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

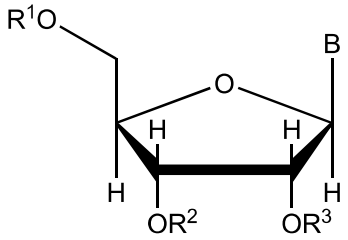
[0003] Modified oligonucleotides are of great value in molecular biological research and in therapeutic applications. While, chemical synthesis of modified oligonucleotides is routine, ease and yield of many modified oligonucleotides is low. For example, commonly used protecting groups are unstable to 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: a. coupling a free 5'-hydroxyl group on a nucleoside or oligonucleotide with a nucleoside phosphoramidite monomer having (i-a) a 2'-phosphoramidite group and (i-b) a triisopropylsilylether (TIPS) protected 3'-hydroxyl group to form a phosphite triester intermediate; b. oxidizing or sulfurizing said phosphite triester intermediate to form a protected intermediate; and c. deprotecting the protected intermediate with a base, wherein

said treating with the base is at an elevated temperature, wherein the nucleoside phosphoramidite monomer has 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 $–P(NR^5R^6)OR^7$;

each R^4 is independently isopropyl;

R^5 and R^6 are independently optionally substituted alkyl, or wherein R^5 and R^6 are linked to form a heterocyclyl; and

R^7 is β-cyanoethyl.

[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

CLAIMS

What is claimed is:

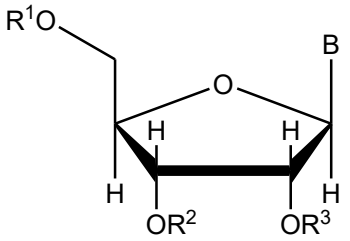
1. A method for synthesizing oligonucleotides having at least one nucleoside with a 3'-OH group, the method comprising:

   a. coupling a free 5'-hydroxyl group on a nucleoside or oligonucleotide with a nucleoside phosphoramidite monomer having (i-a) a 2'-phosphoramidite group and (i-b) a triisopropylsilylether (TIPS) protected 3'-hydroxyl group to form a phosphite triester intermediate;

   b. oxidizing or sulfurizing said phosphite triester intermediate to form a protected intermediate; and.

   c. deprotecting the protected intermediate with a base, wherein said treating with the base is at an elevated temperature,

   wherein the nucleoside phosphoramidite monomer has the structure of Formula (I):



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R^7 is β-cyanoethyl.

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, wherein said base is ammonium hydroxide, methylamine, or a mixture of ammonium hydroxide and methylamine.

10. The method of claim 1, wherein said treating with the base is at a temperature of 30°C or higher.

11. The method of claim 10, wherein said treating with the base is at a temperature of between 32°C and 65°C.

12. The method of claim 11, wherein said treating with the base is at a temperature of 35°C.

13. The method of claim 1, 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 1, 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 6 to 50 nucleotides.

20. The method of claim 19, wherein the oligonucleotide comprises from 10 to 30 nucleotides.

21. The method of claim 1, wherein in the nucleoside monomer of Formula (I), 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).

22. The method of claim 1, wherein R^5 and R^6 are independently optionally substituted C_1-C_6alkyl.

23. The method of claim 1, wherein R^5 and R^6 are isopropyl.

24. The method of claim 1, wherein in the nucleoside monomer of Formula (I), B is adenine, guanine, cytosine, thymine or uracil; R^1 is monomethoxytrityl or dimethoxytrityl;; 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.

25. The method of claim 24, wherein B is adenine, guanine, cytosine or uracil; R^1 is dimethoxytrityl; and R^5 and R^6 are isopropyl.