SIRNA COMPOUNDS COMPRISING TERMINAL SUBSTITUTIONS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications Serial Nos. 61/264668, filed 26-Nov-2009, 61/295721 filed 17-Jan-2010 and 61/372072 filed 9-Aug-2010, which are incorporated herein by reference in their entirety and for all purposes.

FIELD OF THE INVENTION

The present invention relates to modified siRNA compounds, pharmaceutical compositions comprising same and methods of use thereof for the inhibition of gene expression. The compounds and compositions exhibit beneficial properties including knock down activity of target genes and are useful in the treatment of subjects suffering from diseases or conditions and or symptoms associated with such diseases or conditions or at risk of contracting diseases or conditions in which gene expression has adverse consequences.

BACKGROUND OF THE INVENTION

PCT Patent Publication Nos. WO 2008/104978 and WO 2009/044392 to the assignee of the present invention and hereby incorporated by reference in their entirety, disclose novel siRNA structures.

There remains a need for active and effective siRNA therapeutic agents which exhibit enhanced knock down activity, increased stability and or reduced off target effects.

SUMMARY ~~OF THE INVENTION~~

The double stranded RNA (dsRNA) compounds disclosed herein possess structures and modifications which may, for example increase activity, increase stability, reduce immunogenicity, enhance loading into the RISC complex, and or minimize toxicity; the novel modifications of the siRNAs are beneficially applied to double stranded RNA useful in preventing or attenuating target gene expression.

Provided herein are double stranded (duplex) oligonucleotide compounds useful for the down regulation of gene expression. The present disclosure is based in part on the unexpected observation that a double stranded nucleic acid molecule comprising a sense strand and a complementary antisense strand and having a mismatch between the 5' terminal nucleotide of the antisense strand and the nucleotide of the target RNA show potent activity in down regulating target genes. In preferred embodiments the dsRNA comprises an adenosine, deoxyadenosine, uridine, deoxyuridine, ribothymidine or thymidine substituted at position 1 (5' terminus) of the antisense strand rather in place of a cytidine or guanine.

In the first aspect, there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5'	N^1-(N)x-Z	3' (antisense strand)
3'	Z'-N^2-(N')y-z"	5' (sense strand)

wherein each of N^2, N and N' is an unmodified ribonucleotide, a modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;

wherein x=y=18;

wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;

wherein:

N^1 is selected from the group consisting of adenosine and deoxyadenosine when the corresponding nucleotide in the target RNA sequence is adenosine; or

N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is cytidine; or

N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is guanosine; or

N^1 is selected from the group consisting of uridine and deoxyuridine when the corresponding nucleotide in the target RNA sequence is uridine;

wherein z" may be present or absent, but if present is a capping moiety covalently attached at the 5' terminus of N^2—(N')y; and

wherein each of Z and Z' is independently present or absent, but if present is independently 1-5 consecutive nucleotides, 1-5 consecutive non-nucleotide moieties or a combination of 1-5 nucleotides and non-nucleotide moieties, covalently attached at the 3' terminus of the strand in which it is present;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

In the second aspect there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5' N^1-(N)x-Z 3' (antisense strand)
3' Z'-N^2-(N')y-z" 5' (sense strand)

wherein each of N^2, N and N' is an unmodified ribonucleotide, a modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;

wherein x=y=18;

wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;

wherein N^1 is selected from the group consisting of adenosine and deoxyadenosine, and the corresponding nucleotide in the target RNA is adenosine;

wherein z" may be present or absent, but if present is a capping moiety covalently attached at the 5' terminus of N^2—(N')y; and

wherein each of Z and Z' is independently present or absent, but if present is independently 1-5 consecutive nucleotides, 1-5 consecutive non-nucleotide moieties or a combination of 1-5 nucleotides and non-nucleotide moieties, covalently attached at the 3' terminus of the strand in which it is present;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

In the third aspect, there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5' N^1-(N)x-Z 3' (antisense strand)
3' Z'-N^2-(N')y-z" 5' (sense strand)

wherein N, N', (N)x, (N')y, x, y, Z' and z" are defined in the second aspect;

wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;

wherein, Z' is absent or present, Z is present and comprises two alkyl moieties covalently linked to each other via a phosphodiester bond, wherein N^2 comprises uridine or deoxyuridine; and wherein N^1 comprises adenosine when the corresponding nucleotide in the target RNA is adenosine, guanosine or cytidine;

or a pharmaceutically acceptable salt the double stranded nuclei acid.

In the fourth aspect, there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5' N^1-(N)x-Z 3' (antisense strand)
3' Z'-N^2-(N')y-z" 5' (sense strand)

wherein N^2, N, N', (N)x, (N')y, x, y, Z, Z' and z" are as defined in the second aspect;
wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;
wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;
wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine, when the corresponding nucleotide in the target RNA is cytidine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

In the fifth aspect, there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5' N^1-(N)x-Z 3' (antisense strand)
3' Z'-N^2-(N')y-z" 5' (sense strand)

wherein N^2, N, N', (N)x, (N')y, x, y, Z, Z' and z" are as defined in the second aspect;

wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA is guanosine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

In the sixth aspect, there is provided a double-stranded nucleic acid having structure (A) set forth below:

(A) 5' N^1-(N)x-Z 3' (antisense strand)
3' Z'-N^2-(N')y-z" 5' (sense strand)

wherein N^2, N, N', (N)x, (N') y, x, y, Z, Z' and z" are as defined in the second aspect;

wherein the sequence of N^2—(N')y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;
wherein N^1 is uridine or deoxyuridine, when the corresponding nucleotide in the target RNA is uridine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

According to one ~~aspect~~embodiment provided are modified double stranded nucleic acid molecules having structure (A) set forth below:

(A) 5' N^1-(N)x - Z 3' (antisense strand)

3' Z'-N^2-(N')y –z" 5' (sense strand)

wherein each of N^2, N and N' is an unmodified or modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;

wherein each of x and y is independently an integer between 17 and 39;

wherein the sequence of (N')y has complementarity to the sequence of (N)x and (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and is mismatched to the target RNA or is a complementary DNA moiety to the target RNA;

wherein N^1 is a moiety selected from the group consisting of natural or modified uridine, deoxyribouridine, ribothymidine, deoxyribothymidine, adenosine or deoxyadenosine;

wherein z" may be present or absent, but if present is a capping moiety covalently attached at the 5' terminus of N^2- (N')y; and

wherein each of Z and Z' is independently present or absent, but if present is independently 1-5 consecutive nucleotides, consecutive non-nucleotide moieties or a combination thereof covalently attached at the 3' terminus of the strand in which it is present.

(N)x comprises an antisense that is substantially complementary to about 17 to about 39 consecutive nucleotides in a target mRNA. In some embodiments x=y=18.

According to certain preferred embodiments siRNA compounds comprising one or more modified nucleotide, wherein the modified nucleotide possesses a modification in the sugar moiety, in the base moiety or in the internucleotide linkage moiety are provided.

Structure (A) motif is useful with any oligonucleotide pair (sense and antisense strands) to a mammalian or non-mammalian gene. In some embodiments the mammalian gene is a human gene.

In some embodiments a modified siRNA compound having structure (A) exhibits beneficial properties including enhanced activity (e.g. reduced IC50, increased knock down, reduced residual mRNA) when compared to a control compound, i.e. an siRNA compound wherein the antisense oligonucleotide is fully complementary (including 5' terminal nucleotide base paired e.g. A-U, U-A, C-G, G-C) to a nucleotide sequence in a target mRNA. In some embodiments the activity is enhanced by at least 5%, by at least 10%, by at least 20%, by at least 25% or more when compared to a control compound.

In another ~~aspect~~embodiment the present ~~invention~~disclosure provides a method of generating a double stranded RNA molecule consisting of a sense strand and an antisense strand comprising the steps of

a) selecting a consecutive 17 to 25 nucleotide sequence in a target RNA and synthesizing an antisense strand comprising complementarity to the consecutive 17 to 25 nucleotide sequence of the target mRNA wherein the 5' terminal nucleotide of the antisense strand is substituted with uridine, modified uridine, ribothymidine, deoxyribothymidine, adenosine, modified adenosine, deoxyadenosine or modified deoxyadenosine, with the proviso that a rG:rU wobble is not generated between the 5' terminal nucleotide of the antisense strand and the 3' terminal nucleotide of the target mRNA;

b) synthesizing a sense strand of 17 to 25 nucleotides having complementarity to the antisense strand, wherein the 3' terminal nucleotide of the sense strand forms a Watson Crick base pair with the 5' terminal nucleotide of the guide strand; and

c) annealing the antisense and sense strands; thereby generating a double stranded RNA molecule.

According to one ~~aspect~~embodiment, the present ~~invention~~disclosure provides a method of generating a ~~a~~ double stranded RNA molecule consisting of a sense strand and and an antisense strand exhibiting enhanced RNAi activity when compared to an unmodified a double stranded RNA molecule comprising the steps of

a) selecting a consecutive 17 to 25 nucleotide sequence in a target mRNA and synthesizing a sense strand comprising the consecutive 17 to 25 nucleotide sequence of the target mRNA wherein the 3' terminal nucleotide is substituted with adenosine, modified adenosine, deoxyadenosine or modified deoxyadenosine;

b) synthesizing an antisense strand of 17 to 25 nucleotides having complementarity to the sense strand wherein the 5' terminal nucleotide comprises ribouridine, modified ribouridine, deoxyribouridine or modified deoxyribouridine and base pairs with the 3' terminal nucleotide of the passenger strand;

c) annealing the sense strand to the antisense strand;

thereby generating a double stranded RNA molecule having enhanced RNAi activity.

In some embodiments the modified double stranded RNA molecule exhibits enhanced RNAi activity when compared to an unmodified siRNA duplex, i.e. a duplex having full match to the target mRNA.

According to another ~~aspect~~embodiment, the present ~~invention~~disclosure provides a method of generating a modified a double stranded RNA molecule consisting of a sense strand and and antisense strand exhibiting enhanced RNAi activity when compared to an unmodified a double stranded RNA molecule comprising the steps of

a) selecting a consecutive 17 to 25 nucleotide sequence in a target mRNA and synthesizing a sense strand comprising the consecutive 17 to 25 nucleotide sequence of the target mRNA wherein the 3' terminal nucleotide is substituted with adenosine, modified adenosine, deoxyadenosine or modified deoxyadenosine;

b) synthesizing an antisense strand of 17 to 25 nucleotides having complementarity to the sense strand wherein the 5' terminal nucleotide comprises ribouridine, modified ribouridine, deoxyribouridine or modified deoxyribouridine and base pairs with the 3' terminal nucleotide of the sense strand;

c) annealing the sense strand to the antisense strand;

thereby generating a double stranded RNA molecule having enhanced RNAi activity.

In some embodiments step a) includes selecting a consecutive 17 to 25 nucleotide, or 17, 18, 19, 20 , 21, 22, 23, 24 or 25 nucleotide sequence in a target RNA in a target cell wherein the 3' terminal nucleotide is other than adenosine.

In the seventh aspect, there is provided a composition comprising at least one double-stranded nucleic acid or pharmaceutically acceptable salt of such double-stranded nucleic as defined above; and a pharmaceutically acceptable carrier.

In another ~~aspect~~embodiment provided is a pharmaceutical composition comprising a compound according to Structure (A), in an amount effective to inhibit mammalian or non-mammalian gene expression; and a pharmaceutically acceptable carrier. In some embodiments the mammalian gene is a human gene. In some embodiments the non-mammalian gene is involved in a mammalian disease, preferably human disease.

Further provided are methods for treating or preventing the incidence or severity of a disease or condition and/or for reducing the risk or severity of a disease or condition in a subject in need thereof wherein the disease or condition and/or a symptom and/or risk associated therewith is associated with expression of a mammalian or a non-mammalian gene. In a preferred embodiment the subject is a human subject.

In some embodiments the disease or condition is selected from the group hearing loss, acute renal failure (ARF), Delayed Graft Function (DGF) after kidney transplantation, glaucoma, ocular ischemic conditions including anterior ischemic optic neuropathy, age-related macular degeneration (AMD), Ischemic Optic Neuropathy (ION), dry eye syndrome, acute respiratory distress syndrome (ARDS) and other acute lung and respiratory injuries, chronic obstructive pulmonary disease (COPD), primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and/or primary graft dysfunction (PGD) after organ transplantation, in particular in lung transplantation, organ transplantation including lung, liver, heart, pancreas, and kidney transplantation, nephro- and neurotoxicity, spinal cord injury, brain injury, neurodegenerative disease or condition, pressure sores, oral mucositis fibrotic conditions including liver fibrosis, lung fibrosis; and cancer. Such methods involve administering to a mammal in need of such treatment a prophylactically or therapeutically effective amount of one or more such compounds, which inhibit or reduce expression or activity of at least one such gene.

The compounds, methods, materials, and examples that will now be described are illustrative only and are not intended to be limiting; materials and methods similar or

equivalent to those described herein can be used in practice or testing of the ~~invention~~disclosure. Other features and advantages of the ~~invention~~disclosure will be apparent from the following detailed description, and from the claims.

CLAIMS:

1. A double-stranded nucleic acid having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein each of N^2, N and N′ is an unmodified ribonucleotide, a modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N′)y is an oligonucleotide in which each consecutive N or N′ is joined to the adjacent N or N′ by a covalent bond;

wherein x=y=18;
wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;
wherein:

N^1 is selected from the group consisting of adenosine and deoxyadenosine when the corresponding nucleotide in the target RNA sequence is adenosine; or

N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is cytidine; or

N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is guanosine; or

N^1 is selected from the group consisting of uridine and deoxyuridine when the corresponding nucleotide in the target RNA sequence is uridine;

wherein z″ may be present or absent, but if present is a capping moiety covalently attached at the 5′ terminus of N^2—(N′)y; and

wherein each of Z and Z′ is independently present or absent, but if present is independently 1-5 consecutive nucleotides, 1-5 consecutive non-nucleotide moieties or a combination of 1-5 nucleotides and non-nucleotide moieties, covalently attached at the 3′ terminus of the strand in which it is present;

or a pharmaceutically acceptable salt of the double stranded nucleic acid.

2. A double-stranded nucleic acid having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein each of N^2, N and N′ is an unmodified ribonucleotide, a modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N′)y is an oligonucleotide in which each consecutive N or N′ is joined to the adjacent N or N′ by a covalent bond;

wherein x=y=18;

wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;

wherein N^1 is selected from the group consisting of adenosine and deoxyadenosine, and the corresponding nucleotide in the target RNA is adenosine;

wherein z″ may be present or absent, but if present is a capping moiety covalently attached at the 5′ terminus of N^2—(N′)y; and

wherein each of Z and Z′ is independently present or absent, but if present is independently 1-5 consecutive nucleotides, 1-5 consecutive non-nucleotide moieties or a combination of 1-5 nucleotides and non-nucleotide moieties, covalently attached at the 3′ terminus of the strand in which it is present;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

3. The double-stranded nucleic acid of claim 2, wherein N1 is adenosine.

4. The double-stranded nucleic acid of claim 1 wherein z″ is present.

5. The double-stranded nucleic acid of claim 1, wherein Z and Z′ are absent.

6. The double-stranded nucleic acid of claim 1, wherein one of Z or Z′ is present.

7. The double-stranded nucleic acid of claim 6, wherein Z or Z′ comprise non-nucleotide moieties.

8. The double-stranded nucleic acid of claim 1, wherein at least one of N or N′ comprises a 2′O-methyl sugar modified ribonucleotide.

9. The double-stranded nucleic acid of claim 8, wherein (N)x comprises 2′O-methyl sugar modified ribonucleotides.

10. The double-stranded nucleic acid of claim 1, wherein the double-stranded nucleic acid is a siRNA, siNA or a miRNA.

11. A double-stranded nucleic acid having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein N, N′, (N)x, (N′)y, x, y, Z′ and z″ are defined in claim 2;

wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;

wherein, Z′ is absent or present, Z is present and comprises two alkyl moieties covalently linked to each other via a phosphodiester bond, wherein N^2 comprises uridine or deoxyuridine; and wherein N^1 comprises adenosine when the corresponding nucleotide in the target RNA is adenosine, guanosine or cytidine;

or a pharmaceutically acceptable salt the double stranded nuclei acid.

12. The double-stranded nucleic acid of claim 1, wherein the target RNA is an mRNA encoded by a mammalian gene.

13. The double-stranded nucleic acid of claim 12, wherein the target mRNA is an mRNA encoded by a human gene.

14. A composition comprising at least one double-stranded nucleic acid or a pharmaceutically acceptable salt of such double-stranded nucleic acid of claim 1; and a pharmaceutically acceptable carrier.

15. A double-stranded nucleic acid molecule having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein N^2, N, N′, (N)x, (N′)y, x, y, Z, Z′ and z″ are as defined in claim 2;
wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;
wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;
wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine, when the corresponding nucleotide in the target RNA is cytidine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

16. A double-stranded nucleic acid molecule having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein N^2, N, N′, (N)x, (N′)y, x, y, Z, Z′ and z″ are as defined in claim 2;

wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine and deoxythymidine when the corresponding nucleotide in the target RNA is guanosine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

17. A double-stranded nucleic acid molecule having structure (A) set forth below:

(A) 5′ N^1-(N)x-Z 3′ (antisense strand)
3′ Z′-N^2-(N′)y-z″ 5′ (sense strand)

wherein N^2, N, N′, (N)x, (N′) y, x, y, Z, Z′ and z″ are as defined in claim 2;

wherein the sequence of N^2—(N′)y is fully complementary to the sequence of N^1—(N)x and the sequence of (N)x has complementarity to consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and not complementary to the corresponding nucleotide in the target RNA or is an unconventional moiety complementary to the target RNA;
wherein N^1 is uridine or deoxyuridine, when the corresponding nucleotide in the target RNA is uridine;
or a pharmaceutically acceptable salt of the double stranded nucleic acid.

1. A double stranded nucleic acid molecule having structure (A) set forth below:

(A) 5' N^1-(N)x - Z 3'(antisense strand)

3' Z'-N^2-(N')y-z" 5'(sense strand)

wherein each of N^2, N and N' is an unmodified or modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;

wherein each of x and y is independently an integer between 17 and 39;

wherein the sequence of (N')y has complementarity to the sequence of (N)x and the sequence of (N)x has complementarity to a consecutive sequence in a target RNA;

wherein N^1 is covalently bound to (N)x and forms a Watson-Crick base pair with N^2; wherein N^1 is not complementary to the corresponding nucleotide in the target RNA;

wherein

N^1 is selected from the group consisting of natural or modified adenosine, and deoxyadenosine when the corresponding nucleotide in the target RNA sequence is adenosine; or

N^1 is selected from the group consisting of natural or modified adenosine, deoxyadenosine, deoxyuridine, deoxythymidine and ribothymidine when the corresponding nucleotide in the target RNA sequence is cytidine; or

N^1 is selected from the group consisting of natural or modified adenosine, deoxyadenosine, uridine, deoxyuridine, ribothymidine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is guanosine; or

N^1 is selected from the group consisting of natural or modified uridine, deoxyuridine, ribothymidine and deoxythymidine when the corresponding nucleotide in the target RNA sequence is uridine;

wherein z" may be present or absent, but if present is a capping moiety covalently attached at the 5' terminus of N^2- (N')y; and

wherein each of Z and Z' is independently present or absent, but if present is

~~independently 1-5 consecutive nucleotides, consecutive non-nucleotide moieties or a~~ combination thereof covalently attached at the 3' terminus of the strand in which it is present;

or a pharmaceutically acceptable salt of the double stranded nucleic acid molecule.

2. The double stranded nucleic acid molecule of claim 1, wherein the sequence of (N')y is fully complementary to the sequence of (N)x.

3. The double stranded nucleic acid molecule of claim 1, wherein (N)x comprises an antisense oligonucleotide that is fully complementary to about 17 to about 39 consecutive nucleotides in a target RNA.

4. The double stranded nucleic acid molecule of claim 1, wherein (N)x comprises an antisense oligonucleotide that is substantially complementary to about 17 to about 39 consecutive nucleotides in a target RNA.

5. The double stranded nucleic acid molecule of any one of claims 1 to 4 wherein x-y=18, x =y=19 or x=y=20.

6. The double stranded nucleic acid molecule of claim 5 wherein x=y=18.

7. The double stranded nucleic acid molecule of any one of claims 1 to 6, wherein N^1 is selected from the group consisting of adenosine and deoxyadenosine, and the corresponding nucleotide in the target RNA is adenosine.

8. The double stranded nucleic acid molecule of any one of claims 1 to 6, wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine, deoxyuridine, ribothymidine and deoxythymidine and the corresponding nucleotide in the target RNA is cytidine.

9. The double stranded nucleic acid molecule of claim 8, wherein N^1 is selected from the group consisting of adenosine and deoxyadenosine.

10. The double stranded nucleic acid molecule of any one of claims 1 to 6, wherein N^1 is selected from the group consisting of adenosine, deoxyadenosine, uridine, deoxyuridine, ribothymidine and deoxythymidine and the corresponding nucleotide in the target RNA is guanosine.

11. The double stranded nucleic acid molecule of claim 10, wherein N^1 is selected from the group consisting of adenosine and deoxyadenosine.

12. The double stranded nucleic acid molecule of any one of claims 1 to 6, wherein N^1 is selected from the group consisting of uridine, deoxyuridine, ribothymidine and deoxythymidine and the corresponding nucleotide in the target RNA is uridine.

13. The double stranded nucleic acid molecule of claim 12, wherein N^1 is selected from the group consisting of uridine and deoxyuridine.

14. The double stranded acid molecule of any one of claims 1 to 6, wherein N^1 and N^2 form a base pair between uridine or deoxyuridine, and adenosine or deoxyadenosine.

15. The double stranded acid molecule of any one of claims 1 to 6, wherein N^1 and N^2 form a base pair between deoxyuridine and adenosine.

16. The double stranded acid molecule of any one of claims 1 to 15 wherein z" is present.

17. The double stranded nucleic acid molecule of any one of claims 1 to 16, wherein Z and Z' are absent.

18. The double stranded nucleic acid molecule of any one of claims 1 to 16, wherein at least one of Z or Z' is present.

19. The double stranded nucleic acid molecule of claims 1 to 18, wherein at least one of Z or Z' comprise non-nucleotide moieties.

20. The double stranded nucleic acid molecule of any one of claims 1 to 19, wherein at least one of N or N' comprises a 2'OMe sugar-modified ribonucleotide.

21. The double stranded nucleic acid molecule of claim 20 wherein, N comprises single alternating unmodified and 2'OMe sugar modified ribonucleotides.

22. The double stranded nucleic acid molecule of any one of claims 1 to 21, wherein N^2 comprises a 2'OMe sugar-modified ribonucleotide.

23. The double stranded nucleic acid molecule of any one of claims 1 to 22, wherein the double stranded nucleic acid molecule is a siRNA, siNA or a miRNA.

~~24. The double stranded nucleic acid molecule of claim 1 wherein x=y=18, Z' is~~ absent, Z is present and comprises two alkyl moieties covalently linked to each other via a phosphodiester bond, N^2 comprises adenosine and N^1 comprises uridine.

25. The double stranded nucleic acid molecule of claim 1 wherein x=y=18, Z' is absent, Z is present and comprises two alkyl moieties covalently linked to each other via a phosphodiester bond, N^2 comprises uridine or deoxyuridine and N^1 comprises adenosine.

26. The double stranded nucleic acid molecule of any one of claims 1 to 25 wherein the target RNA is an mRNA of a mammalian gene.

27. The double stranded nucleic acid molecule of claim 26 wherein the target mRNA is an mRNA of a human gene.

28. Use of the double stranded nucleic acid molecule of any one of claims 1 to 27, for the preparation of a medicament for use in therapy.

29. A pharmaceutical composition comprising at least one double stranded nucleic acid molecule of any one of claims 1 to 28; and a pharmaceutically acceptable carrier.

30. Use of the double stranded nucleic acid molecule of any one of claims 1 to 27, or the pharmaceutical composition of claim 28, for the preparation of a medicament for treating or preventing the incidence or severity of a disease or condition in a subject in need thereof, wherein the disease or the condition and/or symptoms associated therewith is selected from the group consisting of hearing loss, acute renal failure (ARF), Delayed Graft Function (DGF) after kidney transplantation, glaucoma, ocular ischemic conditions, including non-arteric ischemic optic neuropathy (NAION), anterior ischemic optic neuropathy, age-related macular degeneration (AMD), Ischemic Optic Neuropathy (ION) and dry eye syndrome, acute respiratory distress syndrome (ARDS) and other acute lung and respiratory injuries, chronic obstructive pulmonary disease (COPD), primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and/or primary graft dysfunction (PGD) after organ transplantation, in particular in lung transplantation, organ transplantation including lung, liver, heart, pancreas, and kidney transplantation, nephro- and neurotoxicity, spinal cord injury, brain injury, neurodegenerative disease or condition, pressure sores, oral mucositis, fibrotic disorders and cancer.

31. A method of generating a double stranded RNA molecule consisting of a sense strand and an antisense strand and exhibiting enhanced RNAi activity when compared to an unmodified dsRNA molecule comprising the steps of

a) selecting a consecutive 17 to 25 nucleotide sequence in a target RNA and synthesizing an antisense strand comprising complementarity to the consecutive 17 to 25 nucleotide sequence of the target mRNA, wherein the 5' terminal nucleotide of the antisense strand is substituted with uridine, modified uridine, ribothymidine, deoxyribothymidinc, adenosine, modified adenosine, deoxyadenosine or modified deoxyadenosine, with the proviso that a rG:rU wobble is not generated between the 5' terminal nucleotide of the antisense strand and the 3' terminal nucleotide of the target mRNA;

b) synthesizing a sense strand of 17 to 25 nucleotides having complementarity to the antisense strand, wherein the 3' terminal nucleotide of the sense strand forms a Watson Crick base pair with the 5' terminal nucleotide of the antisense strand; and

c) annealing the sense and antisense strands; thereby generating a double stranded RNA molecule having enhanced RNAi activity.

32. A method of generating a modified siRNA duplex consisting of a sense strand and and an antisense strand exhibiting enhanced RNAi activity when compared to an unmodified siRNA duplex comprising the steps of

a) selecting a consecutive 17 to 25 nucleotide sequence in a target mRNA and synthesizing a sense strand comprising the consecutive 17 to 25 nucleotide sequence of the target mRNA wherein the 3' terminal nucleotide is substituted with adenosine, modified adenosine, deoxyadenosine or modified deoxyadenosine;

b) synthesizing an antisense strand of 17 to 25 nucleotides having complementarity to the sense strand wherein the 5' terminal nucleotide comprises ribouridine, modified ribouridine, deoxyribouridine or modified deoxyribouridine and base pairs with the 3' terminal nucleotide of the passenger strand;

c) annealing the sense strand to the antisense strand;

thereby generating a siRNA duplex having enhanced RNAi activity.