SYNTHESIS OF 3'-RNA OLIGONUCLEOTIDES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/941,153 filed November 27, 2019, the content of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to generally to nucleic acid chemistry and to the chemical synthesis of oligonucleotides. More particularly, the invention relates to monomers and methods for synthesizing oligonucleotides comprising at least one nucleoside comprising a 3'-hydroxyl group.

BACKGROUND

[0003] Modified oligonucleotides are of great value in molecular biological research and in therapeutic applications. While, chemical synthesis of modified oligonucleotides is routine, ease and yield of many modified oligonucleotides is low. For example, commonly used protecting groups are unstable to conditions employed for deprotecting chemically synthesized oligonucleotides. This is especially problematic when preparing oligonucleotides comprising at least one nucleoside comprising a 3'-hydroxyl group. Thus, there remains a need in the art for monomers and methods for preparing such oligonucleotides. The present disclosure addresses, at least partially, this need.

SUMMARY

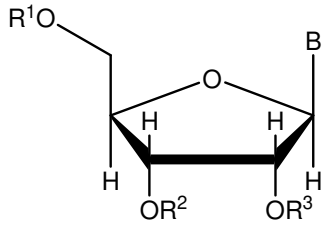
[0004] The disclosure provides monomers and methods for preparing oligonucleotides with improved yields and lower impurities where the oligonucleotide has at least one, e.g., two, three, four or more nucleosides with a 3'-hydroxyl group. Generally, the method comprises coupling a free hydroxyl group on a nucleoside or oligonucleotide with a nucleoside phsphoramidite monomer having a triisopropylsilylether (TIPS) protected 3'-hydroxyl group. The coupling forms a phosphite triester intermediate which can be oxidized or sulfurized to form a phosphate triester or phosphorothioate intermediate.

[0004a] In one aspect, the present disclosure refers to a method for synthesizing oligonucleotides having at least one nucleoside with a 3'-OH group, the method comprising: (i) coupling a free hydroxyl group on a nucleoside or oligonucleotide with a nucleoside phosphoramidite monomer having a triisopropylsilylether (TIPS) protected 3'-hydroxyl group to form a phosphite triester intermediate; and (ii) oxidizing or sulfurizing said phosphite triester intermediate to form a protected intermediate.

[0005] Oligonucleotides having a predetermined length and sequence can be prepared by the method. For example, the oligonucleotides comprising from about 6 to about 50

nucleotides can be prepared using the method and monomers described herein. In some ~~embodiments~~examples, the oligonucleotide comprises from about 10 to about 30 nucleotides.

[0006] In another ~~aspect~~example, the disclosure provides monomers, e.g., nucleoside phosphoramidite monomers having a triisopropylsilylether protected 3'-hydroxyl group. Generally, the monomer is of Formula (I):



FORMULA (I)

[0007] In Formula (I), B is a modified or unmodified nucleobase; R^1 is an acid labile hydroxyl protecting group; R^2 is $-Si(R^4)_3$; R^3 is $-P(NR^5R^6)OR^7$; each R^4 is independently optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl; R^5 and R^6 are independently optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl, or wherein R^5 and R^6 are linked to form a heterocyclyl; and R^7 is optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl.

[0008] In some monomers of Formula (I), B is adenine, guanine, cytosine or uracil; R^1 is dimethoxytrityl; R^4, R^5 and R^6 are isopropyl; and R^7 is β-cyanoethyl.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] This patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0010] **Figure 1** is an HPLC trace of sequence 1 (aUfcaaAf(U-2'-OTBS)CfAfcuuuAfuUfgaguuuc, SEQ ID NO: 1) having U-2'-OTBS at N17 position after deprotection with ammonium hydroxide in ethanol showing the generation of FLP-2'-OTBS, FLP-OH and the cleaved (16mer)

[0011] **Figure 2** is an PLC trace of sequence 2 (aUfcaaAf(U-3'-OTBS)CfAfcuuuAfuUfgaguuuc, SEQ ID NO: 2) having U-3'-OTBS at N17 position after deprotection with ammonium hydroxide in ethanol showing the generation of FLP-3'-OTBS, FLP-OH and the cleaved (16mer)

CLAIMS

What is claimed is:

1. A method for synthesizing oligonucleotides having at least one nucleoside with a 3'-OH group, the method comprising:
   (i) coupling a free hydroxyl group on a nucleoside or oligonucleotide with a nucleoside phosphoramidite monomer having a triisopropylsilylether (TIPS) protected 3'-hydroxyl group to form a phosphite triester intermediate; and
   (ii) oxidizing or sulfurizing said phosphite triester intermediate to form a protected intermediate.
2. The method of claim 1, wherein all synthetic steps are performed on an automated oligonucleotide synthesizer.
3. The method of claim 1, wherein oligonucleotide is synthesized at a large scale.
4. The method of claim 1, wherein said oxidizing is in presence of a weak base.
5. The method of claim 4, wherein said weak base is pyridine, lutidine, picoline or collidine.
6. The method of claim 1, wherein said oxidizing is in presence of I_2/H_2O.
7. The method of claim 1, wherein said sulfurizing is in presence of a sulfur transfer reagent.
8. The method of claim 7, wherein said sulfur transfer reagent is 3-(dimethylaminomethylidene)amino-3H-1,2,4-dithiazole-3-thione (DDTT) or 3*H*-1,2-benzodithiol-3-one 1,1-dioxide.
9. The method of claim 1, further comprising a step of deprotecting the protected intermediate with a base.
10. The method of claim 9, wherein said base is ammonium hydroxide, methylamine, or a mixture of ammonium hydroxide and methylamine.
11. The method of claim 9, wherein said treating with the base is at room temperature or an elevated temperature.
12. The method of claim 11, wherein said treating with the base is at a temperature of 30°C or higher.
13. The method of claim 9, wherein said treating with the base is for at least 30 minutes.
14. The method of claim 13, wherein said treating with the base is for at least 4 hours.

15. The method of claim 9, further comprising treating the base treated intermediate with a deprotecting reagent effective to convert the TIPS-protected hydroxyl group to a free hydroxyl group

16. The method of claim 15, wherein the deprotecting reagent comprises fluoride anions.

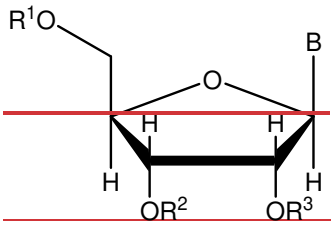
17. The method of claim 15, wherein the deprotecting reagent is HF.pyridine.

18. The method of claim 15, wherein said treating with the deprotecting reagent is at temperature of 30°C or higher.

19. The method of claim 1, wherein the oligonucleotide comprises from ~~about~~ 6 to ~~about~~ 50 nucleotides.

20. The method of claim 10, wherein the oligonucleotide comprises from ~~about~~ 10 to ~~about~~ 30 nucleotides.

~~21. A nucleoside monomer having the structure of Formula (I):~~



~~FORMULA (I)~~

~~wherein:~~

~~B is a modified or unmodified nucleobase;~~

~~R^1 is a hydroxyl protecting group;~~

~~R^2 is $-Si(R^4)_3$;~~

~~R^3 is H or $-P(NR^5R^6)OR^7$;~~

~~each R^4 is independently optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl;~~

~~R^5 and R^6 are independently optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl, or wherein R^5 and R^6 are linked to form a heterocyclyl; and~~

~~R^7 is optionally substituted alkyl, aryl, aralkyl, alkaryl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl or cycloalkynyl.~~

~~22. The nucleoside monomer of claim 21, wherein the hydroxyl protecting group is selected from the group consisting of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-fluorenylmethylcarbonate (Fmoc), o-nitrophenylcarbonyl, p-~~

phenylazophenylcarbonyl, phenylcarbonyl, p-chlorophenylcarbonyl, and 5′ (α-methyl-2-nitropiperonyl)oxycarbonyl (MeNPOC).

23. The nucleoside monomer of claim 21, wherein each R^4 is independently an optionally substituted C_1-C_6alkyl.

24. The nucleoside monomer of claim 21, wherein each R^4 is isopropyl.

25. The nucleoside monomer of claim 21, wherein R^5 and R^6 are independently optionally substituted C_1-C_6alkyl.

26. The nucleoside monomer of claim 21, wherein R^5 and R^6 are isopropyl.

27. The nucleoside monomer of claim 6, wherein R^7 is an optionally substituted C_1-C_6alkyl.

28. The nucleoside monomer of claim 21, wherein R^7 is methyl or β-cyanoethyl.

29. The nucleoside monomer of claim 6, wherein B is adenine, guanine, cytosine, thymine or uracil; R^1 is monomethoxytrityl or dimethoxytrityl; R^4 are independently optionally substituted C_1-C_6alkyl; R^5 and R^6 are independently optionally substituted C_1-C_6alkyl or R^5 and R^5 are linked to form a 4-8 membered heterocyclyl; and R^7 is an optionally substituted C_1-C_6alkyl.

30. The nucleoside monomer of claim 29, wherein B is adenine, guanine, cytosine or uracil; R^1 is dimethoxytrityl; R^4, R^5 and R^6 are isopropyl; and R^7 is β-cyanoethyl.