

BRIEF SUMMARY

[007] To address this need, the disclosure describes nucleotide-based intermediate compounds (*i.e.*, oligonucleotide fragments), as well as methods of making single-stranded (ss) oligonucleotides by ligating a plurality of the oligonucleotide fragments herein and ultimately making double-stranded (ds) therapeutic oligonucleotides such as RNAi agents.

[008] With regard to the intermediate compounds, the disclosure describes oligonucleotide fragments having a nucleotide sequence selected from any one of SEQ ID NOS:5 to 37.

[008a] In an aspect, the disclosure describes an oligonucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:5 to 37, or a pharmaceutically acceptable salt thereof.

[009] With regard to the methods, the disclosure describes methods of making a nucleic acid having a nucleotide sequence of SEQ ID NO:1 that includes a step of ligating the following combination of oligonucleotide fragments having nucleotide sequences selected from:

- (a) SEQ ID NOS:5, 6 and 7;
- (b) SEQ ID NOS:5, 10 and 11;
- (c) SEQ ID NOS:5, 12 and 13;
- (d) SEQ ID NOS:7, 14 and 15;
- (e) SEQ ID NOS:7, 18 and 19;
- (f) SEQ ID NOS: 7, 22 and 23; and
- (g) SEQ ID NOS: 5, 26 and 27.

[009a] In another aspect, the disclosure describes a method of making a nucleic acid of SEQ ID NO:1, the method comprising the step of:

ligating three oligonucleotide fragments selected from the group consisting of:

- (a) SEQ ID NOS:5, 6 and 7,
- (b) SEQ ID NOS:5, 10 and 11,
- (c) SEQ ID NOS:5, 12 and 13,
- (d) SEQ ID NOS:7, 14 and 15,
- (e) SEQ ID NOS:7, 18 and 19,
- (f) SEQ ID NOS: 7, 22 and 23, and
- (g) SEQ ID NOS: 5, 26 and 27.

[0010] In addition, the disclosure describes methods of making a nucleic acid having a nucleotide sequence of SEQ ID NO:2 that includes a step of ligating the following combinations of oligonucleotide fragments having nucleotide sequences selected from:

- (a') SEQ ID NOS:8 and 9;
- (b') SEQ ID NOS:16 and 17;
- (c') SEQ ID NOS:20 and 21; and
- (d') SEQ ID NOS:24 and 25.

[0010a] In another aspect, the disclosure describes a method of making a nucleic acid of SEQ ID NO:2, the method comprising the step of:

ligating two oligonucleotide fragments selected from the group consisting of:

- (a') SEQ ID NOS:8 and 9;
- (b') SEQ ID NOS:16 and 17;
- (c') SEQ ID NOS:20 and 21; and
- (d') SEQ ID NOS:24 and 25.

[0011] The methods above can include an additional step of annealing SEQ ID NO:1 and SEQ ID NO:2 to form a RNAi agent that modulates apolipoprotein(a) gene (*LPA*) expression.

[0011a] In another aspect, the present disclosure describes a method of making a RNAi agent having a sense strand of SEQ ID NO:1 and an antisense strand of SEQ ID NO:2, the method comprising the steps of:

forming the sense strand of SEQ ID NO:1 by ligating three oligonucleotide fragments selected from the group consisting of:

- (a) SEQ ID NOS:5, 6 and 7, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:6 to SEQ ID NO:7,
- (b) SEQ ID NOS:5, 10 and 11, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:10 to SEQ ID NO:11,
- (c) SEQ ID NOS:5, 12 and 13, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:12 to SEQ ID NO:13,
- (d) SEQ ID NOS:7, 14 and 15, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:14 to SEQ ID NO:15 to SEQ ID NO:7,
- (e) SEQ ID NOS:7, 18 and 19, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:18 to SEQ ID NO:19 to SEQ ID NO:7,

(f) SEQ ID NOS: 7, 22 and 23, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:22 to SEQ ID NO:23 to SEQ ID NO:7, and

(g) SEQ ID NOS: 5, 26 and 27, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:26 to SEQ ID NO:27;

forming the antisense strand of SEQ ID NO:2 by ligating two oligonucleotide fragments selected from the group consisting of:

(a') SEQ ID NO:8 and 9, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:9 to SEQ ID NO:8

(b') SEQ ID NOS:16 and 17, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:17 to SEQ ID NO:16,

(c') SEQ ID NOS:20 and 21, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:21 to SEQ ID NO:20, and

(d') SEQ ID NOS:24 and 25, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:25 to SEQ ID NO:24; and

forming the RNAi agent by annealing complementary nucleotides of the sense strand of SEQ ID NO:1 and the antisense strand of SEQ ID NO:2.

[0012] Moreover, the disclosure describes methods of making a nucleic acid having a nucleotide sequence of SEQ ID NO:3 that includes a step of ligating the following combination of oligonucleotide fragments having nucleotide sequences selected from:

(a) SEQ ID NOS:7, 32 and 33.

[0012a] In another aspect, the present disclosure describes a method of making a nucleic acid of SEQ ID NO:3, the method comprising the step of:

ligating three oligonucleotide fragments of (a) SEQ ID NOS:7, 32 and 33, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:32 to SEQ ID NO:33 to SEQ ID NO:7.

[0013] In addition, the disclosure describes methods of making a nucleic acid having a nucleotide sequence of SEQ ID NO:4 that includes a step of ligating the following combination of oligonucleotide fragments having nucleotide sequences selected from:

(a') SEQ ID NOS:34 and 35.

[0013a] In another aspect, the disclosure describes a method of making a nucleic acid of SEQ ID NO:4, the method comprising the step of:

ligating two oligonucleotide fragments of (a') SEQ ID NOS:34 and 35, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:35 to SEQ ID NO:34.

[0014] The methods above can include an additional step of annealing SEQ ID NO:3 and SEQ ID NO:4 to form a RNAi agent that modulates angiopoietin-like 3 gene (*ANGPTL3*) expression.

[0014a] In another aspect, the disclosure describes a method of making a RNAi agent having a sense strand of SEQ ID NO:3 and an antisense strand of SEQ ID NO:4, the method comprising the steps of:

forming the sense strand of SEQ ID NO:3 by ligating three oligonucleotide fragments of (a) SEQ ID NOS:7, 32 and 33, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:32 to SEQ ID NO:33 to SEQ ID NO:7;

forming the antisense strand of SEQ ID NO:4 by ligating two oligonucleotides of (a') SEQ ID NOS:34 and 35, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:35 to SEQ ID NO:34; and

forming the RNAi agent by annealing complementary nucleotides of the sense strand of SEQ ID NO:3 and the antisense strand of SEQ ID NO:4.

[0015] In any of the above methods, the ligating can be mediated by an enzyme. In some instances, the enzyme is a ligase such as a naturally occurring ligase or a non-naturally occurring ligase. In other instances, the ligase is a DNA ligase. In certain instances, the ligase is a RNA ligase.

[0016] An advantage of the methods herein includes process improvements such as, for example, shorter fragments initially produced via solid phase oligonucleotide synthesis (SPOS) allow for increased purity and higher yields. With shorter fragments, more route flexibility is available to incorporate modified nucleotides, and an ability to redesign fragment structures to address more difficult segments of the strands.

[0017] An advantage of the methods herein includes an improved control strategy for impurities during the synthesis, which can include improved detection and characterization of impurities at a fragment stage and an improved final impurity profile for a crude oligonucleotide duplex.

[0018] An advantage of the methods herein includes improved final duplex purity due to rejection of certain classes of fragment impurities based on their inability to participate in the ligation step or reduced adherence to the complementarity principle during fragment self-assembly in the annealing step.

[0019] An advantage of the methods herein includes reduced in unit operations required to produce an oligonucleotide duplex as a single step used to form a duplex material from fragment building blocks allowing to reduce downstream operations to a single chromatography and ultrafiltration (vs need for a separate step for each strand as in a conventional approach).

[0020] An advantage of the methods herein includes that synthesis of shorter fragments via SPOS can allow for reduced washing cycles and for reduced volumes of reagents, leading to a reduced process mass intensity (PMI).

CLAIMS

The invention claimed is:

1. An oligonucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:5 to 37, or a pharmaceutically acceptable salt thereof.
2. A method of making a nucleic acid of SEQ ID NO:1, the method comprising the step of:
ligating three oligonucleotide fragments selected from the group consisting of:
(a) SEQ ID NOS:5, 6 and 7,
(b) SEQ ID NOS:5, 10 and 11,
(c) SEQ ID NOS:5, 12 and 13,
(d) SEQ ID NOS:7, 14 and 15,
(e) SEQ ID NOS:7, 18 and 19,
(f) SEQ ID NOS: 7, 22 and 23, and
(g) SEQ ID NOS: 5, 26 and 27.
3. The method of claim 2, wherein the three oligonucleotide fragments are (a) SEQ ID NOS:5, 6 and 7, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:6 to SEQ ID NO:7.
4. The method of claim 2, wherein the three oligonucleotide fragments are (b) SEQ ID NOS:5, 10 and 11, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:10 to SEQ ID NO:11.
5. The method of claim 2, wherein the three oligonucleotide fragments are (c) SEQ ID NOS:5, 12 and 13, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:12 to SEQ ID NO:13.
6. The method of claim 2, wherein the three oligonucleotide fragments are (d) SEQ ID NOS:7, 14 and 15, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:14 to SEQ ID NO:15 to SEQ ID NO:7.

7. The method of claim 2, wherein the three oligonucleotide fragments are (e) SEQ ID NOS:7, 18 and 19, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:18 to SEQ ID NO:19 to SEQ ID NO:7.
8. The method of claim 2, wherein the three oligonucleotide fragments are (f) SEQ ID NOS: 7, 22 and 23, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:22 to SEQ ID NO:23 to SEQ ID NO:7.
9. The method of claim 2, wherein the three oligonucleotide fragments are (g) SEQ ID NOS: 5, 26 and 27, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:26 to SEQ ID NO:27.
10. A method of making a nucleic acid of SEQ ID NO:2, the method comprising the step of:
ligating two oligonucleotide fragments selected from the group consisting of:
(a') SEQ ID NOS:8 and 9;
(b') SEQ ID NOS:16 and 17;
(c') SEQ ID NOS:20 and 21; and
(d') SEQ ID NOS:24 and 25.
11. The method of claim 10, wherein the two oligonucleotide fragments are (a') SEQ ID NOS:8 and 9, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:9 to SEQ ID NO:8.
12. The method of claim 10, wherein the two oligonucleotide fragments are (b') SEQ ID NOS:16 and 17, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:17 to SEQ ID NO:16.
13. The method of claim 10, wherein the two oligonucleotide fragments are (c') SEQ ID NOS:20 and 21, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:21 to SEQ ID NO:20.

14. The method of claim 10, wherein the two oligonucleotide fragments are (d') SEQ ID NOS:24 and 25, and wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:25 to SEQ ID NO:24.

15. A method of making a nucleic acid of SEQ ID NO:3, the method comprising the step of:

ligating three oligonucleotide fragments of (a) SEQ ID NOS:7, 32 and 33, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:32 to SEQ ID NO:33 to SEQ ID NO:7.

16. A method of making a nucleic acid of SEQ ID NO:4, the method comprising the step of:

ligating two oligonucleotide fragments of (a') SEQ ID NOS:34 and 35, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:35 to SEQ ID NO:34.

17. A method of making a RNAi agent having a sense strand of SEQ ID NO:1 and an antisense strand of SEQ ID NO:2, the method comprising the steps of:

forming the sense strand of SEQ ID NO:1 by ligating three oligonucleotide fragments selected from the group consisting of:

(a) SEQ ID NOS:5, 6 and 7, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:6 to SEQ ID NO:7,

(b) SEQ ID NOS:5, 10 and 11, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:10 to SEQ ID NO:11,

(c) SEQ ID NOS:5, 12 and 13, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:12 to SEQ ID NO:13,

(d) SEQ ID NOS:7, 14 and 15, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:14 to SEQ ID NO:15 to SEQ ID NO:7,

(e) SEQ ID NOS:7, 18 and 19, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:18 to SEQ ID NO:19 to SEQ ID NO:7,

(f) SEQ ID NOS: 7, 22 and 23, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:22 to SEQ ID NO:23 to SEQ ID NO:7, and

(g) SEQ ID NOS: 5, 26 and 27, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:5 to SEQ ID NO:26 to SEQ ID NO:27;

forming the antisense strand of SEQ ID NO:2 by ligating two oligonucleotide fragments selected from the group consisting of:

(a') SEQ ID NO:8 and 9, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:9 to SEQ ID NO:8

(b') SEQ ID NOS:16 and 17, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:17 to SEQ ID NO:16,

(c') SEQ ID NOS:20 and 21, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:21 to SEQ ID NO:20, and

(d') SEQ ID NOS:24 and 25, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:25 to SEQ ID NO:24; and

forming the RNAi agent by annealing complementary nucleotides of the sense strand of SEQ ID NO:1 and the antisense strand of SEQ ID NO:2.

18. A method of making a RNAi agent having a sense strand of SEQ ID NO:3 and an antisense strand of SEQ ID NO:4, the method comprising the steps of:

forming the sense strand of SEQ ID NO:3 by ligating three oligonucleotide fragments of (a) SEQ ID NOS:7, 32 and 33, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:32 to SEQ ID NO:33 to SEQ ID NO:7;

forming the antisense strand of SEQ ID NO:4 by ligating two oligonucleotides of (a') SEQ ID NOS:34 and 35, wherein the fragments are ligated from 5' end to 3' end as SEQ ID NO:35 to SEQ ID NO:34; and

forming the RNAi agent by annealing complementary nucleotides of the sense strand of SEQ ID NO:3 and the antisense strand of SEQ ID NO:4.

19. The method of any one of claims 2 to 18, wherein the ligating step is mediated by an enzyme.

20. The method of claim 19, wherein the enzyme is a deoxyribonucleic acid (DNA) ligase or a ribonucleic acid (RNA) ligase.

21. The method of claim 19, wherein the enzyme is a RNA ligase and is selected from group consisting of RNA Ligase 1 and RNA Ligase 2.