

(ii) the linker L comprises: a 5-carbon to 20-carbon branched or unbranched alkylene chain, or a 4-carbon to 20-carbon branched or unbranched alkylene chain with at least one ether linkage; or

(iii) the linker L comprises: a succinate linker, a PEG3 linker, a PEG4 linker, a sarcosine-glutarate linker, a hydrazone linker, a disulfide linker, a valine-citrulline linker, a valine-alanine linker, a tris-hexylamino linker, a hydroquinone-O,O'-diacetate ("Q") linker, a hexylamine linker, a hexyloxy linker, pentethyleneglycol, or a derivative thereof.

12. The method of any one of claims 9-11, further comprising reacting the oligonucleotide compound of Formula (**II**) with a ligand molecule comprising an amine-reactive group to form a ligand-oligonucleotide conjugate compound of Formula (**IV**):



(**IV**)

or a salt thereof, or a pharmaceutically acceptable salt thereof,

wherein:

L is a divalent linker;

X is S or O;

Y is -SH or -OH;

R^4 is a ligand; and

 represents a covalent bond to the 5'-end of the oligonucleotide.

13. The method of claim 12, wherein (i) the amine-reactive group is selected from an ester, an NHS ester, and a carboxylic acid; and/or (ii) the ligand R^4 is selected from N-

acetylgalactosamine (GalNAc), a triantennary cluster of N-acetylgalactosamine moieties, a lipid molecule, a small molecule peptide, a protein, and an antibody.

14. The method of any one of claims 1-13, wherein

(i) the acid-free aqueous solution is heated to a temperature of at least about 65°C, at least about 70°C, at least about 75°C, at least about 80°C, at least about 85°C, at least about 90°C, or at least about 95°C; and/or

(ii) the acid-free aqueous solution is heated to a temperature of about 60°C to about 100°C, about 60°C to about 80°C, about 60°C to about 75°C, or about 60°C to about 70°C; and/or

(iii) the acid-free aqueous solution is heated for at least about one hour, or at least about two hours; and/or

(iv) the acid-free aqueous solution is heated for about one hour to about two hours; and/or

(v) the acid-free aqueous solution has a pH of about pH 6.5 to about pH 8.0, about pH 6.8 to about pH 7.8, about pH 6.8 to about pH 7.6, about pH 7.0, about pH 7.2, or about pH 7.4; and/or

(vi) the percentage of depurinated oligonucleotide relative to the total deprotected oligonucleotide detected by MS analysis after the deprotection is less than 5%, less than 2%, less than 1%, less than 0.5%, less than 0.1%, or less than 0.01%; and/or

(vii) the oligonucleotide length is from 2-mer to 100-mer; optionally, wherein the oligonucleotide length is from 3-mer to 75-mer, from 5-mer to 50-mer, 15-mer to 50-mer, 15-mer to 40-mer, 15-mer to 30-mer, or 2-mer to 10-mer.

15. The method of any one of claims 9-14, wherein the percentage of oligonucleotide compound of Formula (**I**) converted to oligonucleotide compound of Formula (**II**) is at least 95%, at least 97%, at least 99%, at least 99.5%, or at least 100%.