Extrahepatic Delivery

BACKGROUND

[0001] Efficient delivery of an iRNA agent to cells *in vivo* requires specific targeting and substantial protection from the extracellular environment, particularly serum proteins. RNAi-based therapeutics show promising clinical data for treatment of liver-associated disorders. However, siRNA delivery into extra-hepatic tissues remains an obstacle, limiting the use of siRNA-based therapies.

[0002] One of the factors that limit the experimental and therapeutic application of iRNA agents *in vivo* is the ability to deliver intact siRNA efficiently. Particular difficulties have been associated with non-viral gene transfer into the retina *in vivo*. One of the challenges is to overcome the inner limiting membrane, which impedes the transfection of the retina. Additionally, negatively charged sugars of the vitreous have been shown to interact with positive DNA-transfection reagent complexes, promoting their aggregation, which impedes diffusion and cellular uptake.

[0003] Delivery of oligonucleotides to the central nervous system (CNS) poses particular problems due to the blood brain barrier (BBB) that free oligonucleotides cannot cross. One means to deliver oligonucleotides into the CNS is by intrathecal delivery. However, the oligonucleotides need also to be efficiently internalized into target cells of the CNS to achieve the desired therapeutic effect. Previous work has typically used delivery reagents such as liposomes, cationic lipids, and nanoparticles forming complexes to aid the intracellular internalization of oligonucleotides into cells of neuronal origin.

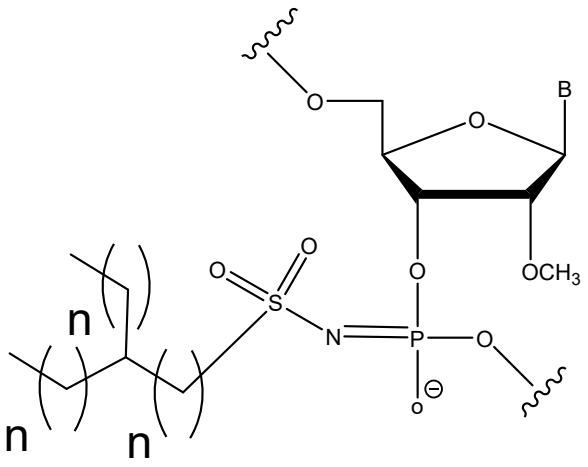
[0004] Thus, there is a continuing need for new and improved methods for delivering siRNA molecules *in vivo*, without the use of tissue delivery reagents, to achieve and enhance the therapeutic potential of iRNA agents.

SUMMARY

[0005] One example of the disclosure provides a compound (e.g., an oligonucleotide that can be either single-stranded or double-stranded) comprising one or more lipophilic monomers, containing one or more lipophilic moieties, conjugated to one or more positions on at least one strand of the oligonucleotide, optionally via a linker or carrier. For instance, some examples of the disclosure provide a compound (e.g., a double-stranded iRNA agent) comprising: an antisense strand which is complementary to a target gene; a sense

strand which is complementary to said antisense strand; and one or more lipophilic monomers, containing one or more lipophilic moieties, conjugated to one or more positions on at least one strand, optionally via a linker or carrier.

[0005a] In one aspect, the present disclosure refers to a compound comprising: an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic monomers, wherein the lipophilic monomer comprises



wherein: n is an integer of 1-21; and B is a modified or unmodified nucleobase.

[0006] In some examples, the lipophilicity of the lipophilic moiety, measured by octanol-water partition coefficient, $\log K_{ow}$, exceeds 0. The lipophilic moiety may possess a $\log K_{ow}$ exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10.

[0007] In some examples, the hydrophobicity of the compound, measured by the unbound fraction in the plasma protein binding assay of the compound, exceeds 0.2. In one example, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the compound, measured by fraction of unbound siRNA in the binding assay, exceeds 0.15, exceeds 0.2, exceeds 0.25, exceeds 0.3, exceeds 0.35, exceeds 0.4, exceeds 0.45, or exceeds 0.5 for an enhanced *in vivo* delivery of siRNA.

[0008] In some examples, the lipophilic moiety is an aliphatic, cyclic such as alicyclic, or polycyclic such as polyalicyclic compound, such as a steroid (e.g., sterol) or a linear or branched aliphatic hydrocarbon. Exemplary lipophilic moieties are lipid, cholesterol, retinoic acid, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-bis-O(hexadecyl)glycerol, geranyloxyhexyanol, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid, O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, ibuprofen, naproxen, dimethoxytrityl, or phenoxazine.

[0009] Suitable lipophilic moieties also include those containing a saturated or unsaturated C_4-C_{30} hydrocarbon chain (e.g., C_4-C_{30} alkyl or alkenyl), and an optional functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne. The functional groups are useful to attach the lipophilic moiety to the iRNA agent. In some examples, the lipophilic moiety contains a saturated or unsaturated C_6-C_{18} hydrocarbon chain (e.g., a linear C_6-C_{18} alkyl or alkenyl). In one example, the lipophilic moiety contains a saturated or unsaturated C_{16} hydrocarbon chain (e.g., a linear C_{16} alkyl or alkenyl). In some examples, the lipophilic moiety contains two or more carbon-carbon double bonds.

[0010] In some examples, the lipophilic moiety is a C_6-C_{30} moiety having a free terminal carboxylic acid functionality (e.g., hexanoic acid, heptanoic acid, octanoic acid,

nonanoic acid, decanoic acid, undecanoic acid, dodcanoic acid, tridecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, arachidonic acid, cis-4,7,10,13,16,19-docosahexaenoic acid).

[0011] In some examples, the lipophilic moiety is a C_6-C_{30} acid (e.g., hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodcanoic acid, tridecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, heptadecanoic acid, octadecanoic acid, oleic acid, linoleic acid, arachidonic acid, cis-4,7,10,13,16,19-docosahexaenoic acid, vitamin A, vitamin E, cholesterol etc.) or a C_6-C_{30} alcohol (e.g., hexanol, heptanol, octanol, nonanol, decanol, undecanol, dodcanol, tridecanol, tetradecanol, pentadecanol, hexadecanol, heptadecanol, octadecanol, oleyl alcohol, linoleyl alcohol, arachidonic alcohol, cis-4,7,10,13,16,19-docosahexanol, retinol, vitamin E, cholesterol etc.).

[0012] The lipophilic monomer may comprise a lipophilic moiety conjugated to any part of the iRNA agent, e.g., a nucleobase, sugar moiety, or internucleosidic linkage. When the lipophilic moiety is conjugated to the iRNA agent via a direct attachment to the nucleobase, ribosugar, or internucleosidic linkage of the iRNA agent, the lipophilic monomer then comprises the nucleobase, ribosugar, or internucleosidic linkage, and the lipophilic moiety. Alternatively, the lipophilic monomer may comprise a lipophilic moiety conjugated to a non-ribose replacement unit, such as a linker or carrier. When the lipophilic moiety is conjugated to the iRNA agent via a non-ribose replacement unit, such as a linker or a carrier, the lipophilic monomer then comprises the non-ribose replacement unit, such as the linker or carrier, and the lipophilic moiety.

[0013] In certain examples, the lipophilic monomer does not contain a nucleobase.

[0014] In certain examples, the lipophilic monomer comprises the lipophilic moiety conjugated to the compound via one or more linkers (tethers).

[0015] In some examples, the lipophilic monomer comprises the lipophilic moiety conjugated to the compound via a linker a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction (e.g., a triazole from the azide-alkyne cycloaddition), or carbamate.

[0016] In some examples, at least one of the linkers (tethers) is a redox cleavable linker (such as a reductively cleavable linker; e.g., a disulfide group), an acid cleavable linker (e.g., a hydrazone group, an ester group, an acetal group, or a ketal group), an esterase

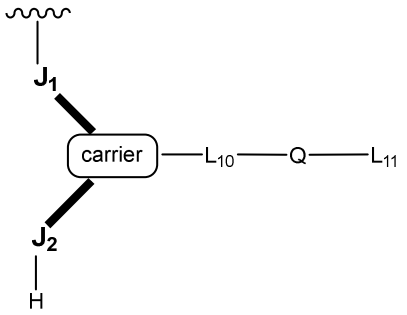
cleavable linker (e.g., an ester group), a phosphatase cleavable linker (e.g., a phosphate group), or a peptidase cleavable linker (e.g., a peptide bond).

[0017] In other examples, at least one of the linkers (tethers) is a bio-cleavable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

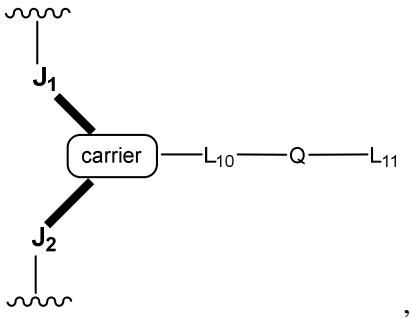
[0018] In certain examples, the lipophilic monomer comprises the lipophilic moiety conjugated to the compound via a non-ribose replacement unit, *i.e.*, a carrier that replaces one or more nucleotide(s). The carrier can be a cyclic group or an acyclic group. In one example, the cyclic group is selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin. In one example, the acyclic group is a moiety based on a serinol backbone, a glycerol backbone, or a diethanolamine backbone.

[0019] In some examples, the carrier replaces one or more nucleotide(s) in the double-stranded iRNA agent. In some examples, the carrier replaces one or more nucleotide(s) in the internal position(s) of the double-stranded iRNA agent. In other examples, the carrier replaces the nucleotides at the terminal end of the sense strand or antisense strand. In one example, the carrier replaces the terminal nucleotide on the 3' end of the sense strand, thereby functioning as an end cap protecting the 3' end of the sense strand. In one example, the carrier is a cyclic group having an amine, for instance, the carrier may be pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, or decalinyl.

[0020] In some examples, the lipophilic monomer may be represented by one of the following formulae:



or



,

wherein:

J_1 and J_2 are each independently O, S, NR^N, optionally substituted alkyl, OC(O)NH, NHC(O)O, C(O)NH, NHC(O), OC(O), C(O)O, OC(O)O, NHC(O)NH, NHC(S)NH, OC(S)NH, $OP(N(R^P)_2)O$, or $OP(N(R^P)_2)$;

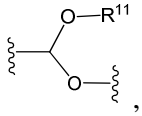
carrier is a cyclic group or an acyclic group;

R^N is H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, optionally substituted aralkyl, optionally substituted heteroaryl, or an amino protecting group;

R^P is independently for each occurrence H, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted cycloalkyl, or optionally substituted heteroaryl;

L_{10} is substituted or unsubstituted, saturated or unsaturated C3-C8 hydrocarbon, (e.g., C3-C8 alkyl, alkenyl, or alkynyl, or C3-C8 hydrocarbon containing two or more double bonds); the substituted groups include those already described herein for "substituted" hydrocarbon, alkyl, alkenyl, or alkynyl;

L_{11} is substituted or unsubstituted, saturated or unsaturated C6-C26 hydrocarbon, (e.g., C6-C26 alkyl, alkenyl, or alkynyl, or C3-C8 hydrocarbon containing two or more double bonds); the substituted groups include those already described herein for "substituted" hydrocarbon, alkyl, alkenyl, or alkynyl; and

Q is absent when there is no nucleobase on the carrier, or a cleavable group that will cleave L_{10} from L_{11} at least 10% *in vivo*. For instance, Q may be a cleavable group that can be cleaved *in vivo* to cleave L_{11} off the lipophilic monomer by about 10-70%, about 15-50%, about 20-40%, or about 20-30%. Exemplary cleavable groups include -OC(O)-, -C(O)O-, -SC(O)-, -C(O)S-, -OC(S)-, -C(S)O-, -S-S-, $-C(R^5)=N-$, $-N=C(R^5)-$, $-C(R^5)=N-O-$, $-O-N=C(R^5)-$, $-C(O)N(R^5)-$, $-N(R^5)C(O)-$, $-C(S)N(R^5)-$, $-N(R^5)C(S)-$, $-N(R^5)C(O)N(R^5)-$, $-N(R^5)C(O)C(R^3)(R^4)OC(O)-$, $-C(O)OC(R^3)(R^4)C(O)N(R^5)-$, –OC(O)O-, $-OSi(R^5)_2O-$, $-C(O)(CR^3R^4)C(O)O-$, $-OC(O)(CR^3R^4)C(O)-$, 

, or combinations thereof, R^{11} is a C_2-C_8 alkyl or alkenyl. For each occurrence, R^3, R^4, and R^5 are each independently H or C_1-C_4 alkyl.

[0021] In one example, the cleavability of Q is determined by the stability of ligands in cerebral spinal fluid (CSF), the stability of ligands in plasma, the stability of ligands in

brain homogenate or tissue homogenate (liver, ocular etc.), or the stability of ligands in vitreous humor.

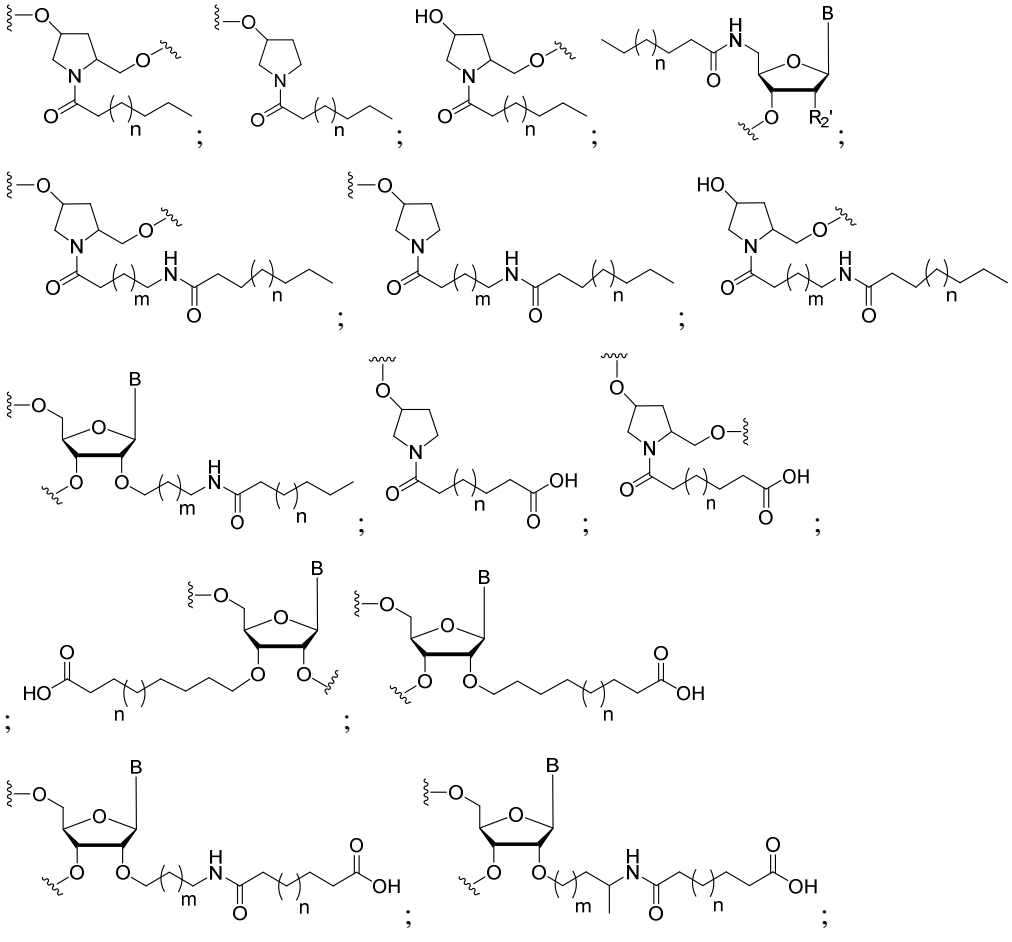
[0022] The cyclic and acyclic groups include those already described herein.

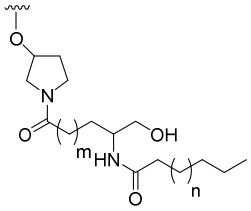
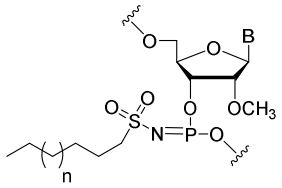
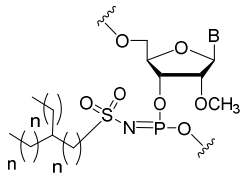
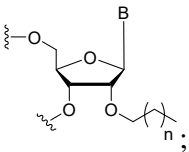
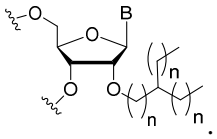
[0023] In one example, the acyclic group is a serinol, glycerol, or diethanolamine backbone.

[0024] In one example, the cyclic group is selected from the group consisting of pyrrolidinyl, hydroxyprolinyl, cyclopentyl, cyclohexyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

[0025] In one example, the cyclic group is a ribose or a ribose analog. Examples of ribose analogs include arabinose, 4'-thio ribose, 2'-O-methyl ribose, GNA, UNA, and LNA analogs.

[0026] In some examples, the lipophilic monomer conjugated to one or more positions of a strand of the compound has a structure of:



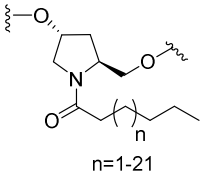

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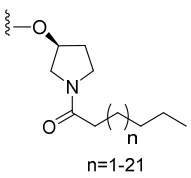
[0027] In the above structures for the lipophilic monomers, the monomers may also contain one or more asymmetric centers and thus occur as racemates and racemic mixtures, single enantiomers, individual diastereomers and diastereomeric mixtures. All such isomeric forms of the monomers are expressly included.

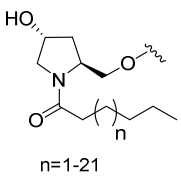
[0028] In the above structures for the lipophilic monomers, the alkylene chain can contain one or more unsaturated bonds.

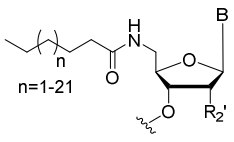
[0029] Integer m is 0-8. Integer n is 1-21. R_2' may be any functional group that is an acceptable 2'-modification for a ribose sugar, such as a 2'-O-methoxyalkyl (*e.g.*, 2'-O-methoxymethyl, 2'-O-methoxyethyl, or 2'-O-2-methoxypropanyl) modification, 2'-O-allyl modification, 2'-C-allyl modification, 2'-fluoro modification, 2'-O-N-methylacetamido (2'-O-NMA) modification, 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE) modification, 2'-O-aminopropyl (2'-O-AP) modification, or 2'-ara-F modification. For instance, R_2' may be H, OH, F, OMe, O-methoxyalkyl, O-allyl, O-N-methylacetamido, O-dimethylaminoethoxyethyl, or O-aminopropyl. B is a modified or unmodified nucleobase. W is an alkyl group such as a C_1-C_4 alkyl (e.g., methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl). R, R', and R'' are each independently H or an alkyl group such as a C_1-C_4 alkyl (e.g., methyl, ethyl, propyl, isopropyl, t-butyl).

[0030] In some examples, the lipophilic monomer conjugated to one or more positions of a strand of the compound has a structure of:

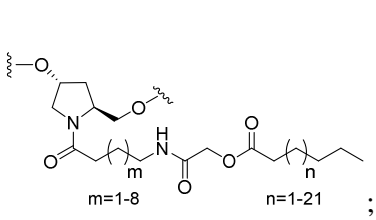


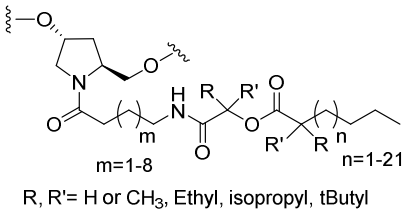
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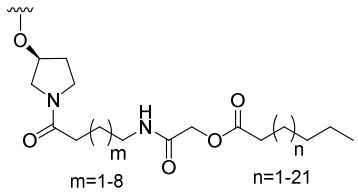
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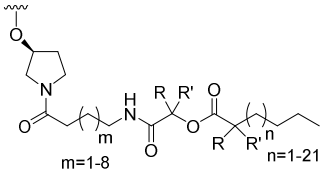
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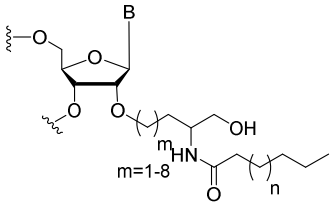
(R$_2$' is 2'-F, 2'-OMe,

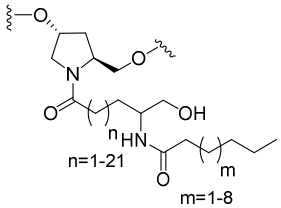


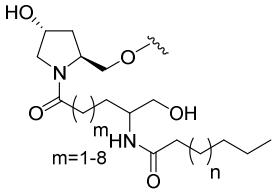
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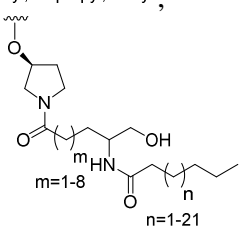
; R, R'= H or CH_3, Ethyl, isopropyl, tButyl ;


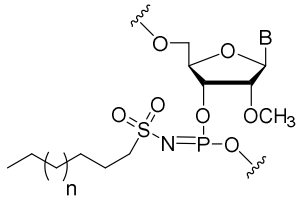
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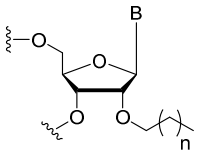
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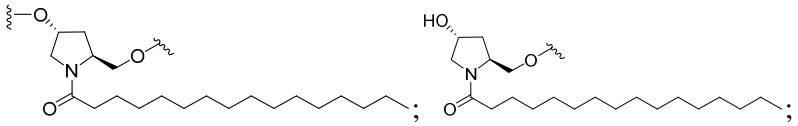
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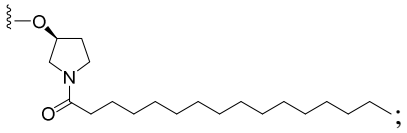
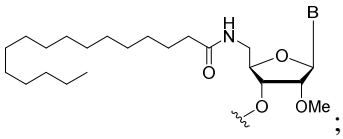
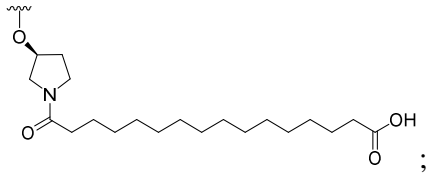
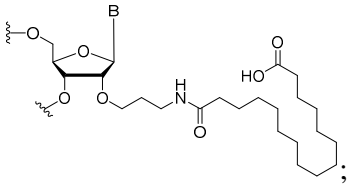
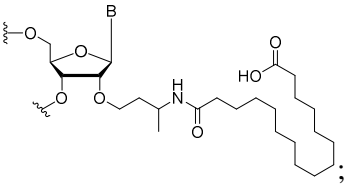
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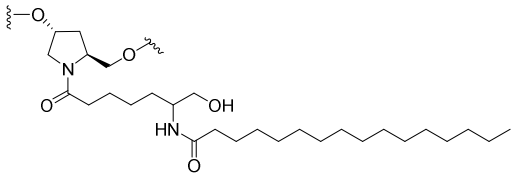
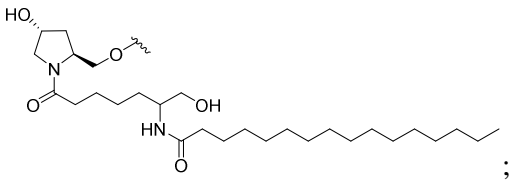
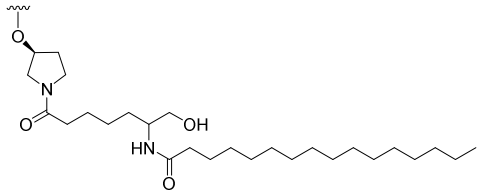
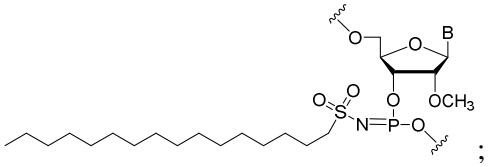
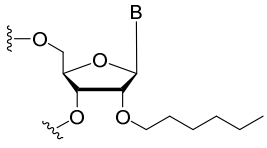
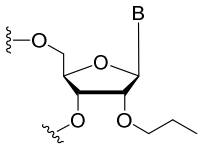
; or


. In these structures, B is a modified or unmodified nucleobase.

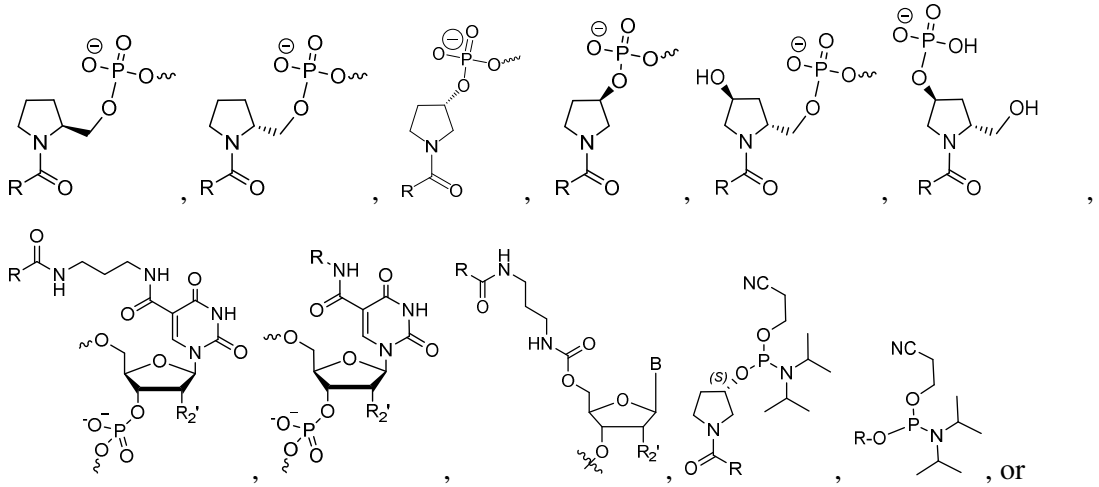
[0031] Specific examples of the lipophilic monomers include:


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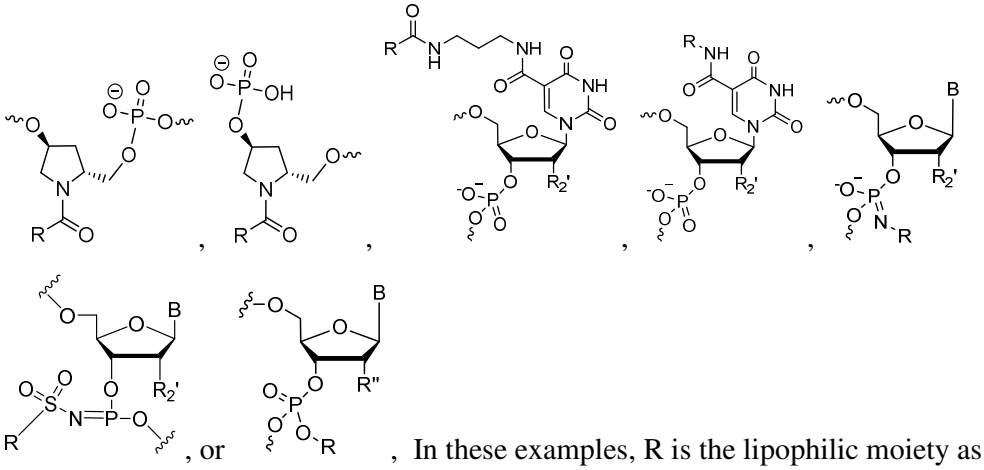

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; and

. In these structures, B is a modified or unmodified nucleobase; and R and R' are each independently H, methyl, ethyl, isopropyl, or t-butyl.

[0032] In some examples, the lipophilic monomer contains a lipophilic moiety conjugated to a strand of the compound (a single strand of a single-stranded oligonucleotide; or sense strand and/or antisense strand of a double-stranded oligonucleotide) via a carrier of:


. In these examples, R is the lipophilic moiety as defined herein. R_2' is H, OH, F, OMe, O-methoxyalkyl, O-allyl, O-N-methylacetamido, O-dimethylaminoethoxyethyl, or O-aminopropyl. B is a modified or unmodified nucleobase.

[0033] In some examples, the lipophilic monomer contains a lipophilic moiety conjugated to an internal position of a strand of the compound (a single strand of a single-

stranded oligonucleotide; or sense strand and/or antisense strand of a double-stranded oligonucleotide) via a carrier of:



, In these examples, R is the lipophilic moiety as defined herein. n is an integer of 1-21. R_2' is H, OH, F, OMe, O-methoxyalkyl, O-allyl, O-N-methylacetamido, O-dimethylaminoethoxyethyl, or O-aminopropyl. B is a modified or unmodified nucleobase.

[0034] Additional examples of the lipophilic monomers can be found in the Examples.

[0035] In some examples, the sense and antisense strands of the compound are each 15 to 30 nucleotides in length.

[0036] In one example, the sense and antisense strands of a compound are each 19 to 25 nucleotides in length.

[0037] In one example, the sense and antisense strands of the compound are each 21 to 23 nucleotides in length.

[0038] In some examples, the compound comprises a single-stranded overhang on at least one of the termini, e.g., 3' and/or 5' overhang(s) of 1-10 nucleotides in length, for instance, an overhang of 1, 2, 3, 4, 5, or 6 nucleotides. In some examples, both strands have at least one stretch of 1-5 (e.g., 1, 2, 3, 4, or 5) single-stranded nucleotides in the double stranded region. In one example, the single-stranded overhang is 1, 2, or 3 nucleotides in length. In some examples, the compound may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand), or vice versa. In one example, the compound comprises a 3' overhang at the 3'-end of the antisense strand, and optionally a blunt end at the 5'-end of the antisense strand. In one example, the compound has a 5' overhang at the 5'-end of the sense strand, and optionally a blunt end at the 5'-end of the antisense strand. In one example, the compound has two blunt ends at both ends of the iRNA duplex.

[0039] In one example, the sense strand of the compound is 21-nucleotide in length, and the antisense strand is 23-nucleotide in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single-stranded overhangs at the 3'-end.

[0040] In some examples, the sense strand further comprises at least one phosphorothioate linkage at the 3'-end. In some examples, the sense strand further comprises at least two phosphorothioate linkages at the 3'-end. In some examples, one or more lipophilic monomers are located on the 3'-end of the sense strand. In one example, one of the phosphorothioate linkages is located between the lipophilic monomer and the first nucleotide from the 3'-end of the sense strand.

[0041] In some examples, the sense strand further comprises at least one phosphorothioate linkage at the 5'-end. In some examples, the sense strand further comprises at least two phosphorothioate linkages at the 5'-end. In some examples, one or more lipophilic monomers are located on the 5'-end of the sense strand. In one example, one of the phosphorothioate linkages is located between the lipophilic monomer and the first nucleotide from the 5'-end of the sense strand.

[0042] In some examples, the antisense strand further comprises at least one phosphorothioate linkage at the 3'-end. In some examples, the antisense strand further comprises at least two phosphorothioate linkages at the 3'-end. In some examples, one or more lipophilic monomers are located on the 3'-end of the antisense strand. In one example, one of the phosphorothioate linkages is located between the lipophilic monomer and the first nucleotide from the 3'-end of the antisense strand.

[0043] In some examples, the compound further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand. In one example, the phosphate mimic is a 5'-vinyl phosphonate (VP).

[0044] In some examples, the 5'-end of the antisense strand of the compound does not contain a 5'-vinyl phosphonate (VP).

[0045] In some examples, the compound further comprises at least one terminal, chiral phosphorus atom.

[0046] A site specific, chiral modification to the internucleotide linkage may occur at the 5' end, 3' end, or both the 5' end and 3' end of a strand. This is being referred to herein as a "terminal" chiral modification. The terminal modification may occur at a 3' or 5' terminal position in a terminal region, *e.g.,* at a position on a terminal nucleotide or within the last 2, 3,

4, 5, 6, 7, 8, 9 or 10 nucleotides of a strand. A chiral modification may occur on the sense strand, antisense strand, or both the sense strand and antisense strand. Each of the chiral pure phosphorus atoms may be in either Rp configuration or Sp configuration, and combination thereof. More details regarding chiral modifications and chirally-modified dsRNA agents can be found in PCT/US18/67103, entitled "Chirally-Modified Double-Stranded RNA Agents," filed December 21, 2018, which is incorporated herein by reference in its entirety.

[0047] In some examples, the compound further comprises a terminal, chiral modification occurring at the first internucleotide linkage at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp configuration or Sp configuration.

[0048] In one example, the compound further comprises a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0049] In one example, the compound further comprises a terminal, chiral modification occurring at the first, second, and third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0050] In one example, the compound further comprises a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occurring at the third internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the antisense strand,

having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0051] In one example, the compound further comprises a terminal, chiral modification occurring at the first and second internucleotide linkages at the 3' end of the antisense strand, having the linkage phosphorus atom in Sp configuration; a terminal, chiral modification occurring at the first, and second internucleotide linkages at the 5' end of the antisense strand, having the linkage phosphorus atom in Rp configuration; and a terminal, chiral modification occurring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

[0052] In some examples, the compound has at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end).

[0053] In some examples, the antisense strand comprises two blocks of one, two, or three phosphorothioate internucleotide linkages separated by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 phosphate internucleotide linkages.

[0054] In some examples, the compound further comprises a targeting ligand that targets a receptor which mediates delivery to a specific CNS tissue. In one example, the targeting ligand is selected from the group consisting of Angiopep-2, lipoprotein receptor related protein (LRP) ligand, bEnd.3 cell binding ligand, transferrin receptor (TfR) ligand, manose receptor ligand, glucose transporter protein, and LDL receptor ligand.

[0055] In some examples, the compound further comprises a targeting ligand that targets a receptor which mediates delivery to an ocular tissue. In one example, the targeting ligand is selected from the group consisting of trans-retinol, RGD peptide, LDL receptor ligand, and carbohydrate-based ligands. In one example, the targeting ligand is a RGD peptide, such as H-Gly-Arg-Gly-Asp-Ser-Pro-Lys-Cys-OH or Cyclo(-Arg-Gly-Asp-D-Phe-Cys).

[0056] In some examples, the compound further comprises a targeting ligand that targets a liver tissue. In some examples, the targeting ligand is a carbohydrate-based ligand. In one example, the targeting ligand is a GalNAc conjugate.

[0057] In some examples, 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35% or 30% of the antisense and sense strand of the compound is modified.

For example, when 50% of the compound is modified, 50% of all nucleotides present in the compound contain a modification as described herein.

[0058] In some examples, the antisense and sense strands of the compound comprise at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or virtually 100% 2'-O-methyl modified nucleotides.

[0059] In one example, the compound is an oligonucleotide, e.g., a double-stranded dsRNA agent, and at least 50% of the nucleotides of the double-stranded dsRNA agent is independently modified with 2'-O-methyl, 2'-O-allyl, 2'-deoxy, or 2'-fluoro.

[0060] In one example, the oligonucleotide is an antisense, and at least 50% of the nucleotides of the antisense is independently modified with LNA, CeNA, 2'-methoxyethyl, or 2'-deoxy.

[0061] In some examples, the sense and antisense strands of the compound comprise less than 12, less than 10, less than 8, less than 6, less than 4, less than 2, or no 2'-F modified nucleotides. In some examples, the compound has less than 12, less than 10, less than 8, less than 6, less than 4, less than 2, or no 2'-F modifications on the sense strand. In some examples, the compound has less than 12, less than 10, less than 8, less than 6, less than 4, less than 2, or no 2'-F modifications on the antisense strand.

[0062] In some examples, the compound has one or more 2'-F modifications on any position of the sense strand or antisense strand.

[0063] In some examples, the compound has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide. Examples of non-natural nucleotide include acyclic nucleotides, LNA, HNA, CeNA, 2'-O-methoxyalkyl (*e.g.*, 2'-O-methoxymethyl, 2'-O-methoxyethyl, or 2'-O-2-methoxypropanyl), 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), 2'-ara-F, L-nucleoside modification (such as 2'-modified L-nucleoside, e.g., 2'-deoxy-L-nucleoside), BNA abasic sugar, abasic cyclic and open-chain alkyl.

[0064] In some examples, the compound has greater than 80%, greater than 85%, greater than 90%, greater than 95%, or virtually 100% natural nucleotides. For the purpose of these examples, natural nucleotides can include those having 2'-OH, 2'-deoxy, and 2'-OMe.

[0065] In some examples, the antisense strand contains at least one unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA) modification, e.g., at the seed region of the

antisense strand. In one example, the seed region is at positions 2-8 (or positions 5-7) of the 5'-end of the antisense strand.

[0066] In one example, the compound comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the compound has less than 20%, less than 15%, less than 10%, less than 5% non-natural nucleotide, or substantially no non-natural nucleotide.

[0067] In one example, the compound comprises a sense strand and antisense strand each having a length of 15-30 nucleotides; at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end); wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); wherein the compound has greater than 80%, greater than 85%, greater than 95%, or virtually 100% natural nucleotides, such as those having 2'-OH, 2'-deoxy, or 2'-OMe.

[0068] One example of the disclosure provides a compound comprising a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five, or six 2'-deoxy modifications on the sense and/or antisense strands; wherein the compound has a double stranded (duplex) region of between 19 to 25 base pairs; wherein the compound comprises a ligand; and wherein the sense strand does not comprise a glycol nucleic acid (GNA).

[0069] It is understood that the antisense strand has sufficient complementarity to a target sequence to mediate RNA interference. In other words, the compound is capable of inhibiting the expression of a target gene.

[0070] In one example, the compound comprises at least three 2'-deoxy modifications. The 2'-deoxy modifications are at positions 2 and 14 of the antisense strand, counting from 5'-end of the antisense strand, and at position 11 of the sense strand, counting from 5'-end of the sense strand.

[0071] In one example, the compound comprises at least five 2'-deoxy modifications. The 2'-deoxy modifications are at positions 2, 12 and 14 of the antisense strand, counting from 5'-end of the antisense strand, and at positions 9 and 11 of the sense strand, counting from 5'-end of the sense strand.

[0072] In one example, the compound comprises at least seven 2'-deoxy modifications. The 2'-deoxy modifications are at positions 2, 5, 7, 12 and 14 of the antisense strand, counting from 5'-end of the antisense strand, and at positions 9 and 11 of the sense strand, counting from 5'-end of the sense strand.

[0073] In one example, the antisense strand comprises at least five 2'-deoxy modifications at positions 2, 5, 7, 12 and 14, counting from 5'-end of the antisense strand. The antisense strand has a length of 18-25 nucleotides, or a length of 18-23 nucleotides.

[0074] In one example, the compound can comprise one or more non-natural nucleotides. For example, the compound can comprise less than 20%, e.g., less than 15%, less than 10%, or less than 5% non-natural nucleotides, or the compound comprises no non-natural nucleotides. For example, the compound comprises all natural nucleotides. Some exemplary non-natural nucleotides include, but are not limited to, acyclic nucleotides, locked nucleic acid (LNA), HNA, CeNA, 2'-methoxyethyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), and 2'-ara-F.

[0075] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; wherein the sense strand does not comprise a glycol nucleic acid (GNA); and wherein the compound comprises less than 20%, e.g., less than 15%, less than 10%, or less than 5% non-natural nucleotides or the compound comprises all natural nucleotides.

[0076] In one example, at least one the sense and antisense strands comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, or at least seven or more, 2'-deoxy modifications in a central region of the sense or antisense strand. Accordingly, in one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; and wherein the sense strand

and/or the antisense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, or at least seven or more, 2'-deoxy modifications in a central region of the sense strand and/or the antisense strand.

[0077] In some example, the sense strand has a length of 18 to 30 nucleotides and comprises at least two 2'-deoxy modifications in the central region of the sense strand. For example, the sense strand has a length of 18 to 30 nucleotides and comprises at least two 2'-deoxy modifications within positions 7, 8, 9, 10, 11, 12, and 13, counting from 5'-end of the sense strand.

[0078] In one example, the antisense strand has a length of 18 to 30 nucleotides and comprises at least two 2'-deoxy modifications in the central region of the antisense strand. For example, the antisense strand has length of 18 to 30 nucleotides and comprises at least two 2'-deoxy modifications within positions 10, 11, 12, 13, 14, 15 and 16, counting from 5'-end of the antisense strand.

[0079] In one example, the compound comprises a sense strand and an antisense strand; wherein the sense strand has a length of 17-30 nucleotide and comprises at least one 2'-deoxy modification in the central region of the sense strand; and wherein the antisense strand independently has a length of 17-30 nucleotides and comprises at least two 2'-deoxy modifications in the central region of the antisense strand.

[0080] In one example, the compound comprises a sense strand and an antisense strand; wherein the sense strand has a length of 17-30 nucleotide and comprises at least two 2'-deoxy modifications in the central region of the sense strand; and wherein the antisense strand independently has a length of 17-30 nucleotides and comprises at least one 2'-deoxy modification in the central region of the antisense strand.

[0081] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; and wherein the sense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, at least seven or more, 2'-deoxy modifications in a central region of the sense strand.

[0082] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two

phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; and wherein the antisense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, at least seven or more, 2'-deoxy modifications in a central region of the antisense strand.

[0083] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; wherein the compound comprises less than 20%, e.g., less than 15%, less than 10%, or less than 5% non-natural nucleotides or the compound comprises all natural nucleotides; and wherein the sense strand and/or the antisense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, at least seven or more, 2'-deoxy modifications in a central region of the sense strand and/or the antisense strand.

[0084] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; wherein the compound comprises less than 20%, e.g., less than 15%, less than 10%, or less than 5% non-natural nucleotides or the compound comprises all natural nucleotides; and wherein the sense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, at least seven or more, 2'-deoxy modifications in a central region of the sense strand.

[0085] In one example, the compound comprises a sense strand and an antisense strand, each strand independently having a length of 15 to 35 nucleotides; at least two phosphorothioate internucleotide linkages between the first five nucleotides counting from the 5' end of the antisense strand; at least three, four, five or six 2'-deoxy nucleotides on the sense and/or antisense strands; and wherein the compound has a duplex region of between 19 to 25 base pairs; wherein the compound comprises a ligand; wherein the compound

comprises less than 20%, e.g., less than 15%, less than 10%, or less than 5% non-natural nucleotides or the compound comprises all natural nucleotides; and wherein the antisense strand comprises at least one, e.g., at least two, at least three, at least four, at least five, at least six, at least seven or more, 2'-deoxy modifications in a central region of the antisense strand.

[0086] In one example, when the compound comprises less than 8 non-2'OMe nucleotides, the antisense stand comprises at least one DNA. For example, in any one of the examples of the disclosure when the compound comprises less than 8 non-2'OMe nucleotides, the antisense stand comprises at least one DNA.

[0087] In one example, when the antisense comprises two deoxy nucleotides and said nucleotides are at positions 2 and 14, counting from the 5'-end of the antisense strand, the compound comprises 8 or less (e.g., 8, 7, 6, 5, 4, 3, 2, 1 or 0) non-2'OMe nucleotides. For example, in any one of the examples of the disclosure when the antisense comprises two deoxy nucleotides and said nucleotides are at positions 2 and 14, counting from the 5'-end of the antisense strand, the compound comprises 0, 1, 2, 3, 4, 5, 6, 7 or 8 non 2'-OMe nucleotides.

[0088] In another example, the disclosure further provides a method for delivering the compound of the disclosure to a specific target in a subject by subcutaneous or intravenous administration. The disclosure further provides the compound of the disclosure for use in a method for delivering said agents to a specific target in a subject by subcutaneous or intravenous administration.

[0089] Another example of the disclosure relates to a method of reducing the expression of a target gene in a cell, comprising contacting said cell with a compound comprising an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic monomers, containing one or more lipophilic moieties, conjugated to one or more positions on at least one strand, optionally via a linker or carrier.

[0090] All the above examples relating to the lipophilic monomers, lipophilic moieties, and their conjugation to the compound in the first example of the disclosure relating to the compound are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a cell.

[0091] In one example, the cell is an extrahepatic cell.

[0092] In one example, the cell is not a hepatocyte.

[0093] Another example of the disclosure relates to a method of reducing the expression of a target gene in a subject, comprising administering to the subject a compound comprising contacting said cell with a compound comprising an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic monomers, containing one or more lipophilic moieties, conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0094] All the above examples relating to the lipophilic monomers, lipophilic moieties, and their conjugation to the compound in the first example of the disclosure relating to the compound are suitable in this example of the disclosure relating to a method of reducing the expression of a target gene in a subject.

[0095] In some examples, the compound is administered extrahepatically.

[0096] In one example, the compound is administered intrathecally or intracerebroventricularly. By intrathecal or intracerebroventricular administration of the compound, the method can reduce the expression of a target gene in a brain or spine tissue, for instance, cortex, cerebellum, cervical spine, lumbar spine, and thoracic spine.

[0097] In some examples, exemplary target genes are APP, ATXN2, C9orf72, TARDBP, MAPT(Tau), HTT, SNCA, FUS, ATXN3, ATXN1, SCA1, SCA7, SCA8, MeCP2, PRNP, SOD1, DMPK, and TTR. To reduce the expression of these target genes in the subject, the compound can be administered directly to the eye(s), e.g., intravitreally. By intravitreal administration of the compound, the method can reduce the expression of the target gene in an ocular tissue.

[0098] Another example of the disclosure relates to a method of treating a subject having a CNS disorder, comprising administering to the subject a therapeutically effective amount of a double-stranded RNAi agent, thereby treating the subject. The double-stranded RNAi agent comprises an antisense strand which is complementary to a target gene; a sense strand which is complementary to said antisense strand; and one or more lipophilic monomers, containing one or more lipophilic moieties conjugated to one or more internal positions on at least one strand, optionally via a linker or carrier.

[0099] All the above examples relating to the lipophilic monomers, lipophilic moieties, and their conjugation to the compound in the first example of the disclosure relating to the compound are suitable in this example of the disclosure relating to a method of treating a subject having a CNS disorder. Exemplary CNS disorders that can be treated by the method

of the disclosure include Alzheimer, amyotrophic lateral sclerosis (ALS), frontotemporal dementia, Huntington, Parkinson, spinocerebellar, prion, and lafora.

BRIEF DESCRIPTION OF THE DRAWINGS

[0100] Figure 1 is a scheme showing the general structure of ceramide.

[0101] Figure 2 is a graph depicting the stability of the siRNA conjugates in rat CSF after incubating the siRNA duplexes with rat CSF for 24 hours.

[0102] Figure 3 is a graph depicting the stability of the siRNA conjugates in the vitreous humor of rabbit and cyno (NHP) for 24 hours. The remaining amounts of ligand-conjugated duplexes were plotted.

[0103] Figure 4 is a graph depicting the stability of the siRNA conjugates in the vitreous humor of rabbit and cyno (NHP) for 24 hours. The remaining amounts of ligand-conjugated duplexes were plotted.

[0104] Figures 5A and 5B are graphs depicting the stability of the siRNA conjugates in rat brain homogenate for 4 hours. The remaining amounts of ligand-conjugated duplexes were plotted in Figure 5A and the stability of PS linkages were plotted in Figure 5B.

[0105] Figure 6 is a graph depicting the stability of the siRNA conjugates having esterase cleavable conjugates in the vitreous humor of rabbit and cyno (NHP) for 24 hours. The percentage of the ligand-conjugated duplexes hydrolyzed were plotted.

[0106] Figure 7 is a graph depicting the stability of the siRNA conjugates having esterase cleavable conjugates in rat plasma, CSF and brain homogenate for 24 hours. The percentage of the hydrolyzed ligand-conjugated duplexes were plotted.

[0107] Figure 8 is a graph depicting human serum albumin binding of siRNA conjugates at different concentrations of HSA. Fraction of bound siRNA was plotted against human serum albumin concentration.

[0108] Figure 9 is a graph depicting human serum albumin binding of siRNA conjugates having exposed carboxylic acids at different concentrations of HSA. Fraction of bound siRNA was plotted against human serum albumin concentration.

[0109] Figure 10 is a graph depicting the inhibition of ocular TTR expression by qPCR in mouse eyes following intravitreal administration of a single 7.5 µg dose of siRNA duplexes compared to PBS control.

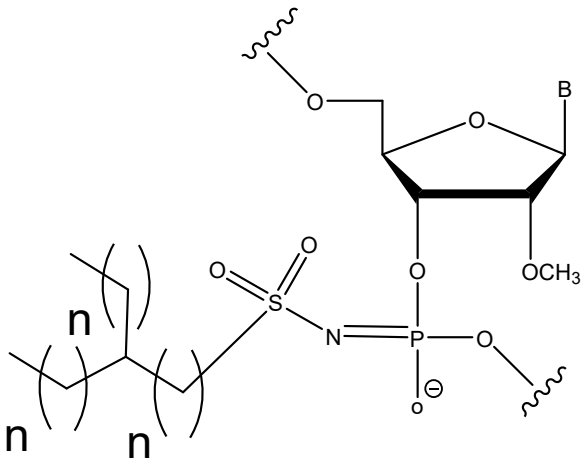
We claim:

1. A compound comprising:

an antisense strand which is complementary to a target gene;

a sense strand which is complementary to said antisense strand; and

one or more lipophilic monomers, wherein the lipophilic monomer comprises



wherein:

n is an integer of 1-21; and

B is a modified or unmodified nucleobase.

2. The compound of claim 1, wherein said sense and antisense strands are each 15 to 30 nucleotides in length.

3. The compound of claim 1, wherein said sense and antisense strands are each 19 to 25 nucleotides in length.

4. The compound of claim 1, wherein said sense and antisense strands are each 21 to 23 nucleotides in length.

5. The compound of claim 4, wherein the sense strand is 21 nucleotides in length, and the antisense strand is 23 nucleotides in length, wherein the strands form a double-stranded region of 21 consecutive base pairs having a 2-nucleotide long single-stranded overhangs at the 3'-end.

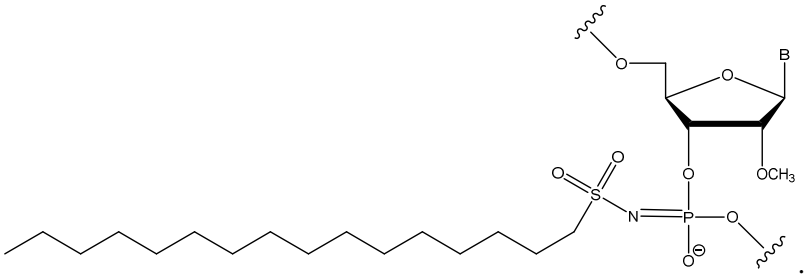
6. The compound of claim 1, wherein said compound comprises a single-stranded overhang on at least one of the termini.

7. The compound of claim 6, wherein said single-stranded overhang is 1, 2 or 3 nucleotides in length.

8. The compound of any one of claims 1-7, wherein the sense and the antisense strands comprise less than ten 2'-fluoro modified nucleotides.

9. The compound of any one of claims 1-7, wherein the sense and antisense strands comprise at least 50%, at least 60%, or least 70% of 2'-OMe modified nucleotides.

10. The compound of claim 1, wherein the lipophilic monomer is



wherein B is a modified or unmodified nucleobase.

11. The compound of any one of claims 1-10, wherein the sense strand comprises at least one phosphorothioate linkage at the 3'-end.

12. The compound of any one of claims 1-10, wherein the sense strand comprises at least two phosphorothioate linkages at the 3'-end.

13. The compound of claim 11 or 12, wherein one of the phosphorothioate linkages is located between the lipophilic monomer and the first nucleotide from the 3'-end of the sense strand.

14. The compound of any one of claims 1-13, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

15. The compound of claim 14, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

16. The compound of any one of claims 1-15, wherein the antisense strand comprises at least one GNA in the seed region.

17. The compound of claim 16, wherein the seed region is at position 5-7 from the 5'-end of the antisense strand.

18. The compound of any one of claims 1-17, further comprising a targeting ligand that targets a receptor which mediates delivery to a CNS tissue.

19. The compound of claim 18, wherein the targeting ligand is selected from the group consisting of Angiopep-2, lipoprotein receptor related protein (LRP) ligand, bEnd.3 cell binding ligand, transferrin receptor (TfR) ligand, manose receptor ligand, glucose transporter protein, and LDL receptor ligand.

20. The compound of any one of claims 1-17, further comprising a targeting ligand that targets a receptor which mediates delivery to an ocular tissue.

21. The compound of claim 20, wherein the targeting ligand is selected from the group consisting of trans-retinol, RGD peptide, LDL receptor ligand, and carbohydrate based ligands.

22. The compound of claim 21, wherein the targeting ligand is a RGD peptide and the RGD peptide is H-Gly-Arg-Gly-Asp-Ser-Pro-Lys-Cys-OH or Cyclo(-Arg-Gly-Asp-D-Phe-Cys).