

WHAT IS CLAIMED IS:

1. A modified small interfering RNA (siRNA), comprising a sense strand having 19 nucleotides and an antisense strand having 21 nucleotides,

wherein (a) the antisense strand contains (i) 7 to 9 2'-fluoro-modified nucleotides, and (ii) 12 to 14 2'-O-methyl-modified nucleotides; and (b) the sense strand contains (i) 2 to 5 2'-fluoro-modified nucleotides, and (ii) 11 to 16 2'-O-methyl-modified nucleotides;

wherein the sense strand, the antisense strand, or both contain one or more phosphothioate (PS) bonds; and

wherein the sense strand and the antisense strand form a double-stranded siRNA, optionally with a two-nucleotide overhang at the 3' end of the antisense strand.

2. The modified siRNA of claim 1, wherein the antisense strand contains a modified 5'-phosphate.

3. The modified siRNA of claim 2, wherein the modified 5'-phosphate is *E*-vinyl phosphonate or phosphorothiorate.

4. The modified siRNA of any one of claims 1-3, wherein the antisense strand contains the 2'-fluoro-modified nucleotides at 7 or more of positions 2-12, 14-16, and 18.

5. The modified siRNA of claim 4, wherein the antisense strand comprises 2'-fluoro-modified nucleotides at position 2 or 3, and position 14, and optionally position 18.

6. The modified siRNA of any one of claims 1-5, wherein in (a), the antisense strand contains the 2'-O-methyl-modified nucleotides at 12 or more of positions 1, 2-13, and 15-21.

7. The modified siRNA of claim 6, wherein the antisense strand comprises 2'-O-methyl-modified nucleotides at positions 1, 13, 17 and 19-21.

8. The modified siRNA of any one of claims 1-7, wherein in (b), the sense strand

contains the 2'-fluoro-modified nucleotides at two or more of positions 5 and 7-11.

9. The modified siRNA of claim 8, wherein the sense strand comprises 2'-fluoro-modified nucleotides at positions 5 and 7-10 or at positions 5, 7, 9, and 11.

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10. The modified siRNA of any one of claims 1-9, wherein the sense strand contains the 2'-O-methyl-modified nucleotides at 11 or more of positions of 1-6, 8, and 10-19.

10 11. The modified siRNA of claim 10, wherein the sense strand comprises 2'-O-methyl-modified nucleotides at positions 4, 6, and 11-19.

12. The modified siRNA of claim 11, wherein the sense strand further comprises 2'-O-methyl-modified nucleotides at positions 8 and 10.

15 13. The modified siRNA of any one of claims 10-12, wherein one or more positions 1-3 in the sense strand contain 2'-O-methyl-modified nucleotides.

14. The modified siRNA of any one of claims 10-12, wherein one or more of positions 1-3 in the sense strand are not modified.

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15. The modified siRNA of any one of claims 1-14, wherein the sense strand contains a 2'-deoxynucleotide, which optionally is located as position 9.

25 16. The modified siRNA of any one of claims 1-15, wherein the antisense strand contains PS bonds between positions 1 and 2, positions 2 and 3, positions 19 and 20, and/or positions 20 and 21.

30 17. The modified siRNA of any one of claims 1-16, wherein the sense strand contains PS bonds between positions 1 and 2, positions 2 and 3, positions 4 and 5, positions 5 and 6, positions 17 and 18, and/or positions 18 and 19.

18. The modified siRNA of claim 1, wherein the antisense strand of (a) and the sense strand of (b) have the following formulas:

- (i) 5'-mffmmffmmffmmffmmfmmm-3'
 5 3'-mmmmmmmmmdffmmmmmm-5';
- (ii) 5'-mmfmfmfmfmfmfmfmfmfm-3'
 3'-mmmmmmmmmmffffmfmmmm-5';
- (iii) 5'-mmfmfmfmfmfmfmfmfmfm-3'
 3'-mmmmmmmmmmffffmfmr-5';
- 10 (iv) 5'-mfmfmfmfmmmmmfmfmfmm-3'
 3'-mmmmmmmmmmffffmfmr-5'
- (v) 5'-mfmmffmmffmmmmmfmfmfmm-3'
 3'-mmmmmmmmmmffffmfmmmm-5';
- (vi) 5'-mfmfmfmfmfmfmfmfmfmm-3'
 15 3'-mmmmmmmmmfmfmfmfmmmm-5';
- (vii) 5'-mfmfmfmfmfmfmfmfmfmm-3'
 3'-mmmmmmmmmfmfmfmfmr-5'' or
- (viii) 5'-mfmfmfmfmfmfmfmfmmmm-3'
 3'-mmmmmmmmmfmfmfmfmr-3'; and

20 wherein m represents 2'-O-methyl nucleotide, f represents 2'-fluoro nucleotide, r represents unmodified nucleotide, and d represents DNA residue.

19. The modified siRNA of claim 18, wherein the antisense strand of (a) and the sense strand of (b) have the following formulas:

- 25 (i) 5'-m*f*fmmffmmffmmffmmfm*m*m-3'
 3'-m*m*mmmmmmmdffmmmm*m*m-5';
- (ii) 5'-m*m*fmfmfmfmfmfmfmfmfm*m*m-3'
 3'-m*m*mmmmmmmmffffmfmm*m*m-5';
- (iii) 5'-m*m*fmfmfmfmfmfmfmfmfm*m*m-3'
 30 3'-m*m*mmmmmmmmffffm*f*mr-5';

- (iv) 5'-m*f*mfmfmffmfmfmfmfmfm*m*m-3'
3'-m*m*mmmmmmmmffffm*f*mrrr-5'
- (v) 5'-m*f*mffmfmffmfmfmfmfmfm*m*m-3'
3'-m*m*mmmmmmmmffffmfm*m*m-5';
- 5 (vi) 5'-m*f*mfmfmfmfmfmfmfmfmfm*m*m-3'
3'-m*m*mmmmmmfmfmfmfmfm*m*m-5';
- (vii) 5'-m*f*mfmfmfmfmfmfmfmfmfm*m*m-3'
3'-m*m*mmmmmmfmfmfm*f*mrrr-5'' or
- (viii) 5'-m*f*mfmfmfmfmfmfmfmfmfm*m*m-3'
10 3'-m*m*mmmmmmfmfmfm*f*mrrr-3'; and

wherein m represents 2'-O-methyl nucleotides, f represents 2'-fluoro nucleotides, r represents unmodified nucleotides, d represents DNA residue, and * represents PS bonds.

20. The modified siRNA of any one of claims 1-19, wherein the sense strand is
15 conjugated to a targeting moiety, which optionally is N-Acetylgalactosamine (GalNAc).

21. A pharmaceutical composition comprising the siRNA of any one of claims 1-20 and a pharmaceutically acceptable carrier.

20 22. A method for modulating gene expression, comprising contacting cells with the siRNA of any one of claims 1-20, or the pharmaceutical composition of claim 21, wherein the siRNA targets a gene of interest.

23. The siRNA of any one of claims 1-20, or the pharmaceutical composition of claim
25 21, for use in treating a disease in a subject, wherein the disease is associated with a gene of interest targeted by the siRNA.