Accordingly, in one aspect, the present invention provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein said antisense strand comprises at least one thermally destabilizing modification of the double stranded region within the first 9 nucleotide positions of the 5′ region or a precursor thereof, wherein said sense strand comprises an ASGPR ligand, and wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length. In another aspect, the present invention refers to a vector containing the dsRNA agent of the present invention. In another aspect, the present invention refers to a cell containing the dsRNA agent of the present invention. In another aspect, the present invention refers to a pharmaceutical composition comprising the dsRNA agent of the present invention. In yet another aspect, the present invention refers to the use of the pharmaceutical composition of the present invention in the manufacture of a medicament for treating a Serpina1 associated disease.

Described herein is a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) target gene sequence, comprising a sense strand and an antisense strand, wherein the antisense strand has sufficient complementarity to the target sequence to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification within the first 9 nucleotide positions of the 5′ region or a precursor thereof, wherein said sense strand comprises an asialoglycoprotein receptor (ASGPR) ligand, and wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length.

In another example, the present disclosure provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein said antisense strand comprises at least one thermally destabilizing modification of the double stranded region within the first 9 nucleotide positions of the 5′ region or a precursor thereof, wherein said sense strand comprises an asialoglycoprotein receptor (ASGPR) ligand, and wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length.

In yet another example, the present disclosure provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand forming a double stranded region, said antisense strand comprising a region of complementarity to an mRNA encoding Serpina1, wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein said antisense strand comprises at least one thermally destabilizing modification within the first 9 nucleotide positions of the 5′ region or a precursor thereof,

wherein said sense strand comprises an asialoglycoprotein receptor (ASGPR) ligand, wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length.

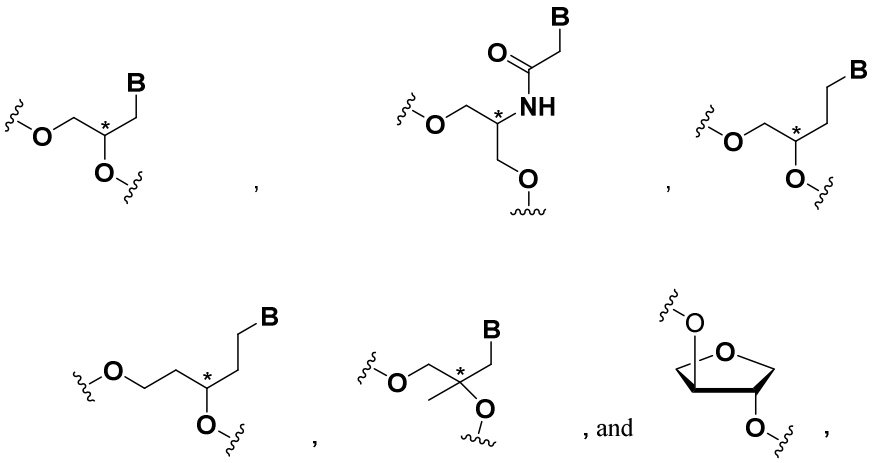
In one example, the dsRNA agent comprises at least four nucleotides comprising a 2'-fluoro modification.

In one example, the dsRNA agent has the following characteristics: a) the thermally destabilizing modification is located in position 4-8 of the 5′ region of the antisense strand; b) and each of the sense and antisense strands independently comprise at least two nucleotides comprising a 2'-fluoro modification; and c) an ASGPR ligand attached to either end of the sense strand.

In another example, the antisense strand has at least two of the following characteristics: a) the thermally destabilizing modification is located in position 4 to 8 of the antisense strand; b) at least two nucleotides comprise a 2'-fluoro modification; c) a phosphorothioate internucleotide linkages between nucleotide positions 1 and 2 (counting from the 5' end); d) a length of 18 to 35 nucleotides.

In one example, the sense strand has at least one of the following characteristics: a) the ASGPR ligand attached to either end of the sense strand; b) at least two nucleotides comprise a 2'-fluoro modification; c) the sense strand and the antisense strand form a double stranded region spanning at least 19 nucleotide positions and wherein the thermally destabilizing modification is located within said double stranded region.

In one example, the thermally destabilizing modification is selected from the group consisting of

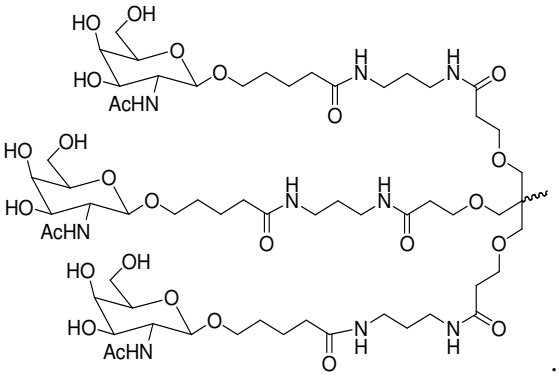


wherein B is nucleobase.

In one example, the destabilizing modification is located in position 7 of the antisense strand.

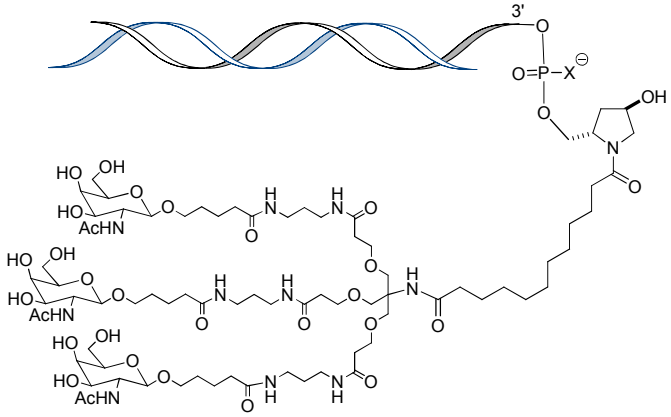
In one example, the ASGPR ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the ASGPR ligand is:



.

In one example, the dsRNA agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the present disclosure provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) target gene sequence, comprising a sense strand and an antisense strand, wherein the antisense strand has sufficient complementarity to a target sequence to mediate RNA interference, wherein the antisense strand comprises at least one thermally destabilizing modification within the first 9 nucleotide positions of the 5′ region, wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length, and wherein the dsRNA has a melting temperature of from about 40°C to about 80°C.

In another example, the present disclosure provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) target gene sequence, comprising a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein the antisense strand comprises at least one thermally destabilizing modification within the first 9 nucleotide positions of the 5′ region, wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length, and wherein the dsRNA has a melting temperature of from about 40°C to about 80°C.

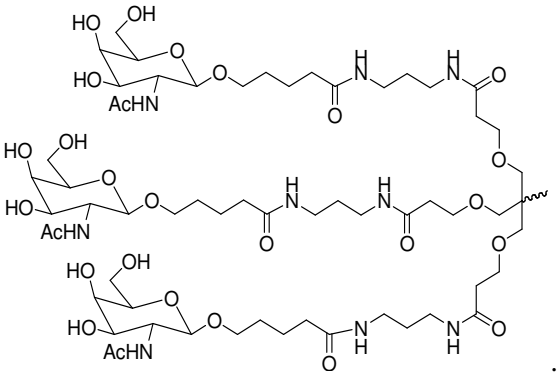
In yet another example, the present disclosure provides a double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) target gene sequence, comprising a sense strand and an antisense strand, said antisense strand comprising a region of complementarity to an mRNA encoding Serpina1, wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15, wherein the antisense strand comprises at least one thermally destabilizing modification within the first 9 nucleotide positions of the 5′ region, wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length, and wherein the dsRNA has a melting temperature of from about 40°C to about 80°C.

In one example, the dsRNA agent has a melting temperature of from about 55°C to about 67°C. In another example, the dsRNA agent has a melting temperature of from about 60°C to about 67°C.

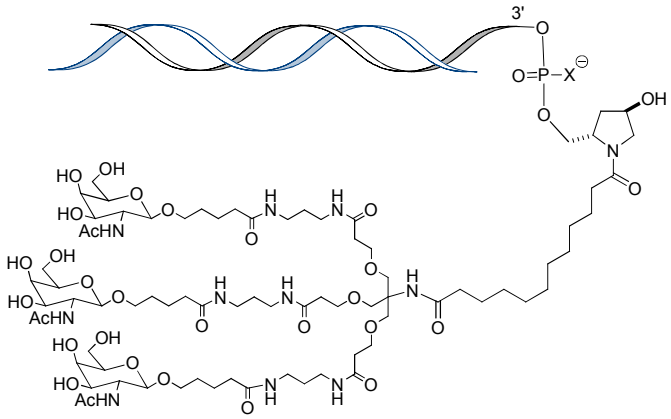
In one example, the dsRNA agent further comprises an asialoglycoprotein receptor (ASGPR) ligand.

In one example, the ASGPR ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In one example, the ASGPR ligand is:



In one example, the dsRNA agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

In one example, the dsRNA agent further has at least one of the following characteristics: (i) the antisense strand comprises 2, 3, 4, 5 or 6 nucleotides comprising a 2'-fluoro modifications (ii) the antisense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 nucleotides comprising a 2'-fluoro modification; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four nucleotides comprising a 2'-fluoro modification; (vii) the dsRNA comprises a double stranded region region of 12-40 nucleotide pairs in length; and (viii) a blunt end at 5'end of the antisense strand.

In some example, each strand of the dsRNA agent may have 15-30 nucleotides, 19-30 nucleotides; or the sense strand may have 21 nucleotides, and the antisense strand may have 23 nucleotides.

In one example, the antisense strand comprises a region of complementarity comprising at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 1440-1480 of SEQ ID NO:1; or nucleotides 1441-1479 of SEQ ID NO:1; or nucleotides 1442-1478 of SEQ ID NO:1; or nucleotides 1443-1477 of SEQ ID NO:1; or nucleotides 1444-1476 of SEQ ID NO:1; or nucleotides 1445-1475 of SEQ ID NO:1; or

nucleotides 1446-1474 of SEQ ID NO:1; or nucleotides 1447-1473 of SEQ ID NO:1; or nucleotides 1448-1473 of SEQ ID NO:1; or nucleotides 1448-1472 of SEQ ID NO:1; or nucleotides 1448-1471 of SEQ ID NO:1; or nucleotides 1448-1470 of SEQ ID NO:1; or nucleotides 1447-1469 of SEQ ID NO:1; or nucleotides 1446-1478 of SEQ ID NO:1; or nucleotides 1449-1471 of SEQ ID NO:1; or nucleotides 1450-1472 of SEQ ID NO:1; or nucleotides 1440-1475 of SEQ ID NO:1; or nucleotides 1445-1480 of SEQ ID NO:1; or nucleotides 1445-1475 of SEQ ID NO:1.

In one example, the region of complemntarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419).

In one example, the sense strand comprises the nucleotide sequence 5' – CUUCUUAAUGAUUGAACAAAA – 3' (SEQ ID NO: 417) and the antisense strand comprises the nucleotide sequence 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419).

In one example, the sense strand comprises the nucleotide sequence 5' - csusucuuAfaUfGfAfuugaacaaaa – 3' (SEQ ID NO: 33) and the antisense strand comprises the nucleotide sequence 5' – usUfsuugu(Tgn)caaucaUfuAfagaagsasc – 3' (SEQ ID NO: 34), wherein a, g, c, and u are 2′-O-methyl (2′-OMe) A, G, C, and U, respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U, respectively; s is a phosphorothioate linkage; and (Tgn) is a thymidine-glycol nucleic acid (GNA) S-Isomer.

In one example, the present disclosure provides a double stranded RNA (dsRNA) molecule that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises the nucleotide sequence 5' - csusucuuAfaUfGfAfuugaacaaaaL96 – 3' (SEQ ID NO: 35) and the antisense strand comprises the nucleotide sequence 5' – usUfsuugu(Tgn)caaucaUfuAfagaagsasc – 3' (SEQ ID NO: 34), wherein a, g, c, and u are 2′-O-methyl (2′-OMe) A, G, C, and U, respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U, respectively; s is a phosphorothioate linkage; (Tgn) is a thymidine-glycol nucleic acid (GNA) S-Isomer; and L96 is N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol.

In one example the disclosure provides a dsRNA agent that inhibits expression of a serine peptidase inhibitor, clade a, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a target Serpina1 sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), and the dsRNA further has at least one (*e.g.*, one, two, three, four, five, six seven, eight or all nine) of the following

characteristics: (i) a melting temperature (T_m) of from about 40°C to about 80°C; (ii) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (iii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iv) the sense strand is conjugated with a ligand; (v) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (vi) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vii) the dsRNA comprises at least four 2'-fluoro modifications; (viii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (ix) a blunt end at 5'end of the antisense strand.

In some examples, the disclosure provides a a dsRNA agent that inhibits expression of a serine peptidase inhibitor, clade a, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a target Serpina1 sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), and the dsRNA further has at least one (*e.g.*, one, two, three, four, five, six seven, eight or all nine) of the following characteristics: (i) a melting temperature (T_m) of from about 40°C to about 80°C; (ii) the antisense comprises 6, 7, 8, 9, 10, 11 or 12 2'-OMe modifications; (iii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iv) the sense strand is conjugated with a ligand; (v) the sense strand comprises 6, 7, 8, 9, 10, 11 or 12 2'-OMe modifications; (vi) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vii) the dsRNA comprises at least 1, 2, 3, 4 or 5 2'-deoxy modification(s); (viii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (ix) a blunt end at 5'end of the antisense strand.

In some examples, the dsRNA has a melting temperature with a lower end of the range from about 40°C, 45°C, 50°C, 55°C, 60°C or 65°C, and upper end of the arranger from about 70°C, 75°C or 80°C. In some examples, the dsRNA has a melting temperature in the range from about 55°C to about 70°C. In some examples, the dsRNA has a melting temperature in the range from about 57°C to about 67°C. In some particular examples, the dsRNA has a melting temperature in the range from about 60°C to about 67°C. In some additional examples, the dsRNA has a melting temperature in the range from about 62°C to about 66°C.

It has also ben discovered that dsRNA agents having a melting temperature of at least 60°C are more effective *in vivo* and *in vitro*. Thus, in some examples, the dsRNA has a melting temperature of at least 60°C.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a target Serpina1 sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one

thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), the dsRNA has a melting temperature (T_m) of from about 40°C to about 80°C, and the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) a blunt end at 5'end of the antisense strand.

In some examples, the dsRNA agent has a duplex region of 12-40 nucleotide pairs in length, wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), and the dsRNA has a T_m of from about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and vii) a blunt end at 5'end of the antisense strand.

In some examples, the dsRNA agent has a duplex region of 19, 21, 22 or 23 nucleotide base pairs in length, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA has a melting temperature of about 40°C to about 80°C.

In some examples, the dsRNA agent has a duplex region of 19, 21, 22 or 23 nucleotide base pairs in length, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA has a melting temperature of about 40°C to about 80°C (*e.g.*, 40°C, 50°C, 60°C, 70°C or 80°C).

In some particular examples, the thermally destabilizing modification of the duplex is at position 5, 6, 7, or 8 of the antisense strand, counting from 5'-end of the antisense strand.

In some particular examples, the thermally destabilizing modification of the duplex is at position 6 of the antisense strand, counting from 5'-end of the antisense strand.

In some particular examples, the thermally destabilizing modification of the duplex is at position 7 of the antisense strand, counting from 5'-end of the antisense strand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and the antisense strand further comprises one or both of the following characteristics:

(i) 2, 3, 4, 5 or 6 2'-fluoro modifications; and

(ii) 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and

the sense strand comprises one, two or three of the following characteristics:

(i) a ligand conjugated with the sense strand;

(ii) 2, 3, 4 or 5 2'-fluoro modifications; and

(iii) 1, 2, 3 or 4 phosphorothioate internucleotide linkages.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions counting from the 5′-end, and a ligand is conjugated with the sense strand, and wherein the dsRNA has a melting temperature of about 40°C to about 80°C.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions counting from the 5′-end, a ligand is conjugated with the sense strand, and the dsRNA comprises at least four 2'-fluoro modifications.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, said sense strand comprises a ligand, and wherein the dsRNA has a melting temperature of about 40°C to about 80°C. In some further examples of this, the ligand is an ASGPR ligand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient

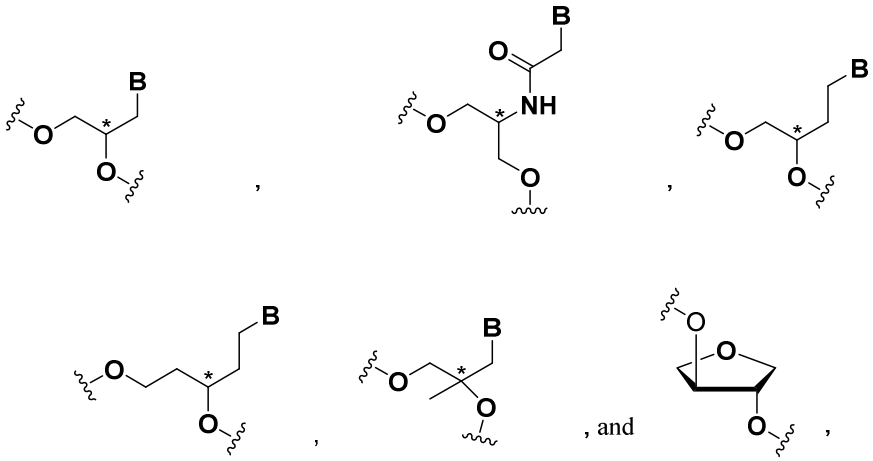
complementarity to a Serina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located in position 4-8, counting from the 5'-end, wherein said sense strand comprises a ligand, wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications, and wherein the dsRNA has a melting temperature of about 40°C to about 80°C. In some further examples of this, the ligand is an ASGPR ligand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the antisense further comprises at least two of the following characteristics: (i) the thermally destabilizing modification of the duplex is located in position 4 to 8 of the antisense strand; (ii) at least two 2'-fluoro modifications; (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2 (counting from the 5' end); and antisense strand has a length of 18 to 35 nucleotides. In some further examples the ligand is an ASGPR ligand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and the sense strand has at least one of the following characteristics: (i) the ligand is attached to either end of the sense strand; (ii) sense strand comprises at least two 2'-fluoro modifications; and (iii) the sense strand and the antisense strand show sufficient complementarity to form a double stranded region spanning at least 19 nucleotide positions and wherein the thermally destabilizing modification of the duplex is located within said double stranded region.

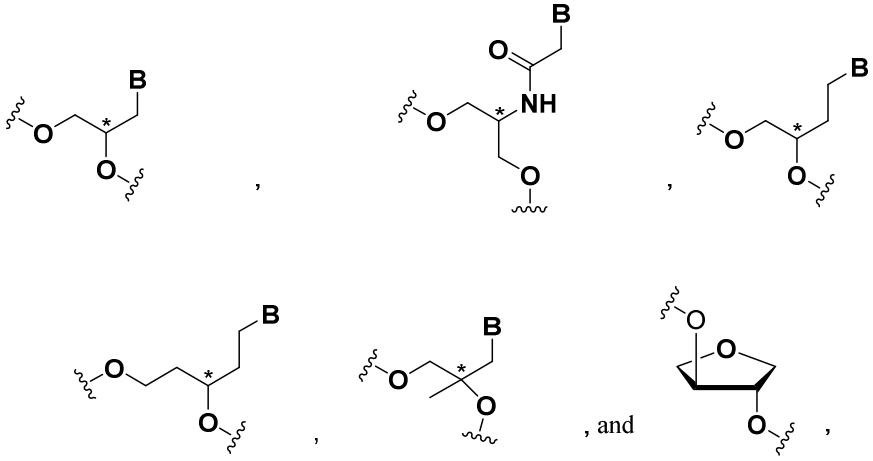
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex

within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:



wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic*..

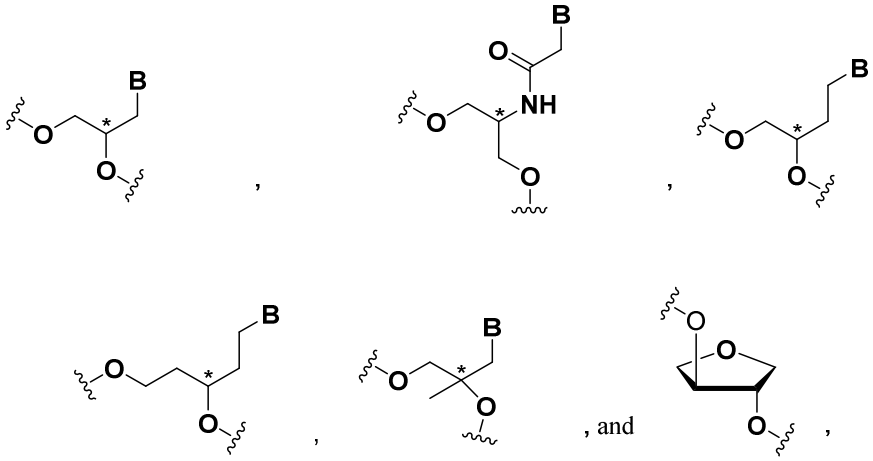
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located in position 4-8, counting from the 5'-end, wherein said sense strand comprises a ligand, and wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:



wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic*..

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located at position 7, counting from the 5'-end of the antisense strand, wherein said sense strand comprises a ligand, and wherein the dsRNA has a melting temperature of about 40°C to about 80°C.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located at position 7, counting from the 5'-end, wherein said sense strand comprises a ligand, and wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:

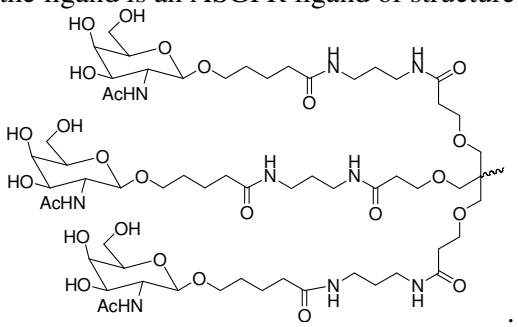


wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic.*

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, wherein said sense strand comprises a ligand, wherein the dsRNA has a melting temperature of about 40°C to about

80°C, and wherein the ligand comprises one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the ligand is an ASGPR ligand of structure:



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In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0, 1 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 3, 4, 5 or 6 2'-fluoro modifications, comprises 2, 3 or 4 phosphorothioate internucleotide linkages; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense strand), and optionally comprises phosphorothioate internucleotide linkages between

nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 3, 4, 5 or 6 2'-fluoro modifications, comprises 2, 3 or 4 phosphorothioate internucleotide linkages; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, between nucleotide positions 22 and 23, between nucleotide positions 1 and 2, between nucleotide positions 2 and 3; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense strand), and optionally comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length, wherein the antisense strand contains at least one thermally destabilizing nucleotide, and where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 18, 19, 21, 22, 23, 24 or 24 nucleotide pairs in length; and (viii) the dsRNA comprises a blunt end at 5'-end of the sense strand. In some particular examples, sense strand is 19, 20 or 21 or 22 nucleotides in length and the antisense strand is 20, 21 or 22 nucleotides in length.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense

strand), and optionally comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, between nucleotide positions 22 and 23, between nucleotide positions 1 and 2, between nucleotide positions 2 and 3; wherein the dsRNA has a melting temperature of about 40°C to about 80°C; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, one end of the dsRNA is a blunt end and the other end has an overhang, wherein the antisense strand contains at least one thermally destabilizing nucleotide, and where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) and the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length. In some examples, the overhang is on the 3'-end of the antisense strand and the blunt end is at the 5'-end of the antisense strand. In some particular examples, the overhang is 2, 3 or 4-nucleotides in length.

In some examples, the dsRNA agent has a duplex region of 19, 21, 22 or 23 nucleotide base pairs in length, wherein one end of the dsRNA is a blunt end and the other end has an overhang, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, five or all six) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications, and optionally the 2 nucleotide overhang is on the 3'-end of the antisense strand and the blunt end is at the 5'-end of the antisense strand.

In some examples, the overhang is on the 3'-end of the antisense strand and the blunt end is at the 5'-end of the antisense strand.

In some examples, the dsRNA agent of the disclosure may also have two blunt ends, at both ends of the dsRNA duplex.

In some examples, the dsRNA has a blunt end at both ends of the duplex, wherein the antisense strand contains at least one thermally destabilizing nucleotide, and where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length.

In some examples, the dsRNA agent has a duplex region of 19, 21, 22 or 23 nucleotide base pairs in length and has a blunt end at both ends of the duplex, wherein one end of the dsRNA is a blunt end and the other end has an overhang, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, five or all six) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications.

In some examples, the dsRNA agent of the disclosure comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide occurs in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein one end of the dsRNA is blunt, while the other end is comprises a 2 nt overhang, wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optinally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense

strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a blunt end at 5'-end of the antisense strand. Preferably, the 2 nt overhang is at the 3'-end of the antisense.

In some examples, the dsRNA agent of the disclosure comprising a sense and antisense strands, wherein: the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1), positions 1 to 23 of said sense strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce Serpina1 target gene expression when said double stranded nucleic acid is introduced into a mammalian cell; and wherein the antisense strand contains at least one thermally destabilizing nucleotide, where at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand), and wherein the dsRNA has a melting temperature of about 40°C to about 80°C. For example, the thermally destabilizing nucleotide occurs between positions opposite or complimentary to positions 14-17 of the 5'-end of the sense strand, and wherein the dsRNA optinally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA comprises a duplex region of 12-30 nucleotide pairs in length.

In some examples, the dsRNA agent of the disclosure comprises a sense and antisense strands, wherein said dsRNA agent comprises a sense strand having a length which is at least 25 and at most 29 nucleotides and an antisense strand having a length which is at most 30 nucleotides with the sense strand comprises a modified nucleotide that is susceptible to enzymatic degradation at position 11 from the 5'end, wherein the 3' end of said sense strand

and the 5' end of said antisense strand form a blunt end and said antisense strand is 1-4 nucleotides longer at its 3' end than the sense strand, wherein the duplex region which is at least 25 nucleotides in length, and said antisense strand is sufficiently complementary to a Serina1 target mRNA along at least 19 nt of said antisense strand length to reduce target gene expression when said dsRNA agent is introduced into a mammalian cell, and wherein dicer cleavage of said dsRNA preferentially results in an siRNA comprising said 3' end of said antisense strand, thereby reducing expression of the target gene in the mammal, wherein the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.* at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and (vi) the dsRNA comprises at least four 2'-fluoro modifications; and (vii) the dsRNA has a duplex region of 12-29 nucleotide pairs in length.

In some examples, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some examples, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40°C to about 80°C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five,

six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some examples, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40^{o}C to about 80^{o}C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six, seven or all eight) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages; (iii) the sense strand is conjugated with a ligand; (iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (v) the sense strand comprises 3 or 4 phosphorothioate internucleotide linkages; (vi) the dsRNA comprises at least four 2'-fluoro modifications; (vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (viii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In some examples, the sense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3, the antisense strand comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23, wherein the antisense strand contains at least one thermally destabilizing modification of the duplex located in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand), wherein the dsRNA has a melting temperature of about 40^{o}C to about 80^{o}C, and wherein the dsRNA optionally further has at least one (*e.g.*, one, two, three, four, five, six or all seven) of the following characteristics: (i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications; (ii) the sense strand is conjugated with a ligand; (iii) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications; (iv) the sense strand comprises 3 or 4 phosphorothioate internucleotide linkages; (v) the dsRNA comprises at least four 2'-fluoro modifications; (vi) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and (vii) the dsRNA has a blunt end at 5'-end of the antisense strand.

In one example the disclosure provides a dsRNA agent capable of inhibiting the expression of a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, comprising a sense strand and an antisense strand, each strand having 14 to 40 nucleotides,

wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), and the dsRNA further has at least one (*e.g.*, one, two, three, four, five, six seven or all eight) of the following characteristics:

(i) the antisense comprises 2, 3, 4, 5 or 6 2'-fluoro modifications;

(ii) the antisense comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages;

(iii) the sense strand is conjugated with a ligand;

(iv) the sense strand comprises 2, 3, 4 or 5 2'-fluoro modifications;

(v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages;

(vi) the dsRNA comprises at least four 2'-fluoro modifications;

(vii) the dsRNA comprises a duplex region of 12-40 nucleotide pairs in length; and

(viii) a blunt end at 5'end of the antisense strand.

In some particular examples, the thermally destabilizing modification of the duplex is at position 7 of the antisense strand, counting frm 5'-end of the antisense strand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the target sequence to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the seed region (*i.e.*, at position 2-9 of the 5'-end of the antisense strand, counting from the 5′-end), and the antisense strand further comprises one or both of the following characteristics:

(iii) 2, 3, 4, 5 or 6 2'-fluoro modifications; and

(iv) 1, 2, 3 or 4 phosphorothioate internucleotide linkages; and

the sense strand comprises one, two or three of the following characteristics:

(iv) a ligand conjugated with the sense strand;

(v) 2, 3, 4 or 5 2'-fluoro modifications; and

(vi) 1, 2, 3 or 4 phosphorothioate internucleotide linkages.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions counting from the 5′-end, and a ligand is conjugated with the sense strand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient

complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference and wherein the antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions counting from the 5′-end, a ligand is conjugated with the sense strand, and the dsRNA comprises at least four 2'-fluoro modifications.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand. In some further examples of this, the ligand is an ASGPR ligand.

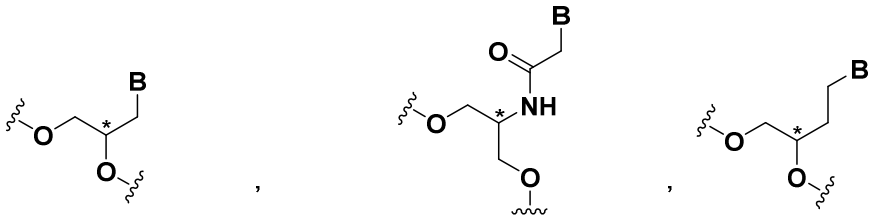
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located in position 4-8, counting from the 5'-end, wherein said sense strand comprises a ligand, and wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications. In some further examples of this, the ligand is an ASGPR ligand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, and wherein the antisense further comprises at least two of the following characteristics: (i) the thermally destabilizing modification of the duplex is located in position 4 to 8 of the antisense strand; (ii) at least two 2'-fluoro modifications; (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2 (counting from the 5' end); and antisense strand has a length of 18 to 35 nucleotides. In some further examples the ligand is an ASGPR ligand.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro,

wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, and the sense strand has at least one of the following characteristics: (i) the ligand is attached to either end of the sense strand; (ii) sense strand comprises at least two 2'-fluoro modifications; and (iii) the sense strand and the antisense strand show sufficient complementarity to form a double stranded region spanning at least 19 nucleotide positions and wherein the thermally destabilizing modification of the duplex is located within said double stranded region.

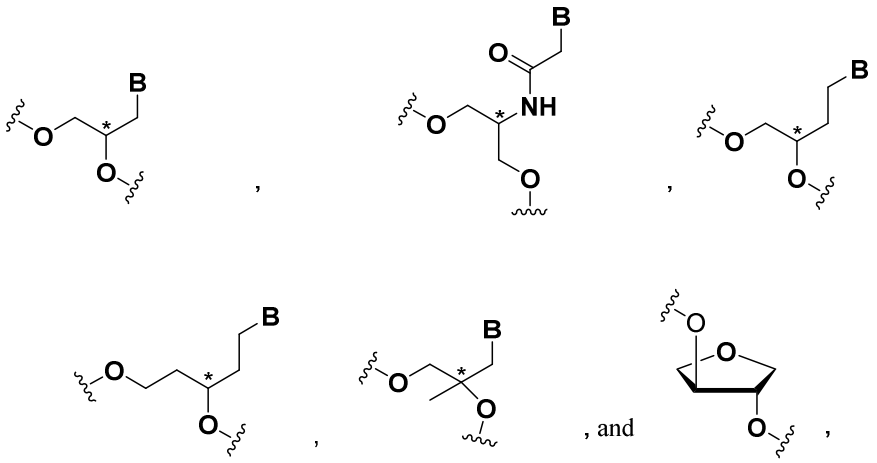
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:



, , , , , and ,

wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic.*

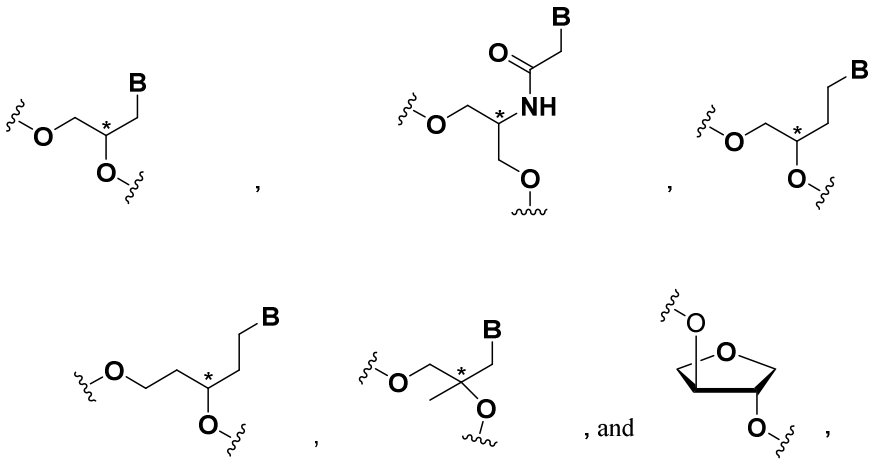
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located in position 4-8, counting from the 5'-end, wherein said sense strand comprises a ligand, and wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:



wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic*.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located at position 7, counting from the 5'-end of the antisense strand, and wherein said sense strand comprises a ligand.

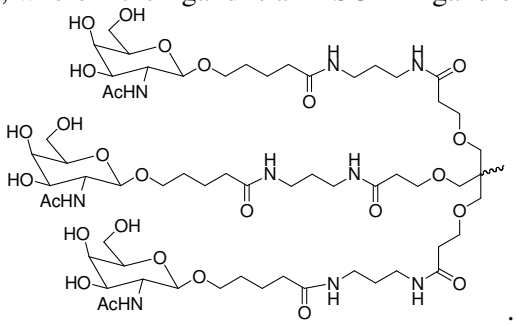
In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to the Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex located at position 7, counting from the 5'-end, wherein said sense strand comprises a ligand, and wherein each of the sense and antisense strands comprise at least two 2'-fluoro modifications, and wherein the thermally destabilizing modification of the duplex is selected from the group consisting of:



wherein B is a modified or unmodified nucleobase and the asteric on each stucture represents either *R*, *S* or *racemic*..

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the ligand comprsies one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some examples, the dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides, wherein the antisense strand has sufficient complementarity to a Serpina1 target sequence, *e.g.,* nucleotides 1440-1480 of SEQ ID NO:1, to mediate RNA interference, wherein the dsRNA comprises at least four 2'-fluoro, wherein said antisense strand comprises at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5'-end, and wherein said sense strand comprises a ligand, wherein the ligand is an ASGPR ligand of structure:



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In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand);

wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0, 1 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 3, 4, 5 or 6 2'-fluoro modifications, comprises 2, 3 or 4 phosphorothioate internucleotide linkages; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense strand), and optionally comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 3, 4, 5 or 6 2'-fluoro modifications, comprises 2, 3 or 4 phosphorothioate internucleotide linkages; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally

destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 3 or 4 2'-fluoro modifications, and comprises 0 or 2 phosphorothioate internucleotide linkages; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, between nucleotide positions 22 and 23, between nucleotide positions 1 and 2, between nucleotide positions 2 and 3; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense strand), and optionally comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6, 14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In some examples, the sense and antisense strands are independently 19, 20, 21, 22, 23, 24 or 25 nucleotides in length; the antisense strand contains at least one thermally destabilizing nucleotide, where the at least one thermally destabilizing nucleotide is in the seed region of the antisense strand (*i.e.*, at position 2-9 of the 5'-end of the antisense strand); wherein the sense strand is conjugated with a ligand, comprises 2'-fluoro modifications at positions 7, 10 and 11 or at positions 7, 9, 10 and 11 (counting from 5'-end of the sense strand), and optionally comprises phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3; wherein the antisense strand comprises 2'-fluoro modifications at positions 2, 6, 8, 9, 14 or 16, or at positions 2, 6,

14 or 16, or at positions 2, 14 and 16; and the antisense comprises phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, between nucleotide positions 22 and 23, between nucleotide positions 1 and 2, between nucleotide positions 2 and 3; and wherein the dsRNA optionally further has at least one (*e.g.*, one, two or all three) of the following characteristics: (i) the dsRNA comprises a duplex region of 12-25 nucleotide pairs in length; (ii) the dsRNA comprises a blunt end at 5'-end of the antisense strand; and (iii) the dsRNA has at least a two nucleotide overhang at the 3'-end of the antisense strand.

In a particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:
  (i) a length of 21 nucleotides;
  (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and
  (iii) 2'-F modifications at positions 7, 10, and 11 (counting from the 5' end);
  and
(b) an antisense strand having:
  (i) a length of 23 nucleotides;
  (ii) 2'-F modifications at positions 2, 6 to 8, 9, 14, and16 (counting from the 5' end);
  (iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end); and
  (iv) a thermally destabilizing modification of the duplex at position 7 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:
  (i) a length of 21 nucleotides;
  (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
  (iii) 2'-F modifications at positions 7, 9, 10, and 11 (counting from the 5' end); and
  (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);
  and
(b) an antisense strand having:
  (i) a length of 23 nucleotides;
  (ii) 2'-F modifications at positions 2, 6, 14, and 16 (counting from the 5' end);
  (iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end); and

(iv) a thermally destabilizing modification of the duplex at position 7 (counting from the 5’ end);

wherein the dsRNA agents have a two nucleotide overhang at the 3’-end of the antisense strand, and a blunt end at the 5’-end of the antisense strand.

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3’-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2’-F modifications at positions 7, 9, 10, and 11 (counting from the 5’ end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5’ end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2’-F modifications at positions 2, 14, and 16 (counting from the 5’ end);

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5’ end); and

(iv) a thermally destabilizing modification of the duplex at position 6 or 7 (counting from the 5’ end);

wherein the dsRNA agents have a two nucleotide overhang at the 3’-end of the antisense strand, and a blunt end at the 5’-end of the antisense strand.

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3’-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2’-F modifications at positions 7, 9, 10, and 11 (counting from the 5’ end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5’ end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2’-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5’ end);

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5’ end); and

(iv) a thermally destabilizing modification of the duplex at position 7 (counting from the 5’ end);

wherein the dsRNA agents have a two nucleotide overhang at the 3’-end of the antisense strand, and a blunt end at the 5’-end of the antisense strand.

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) an ASGPR ligand attached to the 3’-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(iii) 2’-F modifications at positions 7, 9, 10, and 11 (counting from the 5’ end); and

(iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5’ end);

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2’-F modifications at positions 2, 14, and 16 (counting from the 5’ end);

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5’ end); and

(iv) a thermally destabilizing modification of the duplex at position 7 (counting from the 5’ end);

wherein the dsRNA agents have a two nucleotide overhang at the 3’-end of the antisense strand, and a blunt end at the 5’-end of the antisense strand.

In another particular example, the dsRNA agents of the present disclosure comprising an antisense strand having:

(i) 2’-F modifications at positions 2, 14, and 16 (counting from the 5’ end); and

(2) a thermally destabilizing modification of the duplex at position 6 or 7 (counting from the 5’ end).

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:

(i) an ASGPR ligand, wherein said ASGPR ligand;

(ii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5’ end);

and

(b) an antisense strand having:

(i) 2'-F modifications at positions 2, 14, and 16 (counting from the 5' end);

(ii) a thermally destabilizing modification of the duplex at position 6 or 7 (counting from the 5' end);

In another particular example, the dsRNA agents of the present disclosure comprise:

(a) a sense strand having:

(i) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;

(ii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

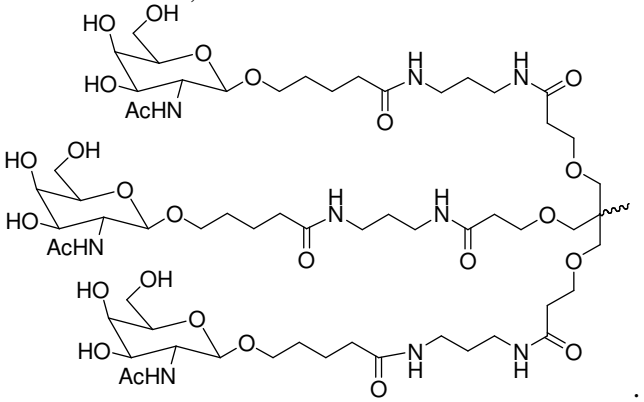
(ii) 2'-F modifications at positions 2, 14, and 16 (counting from the 5' end);

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (counting from the 5' end); and

(iv) a thermally destabilizing modification of the duplex at position 6 or 7 (counting from the 5' end);

wherein the dsRNA agents have a two nucleotide overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some examples, the dsRNA agent further comprises at least one ASGPR ligand. For example, the ASGPR ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker, such as:



.

In one example, the ASGPR ligand is attached to the 3' end of the sense strand.

The region of a Serpina1 mRNA targeted by any of the dsRNA agents described herein may be nucleotides 1440-1480 of SEQ ID NO:1; or nucleotides 1441-1479 of SEQ ID NO:1; or nucleotides 1442-1478 of SEQ ID NO:1; or nucleotides 1443-1477 of SEQ ID NO:1; or nucleotides 1444-1476 of SEQ ID NO:1; or nucleotides 1445-1475 of SEQ ID NO:1; or nucleotides 1446-1474 of SEQ ID NO:1; or nucleotides 1447-1473 of SEQ ID NO:1; or nucleotides 1448-1473 of SEQ ID NO:1; or nucleotides 1448-1472 of SEQ ID

NO:1; or nucleotides 1448-1471 of SEQ ID NO:1; or nucleotides 1448-1470 of SEQ ID NO:1; or nucleotides 1447-1469 of SEQ ID NO:1; or nucleotides 1446-1478 of SEQ ID NO:1; or nucleotides 1449-1471 of SEQ ID NO:1; or nucleotides 1450-1472 of SEQ ID NO:1; or nucleotides 1440-1475 of SEQ ID NO:1; or nucleotides 1445-1480 of SEQ ID NO:1; or nucleotides 1445-1475 of SEQ ID NO:1.

The present disclosure also provides vetors, cells, and pharmaceutical compositions comprising the dsRNA agents of the inevtion.

The pharmaceutical compositions of the disclosure may be an unbuffered solution, *e.g.,* saline or water; or a buffered solution, *e.g.,*a buffered solution c omprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof, or phosphate buffered saline (PBS).

In one example, the present disclosure provides a method of inhibiting Serpina1 expression in a cell. The method includes contacting the cell with any of the foregoing dsRNA agents or pharmaceutical composition of the disclosure, thereby inhibiting expression of the Serpina1 gene in the cell.

In one example, the cell is in a subject, such as a human subject.

In one example, the Serpina1 expression is inhibited by at least about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, or about 100%.

In one example, the present disclosure provides a method of treating a subject having a Serpina1 associated disease. The method includes administering to the subject a therapeutically effective amount of any of the foregoing dsRNA agents or pharmaceutical composition of the disclosure, thereby treating said subject.

In one example, the subject is a human.

In one example, the Serpina1 associated disease is a liver disorder.

In one example, the liver disorder is selected from the group consisting of chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma.

In another example, the present disclosure provides a method of inhibiting development of hepatocellular carcinoma in a subject having a Serpina1 deficiency variant. The method includes administering to the subject a therapeutically effective amount of any of the foregoing dsRNA agents or pharmaceutical composition of the disclosure, thereby inhibiting development of hepatocellular carcinoma in the subject.

In yet another example, the present disclosure provides a method of reducing the accumulation of misfolded Serpina1 in the liver of a subject having a Serpina1 deficiency variant. The method includes administering to the subject a therapeutically effective amount of any of the foregoing dsRNA agents or pharmaceutical composition of the disclosure, thereby reducing the accumulation of misfolded Serpina1 in the liver of the subject.

The dsRNA agent may administered to the subject at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg; or a dose of about 10 mg/kg to about 30 mg/kg.

The dsRNA agent may administered to the subject subcutaneously; or intravenously.

The dsRNA agent may administered to the subject in two or more doses.

The present invention is further illustrated by the following detailed description and drawings.

Brief Description of the Drawings

Figure 1A schematically depicts the modified sense and antisense strand nucleotide sequences of AD-61444; AD-75994; and AD-75995. Figure 1A discloses SEQ ID NOs: 36-37, 36, 38, 36 and 39, respectively, in order of appearance.

Figure 1B is a graph depicting the *in vivo* effect of administration of the indicated dsRNA agents on the level of expression of AAT (Serpina). The level of expression of AAT shown is relative to the pre-bleed level of expression of AAT.

We claim:

1. A double stranded RNA (dsRNA) agent that inhibits expression of a serine peptidase inhibitor, clade A, member 1 (Serpina1) gene, comprising a sense strand and an antisense strand forming a double stranded region,

wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:15,

wherein said antisense strand comprises at least one thermally destabilizing modification of the double stranded region within the first 9 nucleotide positions of the 5′ region or a precursor thereof,

wherein said sense strand comprises an ASGPR ligand, and

wherein each of the sense strand and the antisense strand are independently 14 to 40 nucleotides in length.

2. The dsRNA agent according to claim 1 wherein the dsRNA agent comprises at least four nucleotides comprising a 2'-fluoro modification.

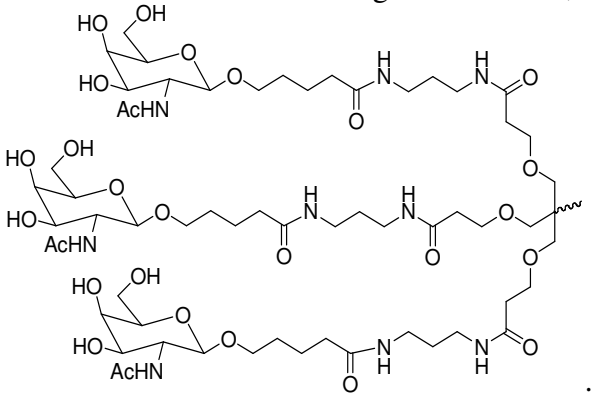
3. The dsRNA agent according to claim 1 wherein the sense strand has at least one of the following characteristics:

a) the ASGPR ligand attached to either end of the sense strand;

b) at least two nucleotides comprise a 2'-fluoro modification;

c) the double stranded region spans at least 19 nucleotide positions and wherein the thermally destabilizing modification is located within said double stranded region.

4. The dsRNA agent of claim 1, wherein the ASGPR ligand is:



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5. The dsRNA of any one of claims 1-4, wherein the dsRNA further has at least one of the following characteristics:
(i) the antisense strand comprises 2, 3, 4, 5 or 6 nucleotides comprising a 2'-fluoro modifications;
(ii) the antisense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages;
(iii) the sense strand is conjugated with a ligand;
(iv) the sense strand comprises 2, 3, 4 or 5 nucleotides comprising a 2'-fluoro modification;
(v) the sense strand comprises 1, 2, 3 or 4 phosphorothioate internucleotide linkages;
(vi) the dsRNA comprises at least four nucleotides comprising a 2'-fluoro modification;
(vii) the dsRNA comprises a double stranded region region of 12-40 nucleotide pairs in length; and
(viii) a blunt end at 5'end of the antisense strand.

6. The dsRNA agent of any one of claims 1-5, wherein each strand has 15-30 nucleotides.

7. The dsRNA agent of any one of claims 1-6, wherein each strand has 19-30 nucleotides.

8. The dsRNA agent of any one of claims 1-7, wherein the sense strand has 21 nucleotides, and the antisense strand has 23 nucleotides.

9. The dsRNA agent of any one of claims 1-8, wherein the antisense strand comprises a region of complementarity comprising at least 15 contiguous nucleotides differing by no more than 3 nucleotides from nucleotides 1440-1480 of SEQ ID NO:1.

10. The dsRNA agent of claim 9, wherein the region of complemntarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419).

11. The dsRNA agent of claim 9, wherein the sense strand comprises the nucleotide sequence 5' – CUUCUUAAUGAUUGAACAAAA – 3' (SEQ ID NO: 417) and the antisense strand comprises the nucleotide sequence 5' – UUUUGUUCAAUCAUUAAGAAGAC – 3' (SEQ ID NO: 419).

12. The dsRNA agent of claim 11, wherein the sense strand comprises the nucleotide sequence 5' - csusucuuAfaUfGfAfuugaacaaaa – 3' (SEQ ID NO: 33) and the antisense strand comprises the nucleotide sequence 5' – usUfsuugu(Tgn)caaucaUfuAfagaagsasc – 3' (SEQ ID NO: 34) ,

wherein a, g, c, and u are 2′-O-methyl (2′-OMe) A, G, C, and U, respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U, respectively; s is a phosphorothioate linkage; and (Tgn) is a thymidine-glycol nucleic acid (GNA) S-Isomer.

13. A vector containing the dsRNA agent of any one of claims 1-12.

14. A cell containing the dsRNA agent of any one of claims 1-12.

15. A pharmaceutical composition comprising the dsRNA agent of any one of claims 1-12.

16. The pharmaceutical composition of claim 15 for use in therapy.

17. Use of the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating a Serpina1 associated disease.

18. The use of claim 17, wherein the Serpina1 associated disease is a liver disorder.

19. The use of claim 18, wherein the liver disorder is selected from the group consisting of chronic liver disease, liver inflammation, cirrhosis, liver fibrosis, and/or hepatocellular carcinoma.

20. The use of claim 17, wherein the Serpina1 associated disease is caused by intracellular accumulations of misfolded Serpina 1 protein, wherein the use comprises that a therapeutically effective amount of the pharmaceutical composition is to be administered to a subject in need thereof, wherein the administration of the pharmaceutical composition reduces the accumulation of misfolded Serpina 1 in the liver of the subject.