EXTRA-HEPATIC DELIVERY IRNA COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Applicaton No. 63/255,984, filed on October 15, 2021, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Efficient delivery of an RNAi 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, RNAi delivery into extra-hepatic tissues remains an obstacle, limiting the use of RNAi-based therapies.

One of the limiting factors is the ability to deliver intact RNAi efficiently to extra-hepatic tissues, such as muscle tissues, *e.g.,* skeletal muscle tissues and cardiac muscle tissues, and adipose tissue.

For example, when administered systemically, RNAi agents naturally accumulate in the liver limiting distribution to extra-hepatic tissues.

Similarly, particular difficulties have been associated when RNAi agents are administered locally, in that although the RNAi agents can achieve significant target gene reduction, there is limited distribution in muscle or adipose tissue and target gene reduction is only observed in a small portion of the tissue, minimizing the potential therapeutic use.

Previous work has used delivery reagents such as liposomes, cationic lipids, and nanoparticles forming complexes to aid the intracellular internalization of RNAi agents into extra-hepatic cells. However, only limited success in delivering RNAi agents to extra-hepatic tissues, like muscle tissue, after systemic administration has been reported. For example although cholesterol-conjugated RNAi agents are delivered to muscles after intravenous injection, a high dose (50 mg/kg) is required to achieve sustainable gene silencing. In addition, cholesterol conjugates are highly toxic at high concentrations, limiting their potential for clinical applications. With respect to adipose tissues, while viral carriers have shown promise for RNAi agent delivery to adipocytes, the delivery process is labor intensive and the high immunogenicity has limited the widespread application. (See, *e.g.,* Biscans, *et al.* (2018) *Nucl Acids Res* 47(3):1082).

Thus, systemic delivery of oligonucleotides to extra-hepatic tissues, like muscle tissue and adipose tissue, remains a challenge and, accordingly, there is a continuing need for new and improved compositions and methods for delivering RNAi agents *in vivo*, without the use of tissue delivery reagents, to achieve and enhance the therapeutic potential of RNAi agents.

SUMMARY OF THE INVENTION

The present invention is based, at least in part, on the surprising discovery that conjugating a C_{22} lipophilic moiety to one or more internal positions on at least one strand of a dsRNA agent, *e.g.,* position 6 on the sense strand, counting from the 5'-end, provides surprisingly efficient *in vivo* delivery to muscle and/or adipose tissue resulting in efficient entry and internalization of the dsRNA agent into muscle tissue, *e.g.,* cardiac and skeletal tissue, and/or adipose tissue, and surpringly good inhibition of target gene expression in muscle tissue and/or adipose tissue, *e.g.,* cardiac and skeletal tissue, and/or adipose tissue.

Accordingly, in one aspect, the present invention provides a dsRNA agent comprising an antisense strand which is complementary to a target gene; a sense strand which is complementary to the antisense strand and forms a double stranded region with the antisense strand; and one or more C_{22} hydrocarbon chain conjugated to one or more internal positions on at least one strand, wherein the dsRNA agent is suitable for delivery to a muscle tissue, *e.g.,* skeletal muscle tissue or cardiac muscle tissue, or an adipose tissue. In some embodiments, the one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand are conjugated to the dsRNA agent *via* a linker or carrier.

In some embodiments, the lipophilicity of the one or more C_{22} hydrocarbon chain, 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.

In some embodiments, the hydrophobicity of the dsRNA agent, measured by the unbound fraction in the plasma protein binding assay of the dsRNA agent, exceeds 0.2. In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the dsRNA agent, measured by fraction of unbound dsRNA 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 dsRNA/

The C_{22} hydrocarbon chain may be saturated or unsaturated.

The C_{22} hydrocarbon chain may be linear or branched

In some embodiments, the internal positions include all positions except the three terminal positions from each end of the at least one strand.

In some embodiments, the internal positions exclude a cleavage site region of the sense strand.

In some embodiments, the internal positions exclude positions 9-12 or positions 11-13, counting from the 5'-end of the sense strand.

In some embodiments, the internal positions exclude a cleavage site region of the antisense strand.

In some embodiments, the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand.

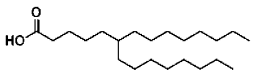
In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

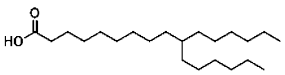
In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand.

In some embodiments, the one or more C_{22} hydrocarbon chains is an aliphatic, alicyclic, or polyalicyclic compound, *e.g.,* the one or more C_{22} hydrocarbon chains contains a functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 acid, *e.g.* the C22 acid is selected from the group consisting of docosanoic acid, 6-octyltetradecanoic acid, 10-hexylhexadecanoic acid, all-cis-7,10,13,16,19-docosapentaenoic acid, all-cis-4,7,10,13,16,19-docosahexaenoic acid, all-cis-13,16-docosadienoic acid, all-cis-7,10,13,16-docosatetraenoic acid, all-cis-4,7,10,13,16-docosapentaenoic acid, and cis-13-docosenoic acid.



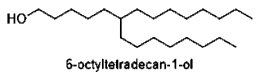
6-octyltetradecanoic acid



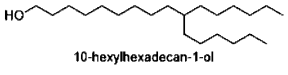
10-hexylhexadecanoic acid

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 alcohol, *e.g.,* the C22 alcohol is selected from the group consisting of 1-docosanol, 6-octyltetradecan-1-ol, 10-hexylhexadecan-1-ol, cis-13-docosen-1-ol, docosan-9-ol, docosan-2-ol, docosan-10-ol, docosan-11-ol, and cis-4,7,10,13,16,19-docosahexanol.

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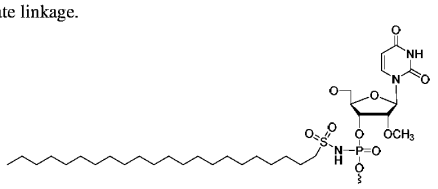
6-octyltetradecan-1-ol



10-hexylhexadecan-1-ol

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 amide, *e.g.,* the C22 amide is selected from the group consisting of (E)-Docos-4-enamide, (E)-Docos-5-enamide, (Z)-Docos-9-enamide, (E)-Docos-11-enamide,12-Docosenamide, (Z)-Docos-13-enamide, (Z)-N-Hydroxy-13-docoseneamide, (E)-Docos-14-enamide, 6-cis-Docosenamide, 14-Docosenamide Docos-11-enamide, (4E,13E)-Docosa-4,13-dienamide, and (5E,13E)-Docosa-5,13-dienamide.

The one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via a direct attachment to the ribosugar of the dsRNA agent. Alternatively, the the one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via a linker or a carrier. In some embodiments, the one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via internucleotide phosphate linkage.



In certain embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via one or more linkers (tethers), *e.g.,* a carrier that replaces one or more nucleotide(s) in the internal position(s).

In some embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent 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.

In some embodiments, 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).

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

In certain embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a carrier that replaces one or more nucleotide(s). The carrier can be a cyclic group or an acyclic group. In one embodiment, 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 embodiment, the acyclic group is a moiety based on a serinol backbone or a diethanolamine backbone.

In some embodiments, the carrier replaces one or more nucleotide(s) in the internal position(s) of the dsRNA agent.

In some embodiments, the sense and antisense strands of the dsRNA agent are each 15 to 30 nucleotides in length.

In one embodiment, the sense and antisense strands of a dsRNA agent are each 19 to 25 nucleotides in length.

In one embodiment, the sense and antisense strands of the dsRNA agent are each 21 to 23 nucleotides in length.

In some embodiments, the dsRNA agent 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 embodiments, 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 embodiment, the single-stranded overhang is 1, 2, or 3 nucleotides in length. In some embodiments, the dsRNA agent 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 embodiment, the dsRNA agent 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 embodiment, the dsRNA agent 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 embodiment, the dsRNA agent has two blunt ends at both ends of the iRNA duplex.

In some embodiments, at least one end of the dsRNA agent is blunt-ended.

In one embodiment, the sense strand of the dsRNA agent 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.

In some embodiments, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic phosphate linkage of the dsRNA agent.

In some embodiments, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand. In one embodiment, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In some embodiments, the 5'-end of the antisense strand of the dsRNA agent does not contain a 5'-vinyl phosphonate (VP).

In some embodiments, the dsRNA agent further comprises at least one terminal, chiral phosphorus atom.

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.

In some embodiments, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring 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.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some embodiments, the dsRNA agent has at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end).

In some embodiments, 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.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a receptor which mediates delivery to a skeletal muscle, cardiac muscle, or adipose tissue. In one embodiment, 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, LDL receptor ligand, trans-retinol, RGD peptide, LDL receptor ligand, CD63 ligand, CD36, and carbohydrate based ligand.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a liver tissue. In some embodiments, the targeting ligand is a carbohydrate-based ligand. In one embodiment, the targeting ligand is a GalNAc conjugate.

In some embodiments, the dsRNA agent further comprises a dual targeting ligand that targets a liver tissue and a receptor which mediates delivery to a skeletal muscle, cardiac muscle, or adipose tissue.

All the above aspects and embodiments would be applicable to an oligonucleotide having one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on the oligonucleotide. In some embodiments, 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35% or 30% of the oligonucleotide is modified. For example, when 50% of the oligonucleotide is modified, 50% of all nucleotides present in the oligonucleotide contain a modification as described herein.

In one embodiment, the oligonucleotide is 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.

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

In some embodiments, the dsRNA agent 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 embodiments, the dsRNA agent 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.

In some embodiments, the dsRNA agent has one or more 2'-F modifications on any position of the sense strand or antisense strand.

In some embodiments, the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

In one embodiment, the target gene is selected from the group consisting of adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4 (KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); or Phosphodiesterase 1 (PDE1).

In one embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, 7B, 7C, 9-16, 19-26, or 28-35.

In one embodiment, the dsRNA agent is any one of the agents in any one of Tables 2, 3, 4, 5, 7B, 7C, 9-16, 19-26, or 28-35.

In one embodiment, the target gene is selected from the group consisting of Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin; folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); and Activin A Receptor Type 1C (ACVR1C).

In one embodiment, the target gene is selected from the group consisting of myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

The present invention also provides cells and pharmaceutical compositions comprising the dsRNA agents of the invention.In another aspect, the present invention provides a method of inhibiting expression of a target gene in a skeletal muscle cell, a cardiac muscle cell, or an adipocyte, or adipose tissue. The method includes contacting the cell with a dsRNA agent that inhibits expression of a target gene, wherein the dsRNA agent comprises an antisense strand which is complementary to the target gene; a sense strand which is complementary to the antisense strand and forms a double stranded region with the antisense strand; and one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand, wherein the dsRNA agent is suitable for delivery to a muscle tissue, *e.g.,* skeletal muscle tissue or cardiac muscle tissue, or an adipose tissue. In some embodiments, the one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand are conjugated to the dsRNA agent *via* a linker or carrier.

In some embodiments, the lipophilicity of the one or more C_{22} hydrocarbon chain, 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.

In some embodiments, the hydrophobicity of the dsRNA agent, measured by the unbound fraction in the plasma protein binding assay of the dsRNA agent, exceeds 0.2. In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the dsRNA agent, measured by fraction of unbound dsRNA 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 dsRNA/

The C_{22} hydrocarbon chain may be saturated or unsaturated.

The C_{22} hydrocarbon chain may be linear or branched

In some embodiments, the internal positions include all positions except the three terminal positions from each end of the at least one strand.

In some embodiments, the internal positions exclude a cleavage site region of the sense strand.

In some embodiments, the internal positions exclude positions 9-12 or positions 11-13, counting from the 5'-end of the sense strand.

In some embodiments, the internal positions exclude a cleavage site region of the antisense strand.

In some embodiments, the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand.

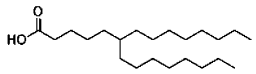
In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

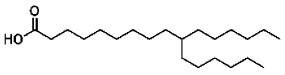
In some embodiments, the one or more C_{22} hydrocarbon chains are conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand.

In some embodiments, the one or more C_{22} hydrocarbon chains is an aliphatic, alicyclic, or polyalicyclic compound, *e.g.,* the one or more C_{22} hydrocarbon chains contains a functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 acid, *e.g.* the C22 acid is selected from the group consisting of docosanoic acid, 6-octyltetradecanoic acid, 10-hexylhexadecanoic acid, all-cis-7,10,13,16,19-docosapentaenoic acid, all-cis-4,7,10,13,16,19-docosahexaenoic acid, all-cis-13,16-docosadienoic acid, all-cis-7,10,13,16-docosatetraenoic acid, all-cis-4,7,10,13,16-docosapentaenoic acid, and cis-13-docosenoic acid.



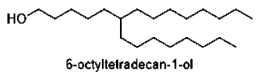
6-octyltetradecanoic acid



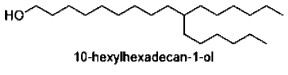
10-hexylhexadecanoic acid

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 alcohol, *e.g.,* the C22 alcohol is selected from the group consisting of 1-docosanol, 6-octyltetradecan-1-ol, 10-hexylhexadecan-1-ol, cis-13-docosen-1-ol, docosan-9-ol, docosan-2-ol, docosan-10-ol, docosan-11-ol, and cis-4,7,10,13,16,19-docosahexanol.

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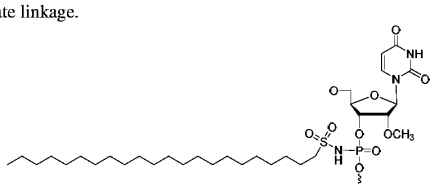
6-octyltetradecan-1-ol



10-hexylhexadecan-1-ol

In some embodiments, the one or more C_{22} hydrocarbon chains is a C22 amide, *e.g.,* the C22 amide is selected from the group consisting of (E)-Docos-4-enamide, (E)-Docos-5-enamide, (Z)-Docos-9-enamide, (E)-Docos-11-enamide,12-Docosenamide, (Z)-Docos-13-enamide, (Z)-N-Hydroxy-13-docoseneamide, (E)-Docos-14-enamide, 6-cis-Docosenamide, 14-Docosenamide Docos-11-enamide, (4E,13E)-Docosa-4,13-dienamide, and (5E,13E)-Docosa-5,13-dienamide.

The one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via a direct attachment to the ribosugar of the dsRNA agent. Alternatively, the the one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via a linker or a carrier. In some embodiments, the one or more C_{22} hydrocarbon chains may be conjugated to the dsRNA agent via internucleotide phosphate linkage.



In certain embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via one or more linkers (tethers), *e.g.,* a carrier that replaces one or more nucleotide(s) in the internal position(s).

In some embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent 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.

In some embodiments, 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).

In other embodiments, at least one of the linkers (tethers) is a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, peptide, *e.g.,* protease cleavable peptide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, mannose, and combinations thereof.

In certain embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a carrier that replaces one or more nucleotide(s). The carrier can be a cyclic group or an acyclic group. In one embodiment, 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 embodiment, the acyclic group is a moiety based on a serinol backbone or a diethanolamine backbone.

In some embodiments, the carrier replaces one or more nucleotide(s) in the internal position(s) of the dsRNA agent.

In some embodiments, the sense and antisense strands of the dsRNA agent are each 15 to 30 nucleotides in length.

In one embodiment, the sense and antisense strands of a dsRNA agent are each 19 to 25 nucleotides in length.

In one embodiment, the sense and antisense strands of the dsRNA agent are each 21 to 23 nucleotides in length.

In some embodiments, the dsRNA agent 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 embodiments, 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 embodiment, the single-stranded overhang is 1, 2, or 3 nucleotides in length. In some embodiments, the dsRNA agent 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 embodiment, the dsRNA agent 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 embodiment, the dsRNA agent 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 embodiment, the dsRNA agent has two blunt ends at both ends of the iRNA duplex.

In some embodiments, at least one end of the dsRNA agent is blunt-ended.

In one embodiment, the sense strand of the dsRNA agent 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.

In some embodiments, the lipophilic moiety is conjugated to a nucleobase, sugar moiety, or internucleosidic phosphate linkage of the dsRNA agent.

In some embodiments, the dsRNA agent further comprises a phosphate or phosphate mimic at the 5'-end of the antisense strand. In one embodiment, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In some embodiments, the 5'-end of the antisense strand of the dsRNA agent does not contain a 5'-vinyl phosphonate (VP).

In some embodiments, the dsRNA agent further comprises at least one terminal, chiral phosphorus atom.

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.

In some embodiments, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring 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.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In one embodiment, the dsRNA agent further comprises a terminal, chiral modification occuring 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 occuring 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 occuring at the first internucleotide linkage at the 5' end of the sense strand, having the linkage phosphorus atom in either Rp or Sp configuration.

In some embodiments, the dsRNA agent has at least two phosphorothioate internucleotide linkages at the first five nucleotides on the antisense strand (counting from the 5' end).

In some embodiments, 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.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a receptor which mediates delivery to a skeletal muscle, cardiac muscle, or adipose tissue. In one embodiment, 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, LDL receptor ligand, trans-retinol, RGD peptide, LDL receptor ligand, CD63 ligand, CD36, and carbohydrate based ligand.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a liver tissue. In some embodiments, the targeting ligand is a carbohydrate-based ligand. In one embodiment, the targeting ligand is a GalNAc conjugate.

In some embodiments, the dsRNA agent further comprises a dual targeting ligand that targets a liver tissue and a receptor which mediates delivery to a skeletal muscle, cardiac muscle, or adipose tissue.

All the above aspects and embodiments would be applicable to an oligonucleotide having one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on the oligonucleotide. In some embodiments, 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35% or 30% of the oligonucleotide is modified. For example, when 50% of the oligonucleotide is modified, 50% of all nucleotides present in the oligonucleotide contain a modification as described herein.

In one embodiment, the oligonucleotide is 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.

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

In some embodiments, the dsRNA agent 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 embodiments, the dsRNA agent 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.

In some embodiments, the dsRNA agent has one or more 2'-F modifications on any position of the sense strand or antisense strand.

In some embodiments, the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

In one embodiment, the target gene is selected from the group consisting of adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4 (KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); or Phosphodiesterase 1 (PDE1).

In one embodiment, the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5, 7B, 7C, 9-16, 19-26, and 28-35.

In one embodiment, the dsRNA agent is any one of the agents in any one of Tables 2, 3, 4, 5, 7B, 7C, 9-16, 19-26, or 28-35.

In one embodiment, the target gene is selected from the group consisting of Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin; folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); and Activin A Receptor Type 1C (ACVR1C).

In another embodiment, the target gene is selected from the group consisting of PPARG, ADIPOQ, CD36, LPL, ADAMTS9, RASD1, GYS2, CAT, DPYS, MLXIPL, VEGFA, HLA-DQA1, LIPA, CTSC, FCGR2A, GBE1, SH2B3, CTSK, CDKN2B, ELN, ARG1, HHEX, TCF7L2, CYP2A6, ALDH2, ACADS, GLYCTK, LDLR, HAL, ACER3, SLC7A7. PTPN22, CDKN1C, LEPR, SNAI2, PGM1, IGF2BP2, TTPA, ATP7B, ASPA, ADRB3, MAN2B1, RCAN1, PIGL, TBX1, LMNB1, FBP1, ETFA, LMNA, LAT2, PRKAG2, SELENBP1, TKT, PCSK1, PSAP, NDN, ACY1, SATB2, CYP21A2, POMC, CDC73, CTSH, CFTR, CTSA, G6PD, EXT1, EXT2, CPT1A, SEMA5A, WFS1, KIT, ACAT1, GGCX, FKBP6, PPARGC1B, DGCR6, HMGCS2, PEPD, WRN, LCAT, KLF13, SLC16A2, DHCR7, ITPR3, CLDN4, FZD9, SLC30A2, APOA5, HADHA, CDKAL1, PTPN2, LIPC, CD226, PON1, MCCC1, EIF2AK3, GYG1, BCL7B, AGL, VKORC1, BAZ1B, NAGS, ASL, STAR, ACP2, POLG, GAA, ALDH3A2, MANBA, ARSA, AGA, CYP27B1, CPS1, DLAT, DCXR, EIF4H, DYRK1A, GTF2I, LAMP2, CTH, EPO, FLAD1, AKT2, WAC, GLB1, RFC2, BACH2, D2HGDH, GHRL, TBL2, RRM2B, PRKACA, DLD, NEU1, ADSL, SLC22A5, ADCY10, INSR, HSD17B10, DGCR8, NPAP1, OXCT1, SDC3, HMGCL, PGAP1, MCCC2, LMF1, PIGM, UCP3, PAH, VPS33A, BCS1L, PDP1, AHCY, ALDH18A1, ENO3, MTTP, MAT1A, GNPTAB, PHGDH, BCAT2, CBS, HDAC4, LIG3, PSAT1, HGD, CTNND2, PDHB, PDHA1, NADK2, UPB1, PKLR, BCKDK, MEN1, GALT, LIMK1, SLC39A4, KCNJ11, PDHX, ACAD8, GSS, CHRNA7, SLC6A9, ERBB3, GLUD1, GSR, OAT, SLC6A8, CLIP2, STX1A, CARTPT, SLC25A15, DGCR2, LIPT2, NR5A1, DNM1L, PHEX, SLC30A9, B3GAT3, SLC34A3, SLC12A3, EPX, SARS2, CAPN10, ASNS, ALDOB, AGRP, MFF, GK, APOC2, CLDN3, HPRT1, PFKM, AMACR, SNRPN, HNF1B, L2HGDH, SORD, IDH2, TPMT, CYP2C19, TERT, MC4R, TMPRSS15, SLCO1B3, FGF23, PAX4, SLC30A8, MTNR1B, SI, SLCO1B1, and NR0B2.

In one embodiment, the target gene is selected from the group consisting of myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

In one embodiment, the cell is within a subject.

In one embodiment, the subject is a human.

In another aspect, the present invention provides a method of treating a subject having a skeletal muscle disorder, a cardiac muscle disorder, or an adipose tissue disorder, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of the invention or a pharmaceutical composition of the invention, thereby treating the subject.

In one embodiment, the cardiac muscle disorder is selected from the group consisting of obstructive hypertrophic cardiomyopathy (HOCM); familial hypertrophic cardiomyopathy (FHC);

Heart failure with preserved ejection fraction (HFPEF); atrial fibrillation (AFIB); ventricular fibrillation (VFIB); angina; myocardial infarction (MI); heart failure or heart failure with reduced ejection fraction (HFREF); supraventricular tachycardia (SVT); and hypertrophic cardiomyopathy (HCM).

In one embodiment, the skeletal muscle disorder is selected from the group consisting of Myostatin-related muscle hypertrophy, congenital myasthenic syndrome, and facioscapulohumeral muscular dystrophy (FSHD).

In one embodiment, the adipose tissue disorder is selected from the group consisting of a metabolic disorder, *e.g.* metabolic syndrome, a disorder of carbohydrates, *e.g.*, type II diabetes, pre-diabetes, a lipid metabolism disorder, *e.g.*, a hyperlipidemia, hypertension, a cardiovascular disease, a disorders of body weight.

The dsRNA agent may be administered to the subject intravenously, subcutaneously or intramuscularly.

In one embodiment, the dsRNA agent is administered to the subject intramuscularly.

In one embodiment, the dsRNA agent is administered to the subject subcutaneoulsy.

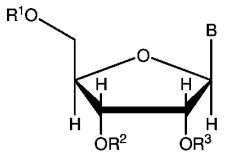
In one embodiment,the methods of the invention further include administering to the subject an additional agent or a therapy suitable for treatment or prevention of a skeletal muscle disorder, cardiac muscle disorder, or an adipose tissue disorder.

In one aspect, the present invention provides an RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of the invention.

In another embodiment, the RNAi agent is a pharmaceutically acceptable salt thereof. "Pharmaceutically acceptable salts" of each of RNAi agents herein include, but are not limited to, a sodium salt, a calcium salt, a lithium salt, a potassium salt, an ammonium salt, a magnesium salt, an mixtures thereof. One skilled in the art will appreciate that the RNAi agent, when provided as a polycationic salt having one cation per free acid group of the optionally modified phosophodiester backbone and/or any other acidic modifications (*e.g.*, 5'-terminal phosphonate groups). For example, an oligonucleotide of "n" nucleotides in length contains n-1 optionally modified phosophodiesters, so that an oligonucleotide of 21 nt in length may be provided as a salt having up to 20 cations (*e.g*, 20 sodium cations). Similarly, an RNAi agentshaving a sense strand of 21 nt in length and an antisense strand of 23 nt in length may be provided as a salt having up to 42 cations (*e.g*, 42 sodium cations). In the preceding example, where the RNAi agent also includes a 5'-terminal phosphate or a 5'-terminal vinylphosphonate group, the RNAi agent may be provided as a salt having up to 44 cations (*e.g*, 44 sodium cations).

In one aspect, the present invention provides a method of synthesizing a nucleoside monomer having the structure of Formula (I):

121301-15320
ALN-396WO01



FORMULA (I)

wherein:

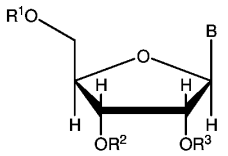
B is a modified or unmodified nucleobase;

R^1 is a hydroxyl protecting group;

R^2 is H or phosphoramidite;

R^3 is $C_{22}H_{45}$

wherein the compound of formula (I) is free or substantially free of a compound of Formula (II)



FORMULA (II)

wherein:

B is a modified or unmodified nucleobase;

R^1 is a hydroxyl protecting group;

R^2 is $C_{22}H_{45}$

R^3 is H

In one embodiment, the hydroxyl protecting group is selected from the group consisting of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-fluorenylmethylcarbonate (Fmoc), o-nitrophenylcarbonyl, p-phenylazophenylcarbonyl, phenylcarbonyl, p-chlorophenylcarbonyl, and 5′-(α-methyl-2-nitropiperonyl)oxycarbonyl (MeNPOC).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph depicting the effect of intravenous administration of a single 5 mg/kg or 20 mg/kg dose of the indicated dsRNA agents at Day 14 post-dose on SOD1 mRNA expression in liver, cardiac, and skeletal muscle tissues in mice.

FIG. 2A is a graph depicting the effect of intravenous administration of a single 1 mg/kg, 5 mg/kg, or 20 mg/kg dose of duplex AD-1615344 at Day 14 and Day 28 post-dose on MALAT1 mRNA expression in skeletal muscle tissue in mice.

FIG. 2B is a graph depicting the effect of intravenous administration of a single 1 mg/kg, 5 mg/kg, or 20 mg/kg dose of duplex AD-1615345 at Day 14 and Day 28 post-dose on MALAT1 mRNA expression in skeletal muscle tissue in mice.

FIG. 3A is a graph depicting the effect of route of administration of a single 5 mg/kg dose of AD-1427062, targeting the mouse SOD1 gene, on SOD1 mRNA expression in skeletal muscle tissue. IV, intravenous administration; IM(I), intramuscular administration of AD-1427062; IM(D); intramuscular administration of distal skeletal muscle with PBS control; SQ, subcutaneous administration; and IP, intraperitoneal administration.

FIG. 3B is a graph depicting the effect of route of administration of a single 5 mg/kg dose of AD-1427062, targeting the mouse SOD1 gene, on SOD1 mRNA expression in cardiac muscle tissue. IV, intravenous administration; IM(I), intramuscular administration; SQ, subcutaneous administration; and IP, intraperitoneal administration.

FIG. 3C is a graph depicting the effect of route of administration of a single 5 mg/kg dose of AD-1640773, targeting the mouse MSTN1 gene, on SOD1 mRNA expression in skeletal muscle tissue. IV, intravenous administration; IM(I), intramuscular administration of AD-1640773; IM(D); intramuscular administration of distal skeletal muscle with PBS control; and SQ, subcutaneous administration.

FIG. 3D is a graph depicting the effect of route of administration of a single 5 mg/kg dose of AD-1427062, targeting the mouse SOD1 gene, on SOD1 mRNA expression in adipose tissue. GAPDH mRNA expression was used as a control. IV, intravenous administration; IM(I), intramuscular administration; SQ, subcutaneous administration; and IP, intraperitoneal administration.

FIG. 3E is a graph depicting the effect of route of administration of a single 5 mg/kg dose of AD-1427062, targeting the mouse SOD1 gene, on SOD1 mRNA expression in adipose tissue. HPRT mRNA expression was used as a control. IV, intravenous administration; IM(I), intramuscular administration; SQ, subcutaneous administration; and IP, intraperitoneal administration.

FIG. 4A is a graph depicting SOD1 mRNA silencing in mouse gonadal adipose tissue at Days 14 and 28 post-dose of a single intravenously administered 0.5 mg/kg, 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting SOD1. GAPDH mRNA expression was used as a control.

FIG. 4B is a graph depicting SOD1 mRNA silencing in mouse subcutaneous adipose tissue at Days 14 and 28 post-dose of a single intravenously administered 0.5 mg/kg, 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting SOD1. GAPDH mRNA expression was used as a control.

FIG. 4C is a graph depicting SOD1 mRNA silencing in mouse brown intrascapular adipose tissue at Days 14 and 28 post-dose of a single intravenously administered 0.5 mg/kg, 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting SOD1. GAPDH mRNA expression was used as a control.

FIG. 5 is a graph summarizing SOD1 mRNA silencing in non-human primates' adipose tissue (brown adipose, white hind limb, white subcutaneous and white uterine) at 30 days post-dose of a single 3 mg/kg IV administered dose of a dsRNA agent targeting SOD1.

FIG. 6A is a graph depicting Leptin serum concentrations in lean female mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 6B is a graph depicting the percent change in leptin concentration in lean female mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean percent change in serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 7A is a graph depicting Leptin serum concentrations in lean male mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 7B is a graph the percent change in leptin concentration in lean male mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean percent change in serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 8A is a graph depicting Leptin serum concentrations in high-fat diet (HFD) fed male mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 8B is a graph depicting the percent change in leptin concentration in high-fat diet (HFD) fed male mice pre-dose (Day 0) and Days 8, 14, and 21 post-dose following subcutaneous administration of a single 5 mg/kg dose of AD-1888031 or AD-1888032. Each bar represents the mean percent change in serum concentration for each treatment group at the indicated timepoint +/- standard error (n=3) compared to PBS control.

FIG. 9A is a graph depicting the average relative mouse myostatin mRNA expression after intravenous administration of single 1, 2.5, or 5 mg/kg dose of lipid conjugated Mstn dsRNA agent at Day 14 post-dose in quadriceps as determined by qPCR.

FIG. 9B is a graph depicting the average relative mouse myostatin mRNA expression after intravenous administration of single 1, 2.5, or 5 mg/kg dose of lipid conjugated Mstn dsRNA agent at Dat 14 and 46 post-dose in quadriceps as determined by qPCR.

FIG. 10 is a graph depicting the average relative mouse SOD1 mRNA expression after intravenous administration of single 2 mg/kg dose of a lipid conjugated SOD1 dsRNA agent (AD-1427062) at Day 14 post-dose in quadriceps (left and right), gastrocnemius, and diaphragm as determined by qPCR.

FIG. 11 is a graph depicting the average relative mouse SOD1 mRNA expression following intravenous or subcutaneous administration of single 2 mg/kg or 1 mg/kg x 2 doses (administered 1 week apart) of AD-1812376 on Day 14 post-dose in quadriceps as determined by qPCR.

FIG. 12 is a graph depicting the average relative mouse SOD1 mRNA expression following single 2 mg/kg or 1 mg/kg x 2 doses of AD-1812376) agent after 21 days in heart administered either intravenously subcutaneously as determined by qPCR.

FIG. 13A is a graph depicting the average relative myostatin mRNA expression following intravenous administration of a single 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting Myostatin and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, at Day 56 post-dose in quadriceps as determined by qPCR and compared to PBS control in non-human primates.

FIG. 13B is a graph depicting the average relative myostatin mRNA expression following intravenous administration of a single 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting Myostatin and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, at Day 56 post-dose in gastrocnemius as determined qPCR and compared to PBS control in non-human primates.

FIG. 13C is a graph depicting the average relative myostatin protein expression following intravenous administration of a single 2 mg/kg or 5 mg/kg dose of a dsRNA agent targeting Myostatin and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, at Day 56 post-dose in quadriceps and gastrocnemius as determined by ELISA and compared to PBS control in non-human primates.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have unexpectedly discovered, *inter alia*, that conjugating a C_{22} lipophilic moiety to one or more internal positions on at least one strand of a dsRNA agent provides surprisingly efficient *in vivo* delivery to muscle and/or adipose tissue resulting in efficient entry and internalization of the dsRNA agent into muscle tissue, *e.g.*, cardiac and skeletal muscle tissue, and/or adipose tissue, and surprisingly good inhibition of target gene expression in muscle tissue, *e.g.*, cardiac and skeletal muscle tissue, and/or adipose tissue.

Accordingly, in one aspect, the present invention provides a dsRNA agent comprising an antisense strand which is complementary to the target gene; a sense strand which is complementary to the antisense strand and forms a double stranded region with the antisense strand; and one or more C_{22} hydrocarbon chains, *e.g.*, saturated or unsaturated, conjugated to one or more internal positions on at least one strand, wherein the dsRNA agent is suitable for delivery to a muscle tissue or an adipose tissue. In some embodiments, the one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand are conjugated to the dsRNA agent *via* a linker or carrier.

The following detailed description discloses how to make and use compositions containing dsRNA agents comprising one or more C_{22} hydrocarbon chains to inhibit the expression of a target gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of the target gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element, *e.g.*, a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, "sense strand or antisense strand" is understood as "sense strand or antisense strand or sense strand and antisense strand."

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means ±10%. In certain embodiments, about means ±5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least", "no less than", or "or more" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 19 nucleotides of a 21 nucleotide nucleic acid molecule" means that 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "or less" is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range. As used herein, ranges include both the upper and lower limit.

As used herein, methods of detection can include determination that the amount of analyte present is below the level of detection of the method.

In the event of a conflict between an indicated target site and the nucleotide sequence for a sense or antisense strand, the indicated sequence takes precedence.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

As used herein, "target sequence" or "target nucleic acid" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a target gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for RNAi-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a target gene. In one embodiment, the target sequence is within the protein coding region of the target gene. In another embodiment, the target sequence is within the 3' UTR of the target gene. The target nucleic acid can be a cellular gene (or mRNA transcribed from the gene) whose expression is associated with a particular disorder or disease state.

The target sequence may be from about 9-36 nucleotides in length, *e.g.*, about 15-30 nucleotides in length. For example, the target sequence can be about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. In some embodiments, the target sequence is about 19 to about 30 nucleotides in length. In other embodiments, the target sequence is about 19 to about 25 nucleotides in length. In still other embodiments, the target sequence is about 19 to about 23 nucleotides in length. In some embodiments, the target sequence is about 21 to about 23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.*, Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. It is understood that when a cDNA sequence is provided, the corresponding mRNA or RNAi agent would include a U in place of a T. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another

example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention. Further, one of skill in the art that a T is a target gene sequence, or reverse complement thereof, would often be replaced by a U in an RNAi agent of the invention.

The terms "iRNA", "RNAi agent," "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript *via* an RNA-induced silencing complex (RISC) pathway. RNA interference (RNAi) is a process that directs the sequence-specific degradation of mRNA. RNAi modulates, *e.g.,* inhibits, the expression of a target gene in a cell, *e.g.,* a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the disclosure includes a single stranded RNAi that interacts with a target RNA sequence, *e.g.,* a target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double-stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). Thus, in one aspect the disclosure relates to a single stranded RNA (ssRNA) (the antisense strand of a siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded RNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150:883-894.

In another embodiment, an "RNAi agent" for use in the compositions and methods of the disclosure is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA" refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel

and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, *i.e.,* a target mRNA sequence. In some embodiments of the disclosure, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, *e.g.,* an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, a dsRNA molecule can include ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.,* a deoxyribonucleotide, a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides.

As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or a modified nucleobase. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, *e.g.,* a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the disclosure include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide – which is acknowledged as a naturally occurring form of nucleotide – if present within a RNAi agent can be considered to constitute a modified nucleotide.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, *e.g.,* about 15-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 20, at least 23 or more unpaired nucleotides or nucleotides not directed to the target site of the dsRNA. In some embodiments, the hairpin loop can be

10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

In certain embodiment, the two strands of double-stranded oligomeric compound can be linked together. The two strands can be linked to each other at both ends, or at one end only. By linking at one end is meant that 5'-end of first strand is linked to the 3'-end of the second strand or 3'-end of first strand is linked to 5'-end of the second strand. When the two strands are linked to each other at both ends, 5'-end of first strand is linked to 3'-end of second strand and 3'-end of first strand is linked to 5'-end of second strand. The two strands can be linked together by an oligonucleotide linker including, but not limited to, (N)n; wherein N is independently a modified or unmodified nucleotide and n is 3-23. In some embodiemtns, n is 3-10, *e.g.,* 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the oligonucleotide linker is selected from the group consisting of GNRA, (G)4, (U)4, and (dT)4, wherein N is a modified or unmodified nucleotide and R is a modified or unmodified purine nucleotide. Some of the nucleotides in the linker can be involved in base-pair interactions with other nucleotides in the linker. The two strands can also be linked together by a non-nucleosidic linker, *e.g.* a linker described herein. It will be appreciated by one of skill in the art that any oligonucleotide chemical modifications or variations describe herein can be used in the oligonucleotide linker.

Hairpin and dumbbell type oligomeric compounds will have a duplex region equal to or at least 14, 15, 15, 16, 17, 18, 19, 29, 21, 22, 23, 24, or 25 nucleotide pairs. The duplex region can be equal to or less than 200, 100, or 50, in length. In some embodiments, ranges for the duplex region are 15-30, 17 to 23, 19 to 23, and 19 to 21 nucleotides pairs in length.

The hairpin oligomeric compounds can have a single strand overhang or terminal unpaired region, in some embodiments at the 3', and in some embodiments on the antisense side of the hairpin. In some embodiments, the overhangs are 1-4, more generally 2-3 nucleotides in length. The hairpin oligomeric compounds that can induce RNA interference are also referred to as "shRNA" herein.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In one embodiment, an RNAi agent of the invention is a dsRNA, each strand of which is 24-30 nucleotides in length, that interacts with a target RNA sequence, *e.g.,* a target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA

into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188).

In one embodiment, an RNAi agent of the invention is a dsRNA agent, each strand of which comprises 19-23 nucleotides that interacts with a target mRNA sequence to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp *et al.* (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, *et al.*, (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, *et al.*, (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, *et al.*, (2001) *Genes Dev.* 15:188). In one embodiment, an RNAi agent of the invention is a dsRNA of 24-30 nucleotides that interacts with a target mRNA sequence to direct the cleavage of the target RNA.

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a RNAi agent, *e.g.,* a dsRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively, the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment of the dsRNA, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.,* 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In one embodiment, the antisense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* 0-3, 1-3, 2-4, 2-5, 4-10, 5-10, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end.

In one embodiment, the sense strand of a dsRNA has a 1-10 nucleotide, *e.g.,* a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, *e.g.,* 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3'end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5'end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate. In certain embodiments, the overhang includes a self-complementary portion such that the overhang is capable of forming a hairpin structure that is stable under physiological conditions.

The terms "blunt" or "blunt ended" as used herein in reference to a dsRNA mean that there are no unpaired nucleotides or nucleotide analogs at a given terminal end of a dsRNA, *i.e.*, no nucleotide overhang. One or both ends of a dsRNA can be blunt. Where both ends of a dsRNA are blunt, the dsRNA is said to be blunt ended. To be clear, a "blunt ended" dsRNA is a dsRNA that is blunt at both ends, *i.e.*, no nucleotide overhang at either end of the molecule. Most often such a molecule will be double stranded over its entire length.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, *e.g.,* a dsRNA, which includes a region that is substantially complementary to a target sequence, *e.g.,* a target mRNA sequence.

As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, *e.g.,* a target nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, *e.g.,* within 5, 4, 3, or 2 nucleotides of the 5'- or 3'-terminus of the RNAi agent.

In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, the antisense strand of the double stranded RNA agent of the invention includes no more than 4 mismatches with the target mRNA, *e.g.,* the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the target mRNA. In some embodiments, the antisense strand double stranded RNA agent of the invention includes no more than 4 mismatches with the sense strand, *e.g.,* the antisense strand includes 4, 3, 2, 1, or 0 mismatches with the sense strand. In some embodiments, a double stranded RNA agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the sense strand of the double stranded RNA

agent of the invention includes no more than 4 mismatches with the antisense strand, *e.g.,* the sense strand includes 4, 3, 2, 1, or 0 mismatches with the antisense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA agent. In some embodiments, the mismatch(s) is not in the seed region.

Thus, an RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, a RNAi agent as described herein contains no more than 3 mismatches (*i.e.,* 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a target gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a target gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a target gene is important, especially if the particular region of complementarity in a target gene is known to vary.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of a RNAi agent that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can be, for example, "stringent conditions", where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50 oC or 70 oC for 12-16 hours followed by washing (see, *e.g.,*

"Molecular Cloning: A Laboratory Manual, Sambrook, *et al.* (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an RNAi agent, *e.g.,* within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.,* inhibition of gene expression , *in vitro* or *in vivo*. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogsteen base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between two oligonucleotides or polynucleotides, such as the antisense strand of a RNAi agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) or target sequence refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest or target sequence (*e.g.,* an mRNA encoding a target gene). For example, a polynucleotide is complementary to at least a part of a target RNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a target gene.

Accordingly, in some embodiments, the antisense strand polynucleotides disclosed herein are fully complementary to the target gene sequence.

Exemplary target genes include, for example, adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4 (KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); Phosphodiesterase 1 (PDE1); myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); Double Homeobox 4 (DUX4); Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin; folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); and Activin A Receptor Type 1C (ACVR1C).

As used herein, "adrenoceptor beta 1," used interchangeably with the term "ADRB1," refers to a member of the adrenergic receptor family. The adrenergic receptors are a prototypic family of guanine nucleotide binding regulatory protein-coupled receptors that mediate the physiological effects of the hormone epinephrine and the neurotransmitter norepinephrine. Beta-1 adrenoceptors are predominately located in the heart. Specific polymorphisms in this gene have been shown to affect the resting heart rate and can be involved in heart failure. ADRB1 is also known as ADRB1R, beta-1 adrenergic receptor, B1AR, BETA1AR, FNSS2, or RHR

An exemplary sequence of a human ADRB1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1653960731 (NM_000684.3; SEQ ID NO:1; reverse complement, SEQ ID NO: 5). The sequence of mouse ADRB1 mRNA can be found at, for example, GenBank Accession No. GI: 1693744501 (NM_007419.3; SEQ ID NO:2; reverse complement, SEQ ID NO: 6). The sequence of rat ADRB1 mRNA can be found at, for example, GenBank Accession No. GI: 6978458 (NM_012701.1; SEQ ID NO:3; reverse complement, SEQ ID NO: 7). The sequence of Macaca mulatta ADRB1 mRNA can be found at, for example, GenBank Accession No. GI: 577861029 (NM_001289866.1; SEQ ID NO: 4; reverse complement, SEQ ID NO: 8). The sequence of Macaca fascicularis ADRB1 mRNA can be found at, for example, GenBank Accession No. GI: 985482105 (NM_001319353.1; SEQ ID NO: 9; reverse complement, SEQ ID NO: 10).

Additional examples of ADRB1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on ADRB1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=ADRB1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term ADRB1, as used herein, also refers to variations of the ADRB1 gene including variants provided in the SNP database. Numerous sequence variations within the ADRB1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=ADRB1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

In one embodiment, the target gene is calcium voltage-gated channel subunit alpha1 C (CACNA1C).

As used herein, "calcium voltage-gated channel subunit alpha1 C," used interchangeably with the term "CACNA1C," refers to an alpha-1 subunit of a voltage-dependent calcium channel. Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization. The alpha-1 subunit consists of 24 transmembrane segments and forms the pore through which ions pass into the cell. The calcium channel consists of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio. There are multiple isoforms of each of these proteins, either encoded by different genes or the result of alternative splicing of transcripts. The protein encoded by this gene binds to and is inhibited by dihydropyridine. CACNA1C is also known as calcium channel, voltage-dependent, L type, alpha 1C subunit; voltage-dependent L-type calcium channel subunit alpha-1C; voltage-gated L-type calcium channel Cav1.2 alpha 1 subunit, splice variant 10; calcium channel, L type, alpha-1 polypeptide, isoform 1, cardiac muscle; calcium channel, cardic dihydropyridine-sensitive, alpha-1 subunit; voltage-dependent L-type Ca2+ channel alpha 1 subunit; voltage-gated calcium channel subunit alpha CaV1.2; DHPR, alpha-1 subunit; CACH2, CACN2, CACNL1A1, CCHL1A1, CaV1.2, LQT8, TS, or TS. LQT8

An exemplary sequence of a human CACNA1C mRNA transcript can be found at, for example, GenBank Accession No. GI: 1890333913 (NM_199460.4; SEQ ID NO:11; reverse complement, SEQ ID NO: 12). The sequence of mouse CACNA1C mRNA can be found at, for example, GenBank Accession No. GI: 594140631 (NM_009781.4; SEQ ID NO:13; reverse complement, SEQ ID NO: 14). The sequence of rat CACNA1C mRNA can be found at, for example, GenBank Accession No. GI: 158186632 (NM_012517.2; SEQ ID NO:15; reverse complement, SEQ ID NO: 16). The sequence of Macaca mulatta CACNA1C mRNA can be found at, for example, GenBank Accession No. GI: 1622843324 (XM_028829106.1; SEQ ID NO: 17; reverse complement, SEQ ID NO: 18).

Additional examples of CACNA1C mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on CACNA1C can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CACNA1C.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CACNA1C, as used herein, also refers to variations of the CACNA1C gene including variants provided in the SNP database. Numerous sequence variations within the CACNA1C gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=CACNA1C, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "calcium voltage-gated channel subunit alpha1 G," used interchangeably with the term "CACNA1G," refers to a T-type, low-voltage activated calcium channel. Voltage-sensitive calcium channels mediate the entry of calcium ions into excitable cells, and are also involved in a variety of calcium-dependent processes, including muscle contraction, hormone or neurotransmitter release, gene expression, cell motility, cell division, and cell death. The T-type channels generate currents that are both transient, owing to fast inactivation, and tiny, owing to small conductance. T-type channels are thought to be involved in pacemaker activity, low-threshold calcium spikes, neuronal oscillations and resonance, and rebound burst firing. CACNA1G is also known as calcium channel, voltage-dependent, T type, alpha 1G subunit; voltage-dependent T-type calcium channel subunit alpha-1G; voltage-gated calcium channel subunit alpha Cav3.1; NBR13 ; Cav3.1c; Ca(V)T.1; KIAA1123; SCA42ND; or SCA42.

An exemplary sequence of a human CACNA1G mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519244109 (NM_018896.5; SEQ ID NO: 21; reverse complement, SEQ ID NO: 22). The sequence of mouse CACNA1G mRNA can be found at, for example, GenBank Accession No. GI: 295444826 (NM_009783.3; SEQ ID NO: 23; reverse complement, SEQ ID NO: 24). The sequence of rat CACNA1G mRNA can be found at, for example, GenBank Accession No. GI: 1995160279 (NM_001308302.2; SEQ ID NO: 25; reverse complement, SEQ ID NO: 26). The sequence of Macaca mulatta CACNA1G mRNA can be found at, for example, GenBank Accession No. GI: 1622879013 (XM_015119270.2; SEQ ID NO: 27; reverse complement, SEQ ID NO: 28). The sequence of Macaca fascicularis CACNA1G mRNA can be found at, for example, GenBank Accession No. GI: 982305044 (XM_005583707.2; SEQ ID NO: 29; reverse complement, SEQ ID NO: 30).

Additional exemplary examples of CACNA1G mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on CACNA1G can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CACNA1G.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CACNA1G, as used herein, also refers to variations of the CACNA1G gene including variants provided in the SNP database. Numerous sequence variations within the CACNA1G gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=CACNA1G, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "angiotensin II receptor type 1," used interchangeably with the term "AGTR1," refers to a receptor for the vasoconstricting peptide angiotensin II. Angiotensin II is a potent vasopressor hormone and a primary regulator of aldosterone secretion. AGTR1 is activated by angiotensin II. The activated receptor in turn couples to G protein and, thus, activates phospholipase C and increases the cytosolic Ca2+ concentrations, which in turn triggers cellular responses such as stimulation of protein kinase C. AGTR1 plays an integral role in blood pressure control, and is implicated in the pathogenesis of hypertension. AGTR1 is also known as angiotensin receptor 1B, AT1, AT2R1, AGTR1A, AT2R1B, AGTR1B, HAT1R, AG2S, AT1B, AT2R1A, AT1AR, AT1BR, or AT1R.

An exemplary sequence of a human AGTR1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1820101583 (NM_000685.5; SEQ ID NO: 31; reverse complement, SEQ ID NO: 32). The sequence of mouse AGTR1 mRNA can be found at, for example, GenBank Accession No. GI: 158937294 (NM_177322.3; SEQ ID NO: 33; reverse complement, SEQ ID NO: 34). The sequence of rat AGTR1 mRNA can be found at, for example, GenBank Accession No. GI: 140969764 (NM_030985.4; SEQ ID NO: 35; reverse complement, SEQ ID NO: 36). The sequence of Macaca mulatta AGTR1 mRNA can be found at, for example, GenBank Accession No. GI: 1622904093 (XM_028843763.1; SEQ ID NO: 37; reverse complement, SEQ ID NO: 38). The sequence of Macaca fascicularis AGTR1 mRNA can be found at, for example, GenBank Accession No. GI: 544411901 (XM_005546040.1; SEQ ID NO: 39; reverse complement, SEQ ID NO: 40).

Additional examples of AGTR1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on AGTR1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=AGTR1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term AGTR1, as used herein, also refers to variations of the AGTR1 gene including variants provided in the SNP database. Numerous sequence variations within the AGTR1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*,

www.ncbi.nlm.nih.gov/gene/?term=AGTR1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Sodium Voltage-Gated Channel Alpha Subunit 2," used interchangeably with the term "SCN2A," refers to a member of the voltage-gated sodium channel family. Voltage-gated sodium channels are transmembrane glycoprotein complexes composed of a large alpha subunit with four repeat domains, each of which is composed of six membrane-spanning segments, and one or more regulatory beta subunits. Voltage-gated sodium channels function in the generation and propagation of action potentials in neurons and muscle. Specifically, SCN2A permits the sodium influx from the extracellular space into the cytosol after depolarization of the nerve membrane. Allelic variants of SCN2A are associated with seizure disorders and autism spectrum disorders. SCN2A is also known as Nav1.2, HBSCII, SCN2A1, SCN2A2, HBSCI, EIEE11, BFIC3, BFIS3, BFNIS, DEE11, EA9, or HBA.

An exemplary sequence of a human SCN2A mRNA transcript can be found at, for example, GenBank Accession No. GI: 1697699196 (NM_021007.3; SEQ ID NO: 41; reverse complement, SEQ ID NO: 42). The sequence of mouse SCN2A mRNA can be found at, for example, GenBank Accession No. GI: 1114439824 (NM_001099298.3; SEQ ID NO: 43; reverse complement, SEQ ID NO: 44). The sequence of rat SCN2A mRNA can be found at, for example, GenBank Accession No. GI: 1937915892 (NM_012647.2; SEQ ID NO: 45; reverse complement, SEQ ID NO: 46). The sequence of Macaca mulatta SCN2A mRNA can be found at, for example, GenBank Accession No. GI: 1622850108 (XM_001100368.4; SEQ ID NO: 47; reverse complement, SEQ ID NO: 48). The sequence of Macaca fascicularis SCN2A mRNA can be found at, for example, GenBank Accession No. GI: 544475515 (XM_005573351.1; SEQ ID NO: 49; reverse complement, SEQ ID NO: 50).

Additional examples of SCN2A mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on SCN2A can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=SCN2A.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term SCN2A, as used herein, also refers to variations of the SCN2A gene including variants provided in the SNP database. Numerous sequence variations within the SCN2A gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=SCN2A, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1," used interchangeably with the term "HCN1," refers to a member of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel family. These channels are primarily expressed in the heart and in the central and peripheral nervous systems. HCN channels mediate rhythmic electrical

activity of cardiac pacemaker cells, and in neurons play important roles in setting resting membrane potentials, dendritic integration, neuronal pacemaking, and establishing action potential threshold. The HCN1 protein can homodimerize or heterodimerize with other pore-forming subunits to form a potassium channel. HCN1 is also known as potassium channel 1, BCNG-1, HAC-2, BCNG1, Potassium/Sodium Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel 1; Brain Cyclic Nucleotide-Gated Channel 1; Hyperpolarization Activated Cyclic Nucleotide-Gated Potassium Channel 1; GEFSP10, EIEE24, or DEE24.

An exemplary sequence of a human HCN1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519313076 (NM_021072.4; SEQ ID NO: 51; reverse complement, SEQ ID NO: 52). The sequence of mouse HCN1 mRNA can be found at, for example, GenBank Accession No. GI: 283837798 (NM_010408.3; SEQ ID NO: 53; reverse complement, SEQ ID NO: 54). The sequence of rat HCN1 mRNA can be found at, for example, GenBank Accession No. GI: 2000186052 (NM_053375.2; SEQ ID NO: 55; reverse complement, SEQ ID NO: 56). The sequence of Macaca mulatta HCN1 mRNA can be found at, for example, GenBank Accession No. GI: 1622944535 (XM_015140004.2; SEQ ID NO: 57; reverse complement, SEQ ID NO: 58). The sequence of Macaca fascicularis HCN1 mRNA can be found at, for example, GenBank Accession No. GI: 982252681 (XM_005556858.2; SEQ ID NO: 59; reverse complement, SEQ ID NO: 60).

Additional examples of HCN1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on HCN1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=HCN1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term HCN1, as used herein, also refers to variations of the HCN1 gene including variants provided in the SNP database. Numerous sequence variations within the HCN1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=HCN1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4," used interchangeably with the term "HCN4," refers to a member of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel family. The HCN4 channel transports positively charged ions into heart muscle cells. This channel is located primarily in the sino-atrial (SA) node, which is an area of specialized cells in the heart that functions as a natural pacemaker. The HCN4 channel allows potassium and sodium ions to flow into cells of the SA node. This ion flow is often called the "pacemaker current" because it generates electrical impulses that start each heartbeat and is involved in maintaining a regular heart rhythm. HCN4 is also known as Potassium/Sodium Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel 4, Hyperpolarization Activated Cyclic

Nucleotide-Gated Potassium Channel 4, Hyperpolarization Activated Cyclic Nucleotide-Gated Cation Channel 4 or SSS2.

An exemplary sequence of a human HCN4 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519312820 (NM_005477.3; SEQ ID NO: 61; reverse complement, SEQ ID NO: 62). The sequence of mouse HCN4 mRNA can be found at, for example, GenBank Accession No. GI: 1686254400 (NM_001081192.3; SEQ ID NO: 63; reverse complement, SEQ ID NO: 64). The sequence of rat HCN4 mRNA can be found at, for example, GenBank Accession No. GI: 1937893976 (NM_021658.2; SEQ ID NO: 65; reverse complement, SEQ ID NO: 66). The sequence of Macaca mulatta HCN4 mRNA can be found at, for example, GenBank Accession No. GI: 1622953870 (XM_002804859.3; SEQ ID NO: 67; reverse complement, SEQ ID NO: 68). The sequence of Macaca fascicularis HCN4 mRNA can be found at, for example, GenBank Accession No. GI: 982258526 (XM_005559993.2; SEQ ID NO: 69; reverse complement, SEQ ID NO: 70).

Additional examples of HCN4 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on HCN4 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=HCN4.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term HCN4, as used herein, also refers to variations of the HCN4 gene including variants provided in the SNP database. Numerous sequence variations within the HCN4 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=HCN4, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3," used interchangeably with the term "HCN3," refers to a member of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel family. A study conducted in the mouse suggested that HCN3 channels might be involved in the regulation of the circadian system. HCN3 channels have also been reported to be present in the intergeniculate leaflet of the hypothalamus. HCN3 is also known as Potassium/Sodium Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel 3, Hyperpolarization Activated Cyclic Nucleotide-Gated Potassium Channel 3, or KIAA1535.

An exemplary sequence of a human HCN3 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519312303 (NM_020897.3; SEQ ID NO: 71; reverse complement, SEQ ID NO: 72). The sequence of mouse HCN3 mRNA can be found at, for example, GenBank Accession No. GI: 6680190 (NM_008227.1; SEQ ID NO: 73; reverse complement, SEQ ID NO: 74). The sequence of rat HCN3 mRNA can be found at, for example, GenBank Accession No. GI: 16758501 (NM_053685.1; SEQ ID NO: 75; reverse complement, SEQ ID NO: 76). The sequence of Macaca mulatta HCN3 mRNA can be found at, for example, GenBank Accession No. GI:

1622829938 (XM_001115891.4; SEQ ID NO: 77; reverse complement, SEQ ID NO: 78). The sequence of Macaca fascicularis HCN3 mRNA can be found at, for example, GenBank Accession No. GI: 982225310 (XM_005541549.2; SEQ ID NO: 79; reverse complement, SEQ ID NO: 80).

Additional examples of HCN3 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on HCN3 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=HCN3.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term HCN3, as used herein, also refers to variations of the HCN3 gene including variants provided in the SNP database. Numerous sequence variations within the HCN3 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=HCN3, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Potassium Voltage-Gated Channel Subfamily A Member 5," used interchangeably with the term "KCNA5," refers to a member of the voltage-gated potassium channel family. The Voltage-gated potassium channels mediate transmembrane potassium transport in excitable membranes. These channels form tetrameric potassium-selective channels through which potassium ions pass in accordance with their electrochemical gradient, and alternate between opened and closed conformations in response to the voltage difference across the membrane. KCNA5 contains six membrane-spanning domains with a shaker-type repeat in the fourth segment. It belongs to the delayed rectifier class, the function of which could restore the resting membrane potential of beta cells after depolarization and thereby contribute to the regulation of insulin secretion. KCNA5 is also known as HPCN1, HK2, Potassium Voltage-Gated Channel, Shaker-Related Subfamily, Member 5; Voltage-Gated Potassium Channel Subunit Kv1.5; Voltage-Gated Potassium Channel HK2; Kv1.5; Insulinoma And Islet Potassium Channel; Cardiac Potassium Channel; Potassium Channel 1; ATFB7, HCK1 or PCN1.

An exemplary sequence of a human KCNA5 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1653961222 (NM_002234.4; SEQ ID NO: 81; reverse complement, SEQ ID NO: 82). The sequence of mouse KCNA5 mRNA can be found at, for example, GenBank Accession No. GI: 158937280 (NM_145983.2; SEQ ID NO: 83; reverse complement, SEQ ID NO: 84). The sequence of rat KCNA5 mRNA can be found at, for example, GenBank Accession No. GI: 6981117 (NM_012972.1; SEQ ID NO: 85; reverse complement, SEQ ID NO: 86). The sequence of Macaca mulatta KCNA5 mRNA can be found at, for example, GenBank Accession No. GI: 1622843572 (XM_001102294.4; SEQ ID NO: 87; reverse complement, SEQ ID NO: 88). The sequence of Macaca fascicularis KCNA5 mRNA can be found at, for example, GenBank Accession No. GI: 982279162 (XM_005569870.2; SEQ ID NO: 89; reverse complement, SEQ ID NO: 90).

Additional examples of KCNA5 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on KCNA5 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=KCNA5.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term KCNA5, as used herein, also refers to variations of the KCNA5 gene including variants provided in the SNP database. Numerous sequence variations within the KCNA5 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=KCNA5, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Potassium Inwardly Rectifying Channel Subfamily J Member 3," used interchangeably with the term "KCNJ3," refers to an integral membrane protein and an inward-rectifier type potassium channel. The inward-rectifier type potassium channels have a greater tendency to allow potassium to flow into a cell rather than out of a cell. This asymmetry in potassium ion conductance plays a key role in the excitability of muscle cells and neurons. KCNJ3 is controlled by G-proteins and plays an important role in regulating heartbeat. It associates with three other G-protein-activated potassium channels to form a heteromultimeric pore-forming complex, which also couples to neurotransmitter receptors in the brain. These multimeric G-protein-gated inwardly-rectifying potassium (GIRK) channels have a wide range of physiological roles, including the regulation of heartbeat, reward mechanisms, learning and memory functions, blood platelet aggregation, insulin secretion, and lipid metabolism. KCNJ3 is also known as GIRK1, G Protein-Activated Inward Rectifier Potassium Channel 1, KGA; Potassium Channel, Inwardly Rectifying Subfamily J Member 3; Inward Rectifier K(+) Channel Kir3.1; or Potassium Inwardly-Rectifying Channel Subfamily J Member 3 Splice Variant 1e.

An exemplary sequence of a human KCNJ3 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519246021 (NM_002239.4; SEQ ID NO: 91; reverse complement, SEQ ID NO: 92). The sequence of mouse KCNJ3 mRNA can be found at, for example, GenBank Accession No. GI: 756398330 (NM_008426.2; SEQ ID NO: 93; reverse complement, SEQ ID NO: 94). The sequence of rat KCNJ3 mRNA can be found at, for example, GenBank Accession No. GI: 148747456 (NM_031610.3; SEQ ID NO: 95; reverse complement, SEQ ID NO: 96). The sequence of Macaca mulatta KCNJ3 mRNA can be found at, for example, GenBank Accession No. GI: 387849010 (NM_001261696.1; SEQ ID NO: 97; reverse complement, SEQ ID NO: 98). The sequence of Macaca fascicularis KCNJ3 mRNA can be found at, for example, GenBank Accession No. GI: 982285759 (XM_005573205.2; SEQ ID NO: 99; reverse complement, SEQ ID NO: 100).

Additional examples of KCNJ3 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on KCNJ3 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=KCNJ3.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term KCNJ3, as used herein, also refers to variations of the KCNJ3 gene including variants provided in the SNP database. Numerous sequence variations within the KCNJ3 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=KCNAJ3, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Potassium Inwardly Rectifying Channel Subfamily J Member 4," used interchangeably with the term "KCNJ4," refers to an integral membrane protein and inward-rectifier type potassium channel. The inward-rectifier type potassium channels have a greater tendency to allow potassium to flow into a cell rather than out of a cell. This asymmetry in potassium ion conductance plays a key role in the excitability of muscle cells and neurons. KCNJ4 can tetramerize to form functional inwardly rectifying channels, in which each monomer contains two transmembrane helix domains, an ion-selective P-loop, and cytoplasmic N- and C-terminal domains. The distribution of KCNJ4 is predominantly focused in both heart and brain, especially in the cardiac myocytes and forebrain region. KCNJ4 may play important roles in the regulation of resting membrane potential, cellular excitability and potassium homeostasis in the nervous system and various peripheral tissues. KCNJ4 is also known as HIRK2, HRK1, IRK3, HIR, Kir2.3, inward rectifier potassium channel 4; Inward Rectifier K(+) Channel Kir2.3; Potassium Voltage-Gated Channel Subfamily J Member 4; Hippocampal Inward Rectifier Potassium Channel; or Hippocampal Inward Rectifier.

An exemplary sequence of a human KCNJ4 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1732746379 (NM_152868.3; SEQ ID NO: 101; reverse complement, SEQ ID NO: 102). The sequence of mouse KCNJ4 mRNA can be found at, for example, GenBank Accession No. GI: 1720383422 (XM_006520486.4; SEQ ID NO: 103; reverse complement, SEQ ID NO: 104). The sequence of rat KCNJ4 mRNA can be found at, for example, GenBank Accession No. GI: 1937901561 (NM_053870.3; SEQ ID NO: 105; reverse complement, SEQ ID NO: 106). The sequence of Macaca mulatta KCNJ4 mRNA can be found at, for example, GenBank Accession No. GI: 1622838042 (XM_015150354.2; SEQ ID NO: 107; reverse complement, SEQ ID NO: 108). The sequence of Macaca fascicularis KCNJ4 mRNA can be found at, for example, GenBank Accession No. GI: 544461851 (XM_005567299.1; SEQ ID NO: 109; reverse complement, SEQ ID NO: 110).

Additional examples of KCNJ4 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on KCNJ4 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=KCNJ4.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term KCNJ4, as used herein, also refers to variations of the KCNJ4 gene including variants provided in the SNP database. Numerous sequence variations within the KCNJ4 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=KCNAJ4, the entire contents of which is incorporated herein by reference as of the date of filing this application. As used herein, "Phosphodiesterase 1," used interchangeably with the term "PDE1," refers to a member of the cyclic nucleotide phosphodiesterases families. Cyclic nucleotide phosphodiesterases (PDEs) are superfamily of enzymes that regulate the spatial and temporal relationship of second messenger signaling in the cellular system. Among the 11 different families of PDEs, phosphodiesterase 1 (PDE1) sub-family of enzymes hydrolyze both 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) in a mutually competitive manner. The catalytic activity of PDE1 is stimulated by their binding to Ca2+/calmodulin (CaM), resulting in the integration of Ca2+ and cyclic nucleotide-mediated signaling in various diseases. The PDE1 family includes three subtypes, PDE1A, PDE1B and PDE1C, which differ for their relative affinities for cAMP and cGMP. These isoforms are differentially expressed throughout the body, including the cardiovascular, central nervous system and other organs. Thus, PDE1 enzymes play a critical role in the pathophysiology of diseases through the fundamental regulation of cAMP and cGMP signaling. PDE1 is also known as Calcium/Calmodulin-Dependent 3',5'-Cyclic Nucleotide Phosphodiesterase 1; Calcium/Calmodulin-Stimulated Cyclic Nucleotide Phosphodiesterase; CAM-PDE 1, HSPDE1, HCAM1, or EC 3.1.4.

An exemplary sequence of a human PDE1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 2062580163 (NM_005019.7; SEQ ID NO: 111; reverse complement, SEQ ID NO: 112). The sequence of mouse PDE1 mRNA can be found at, for example, GenBank Accession No. GI: 227330628 (NM_001159582.1; SEQ ID NO: 113; reverse complement, SEQ ID NO: 114). The sequence of rat PDE1 mRNA can be found at, for example, GenBank Accession No. GI: 13540702 (NM_030871.1; SEQ ID NO: 115; reverse complement, SEQ ID NO: 116). The sequence of Macaca mulatta PDE1 mRNA can be found at, for example, GenBank Accession No. GI: 383872283 (NM_001257584.1; SEQ ID NO: 117; reverse complement, SEQ ID NO: 118). The sequence of Macaca fascicularis PDE1 mRNA can be found at, for example, GenBank Accession No. GI: 982286500 (XR_001483985.1; SEQ ID NO: 119; reverse complement, SEQ ID NO: 120).

Additional examples of PDE1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site.

Further information on PDE1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=PDE1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term PDE1, as used herein, also refers to variations of the PDE1 gene including variants provided in the SNP database. Numerous sequence variations within the PDE1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/gene/?term=PDE1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Delta 4-Desaturase, Sphingolipid 1," used interchangeably with the term "DEGS1," refers to a member of the membrane fatty acid desaturase family which is responsible for inserting double bonds into specific positions in fatty acids. DEGS1 is an enzyme that catalyzes the final step in the ceramide biosynthesis pathway. Ceramides have emerged as important regulators of tissue metabolism that play essential roles in cardiometabolic disease. They are potent biomarkers of diabetes and heart disease and are now being measured clinically as predictors of major adverse cardiac events. Moreover, studies in rodents reveal that inhibitors of ceramide synthesis prevent or reverse the pathogenic features of type 2 diabetes, nonalcoholic fatty liver disease, atherosclerosis, and cardiomyopathy. Therefore, inhibition of DEGS1 is considered as a potential therapeutic approach to lower ceramides and combat cardiometabolic disease.

DEGS1 is also known as MLD, DES-1, FADS7, Cell Migration-Inducing Gene 15 Protein, Sphingolipid Delta(4)-Desaturase DES1, Dihydroceramide Desaturase 1, Membrane Lipid Desaturase, Degenerative Spermatocyte Homolog 1, Lipid Desaturase, Membrane Fatty Acid (Lipid) Desaturase, Migration-Inducing Gene 15 Protein, Sphingolipid Delta 4 Desaturase, EC 1.14.19.17, HLD18, MIG15 and DEGS.

An exemplary sequence of a human DEGS1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519243257 (NM_003676.4; SEQ ID NO:121; reverse complement, SEQ ID NO: 122). The sequence of mouse DEGS1 mRNA can be found at, for example, GenBank Accession No. GI: 1343071492 (NM_007853.5; SEQ ID NO:123; reverse complement, SEQ ID NO: 124). The sequence of rat DEGS1 mRNA can be found at, for example, GenBank Accession No. GI: 162287183 (NM_053323.2; SEQ ID NO:125; reverse complement, SEQ ID NO: 126). The sequence of Macaca fascicularis DEGS1 mRNA can be found at, for example, GenBank Accession No. GI: 982223631 (XM_005540946.2; SEQ ID NO: 127; reverse complement, SEQ ID NO: 128). The sequence of Macaca mulatta DEGS1 mRNA can be found at, for example, GenBank Accession No. GI: 388452769 (NM_001266006.1; SEQ ID NO: 129; reverse complement, SEQ ID NO: 130).

Additional examples of DEGS1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on DEGS1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=DEGS1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term DEGS1, as used herein, also refers to variations of the DEGS1 gene including variants provided in the SNP database. Numerous sequence variations within the DEGS1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=DEGS1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "leptin," used interchangeably with the term "LEP," refers to a protein that is secreted by white adipocytes into the circulation and plays a major role in the regulation of energy homeostasis. Circulating leptin binds to the leptin receptor in the brain, which activates downstream signaling pathways that inhibit feeding and promote energy expenditure. This protein also has several endocrine functions, and is involved in the regulation of immune and inflammatory responses, hematopoiesis, angiogenesis, reproduction, bone formation and wound healing. Mutations in this gene and its regulatory regions cause severe obesity and morbid obesity with hypogonadism in human patients. A mutation in this gene has also been linked to type 2 diabetes mellitus development. Leptin is also known as OBS, OB, obese, obesity factor, or LEPD.

An exemplary sequence of a human leptin mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519312816 (NM_000230.3; SEQ ID NO:131; reverse complement, SEQ ID NO: 132). The sequence of mouse leptin mRNA can be found at, for example, GenBank Accession No. GI: 34328437 (NM_008493.3; SEQ ID NO:133; reverse complement, SEQ ID NO: 134). The sequence of rat leptin mRNA can be found at, for example, GenBank Accession No. GI: 291463266 (NM_013076.3; SEQ ID NO:135; reverse complement, SEQ ID NO: 136). The sequence of Macaca fascicularis leptin mRNA can be found at, for example, GenBank Accession No. GI: 982241369 (XM_005550685.2; SEQ ID NO: 137; reverse complement, SEQ ID NO: 138). The sequence of Macaca mulatta leptin mRNA can be found at, for example, GenBank Accession No. GI: 112363108 (NM_001042755.1; SEQ ID NO: 139; reverse complement, SEQ ID NO: 140).

Additional examples of leptin mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on leptin can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=leptin.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term leptin, as used herein, also refers to variations of the leptin gene including variants provided in the SNP database. Numerous sequence variations within the leptin gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=leptin, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "folliculin," used interchangeably with the term "FLCN," refers to a protein that is related to Birt-Hogg-Dubé syndrome, primary spontaneous pneumothorax and some types of nonhereditary (sporadic) tumors. The folliculin protein is present in many of the body's tissues,

including the brain, heart, placenta, testis, skin, lung, and kidney. Folliculin may be important for cells' uptake of foreign particles (endocytosis or phagocytosis). The protein may also play a role in the structural framework that helps to define the shape, size, and movement of a cell (the cytoskeleton) and in interactions between cells. FLCN is also known as BHD, DENND8B, BHD Skin Lesion Fibrofolliculoma Protein, Birt-Hogg-Dube Syndrome Protein, MGC17998, MGC23445 or FLCL.

An exemplary sequence of a human FLCN mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519312711 (NM_144997.7; SEQ ID NO:141; reverse complement, SEQ ID NO: 142). The sequence of mouse FLCN mRNA can be found at, for example, GenBank Accession No. GI: 405778334 (NM_001271356.1; SEQ ID NO:143; reverse complement, SEQ ID NO: 144). The sequence of rat FLCN mRNA can be found at, for example, GenBank Accession No. GI: 55742811 (NM_199390.2; SEQ ID NO:145; reverse complement, SEQ ID NO: 146). The sequence of Macaca fascicularis FLCN mRNA can be found at, for example, GenBank Accession No. GI: 982303338 (XM_005583008.2; SEQ ID NO: 147; reverse complement, SEQ ID NO: 148). The sequence of Macaca mulatta FLCN mRNA can be found at, for example, GenBank Accession No. GI: 388490399 (NM_001266691.1; SEQ ID NO: 149; reverse complement, SEQ ID NO: 150).

Additional examples of FLCN mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on FLCN can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=FLCN.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term FLCN, as used herein, also refers to variations of the FLCN gene including variants provided in the SNP database. Numerous sequence variations within the FLCN gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=FLCN, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Zinc Finger Protein 423," used interchangeably with the term "ZFP423," refers to a nuclear protein that belongs to the family of Kruppel-like C2H2 zinc finger proteins. It functions as a DNA-binding transcription factor by using distinct zinc fingers in different signaling pathways. Thus, it is thought that this gene may have multiple roles in signal transduction during development. Mutations in this gene are associated with nephronophthisis-14 and Joubert syndrome-19.

ZFP423 is also known as NPHP14, HOAZ, OAZ, KIAA0760, Zfp104, JBTS19, Ebfaz, Early B-Cell Factor Associated Zinc Finger Protein, Smad- And Olf-Interacting Zinc Finger Protein, Olf1/EBF-Associated Zinc Finger Protein, or Roaz.

An exemplary sequence of a human ZFP423 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1889411210 (NM_015069.5; SEQ ID NO:151; reverse complement, SEQ ID NO: 152). The sequence of mouse ZFP423 mRNA can be found at, for example, GenBank

Accession No. GI: 46359076 (NM_033327.2; SEQ ID NO:153; reverse complement, SEQ ID NO: 154). The sequence of rat ZFP423 mRNA can be found at, for example, GenBank Accession No. GI: 1997589018 (NM_001393718.1; SEQ ID NO:155; reverse complement, SEQ ID NO: 156). The sequence of Macaca fascicularis ZFP423 mRNA can be found at, for example, XM_005591872.2; (SEQ ID NO: 157; reverse complement, SEQ ID NO: 158). The sequence of Macaca mulatta ZFP423 mRNA can be found at, for example, XM_015126090.2; SEQ ID NO: 159; reverse complement, SEQ ID NO: 160).

Additional examples of ZFP423 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on ZFP423 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=ZFP423.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term ZFP423, as used herein, also refers to variations of the ZFP423 gene including variants provided in the SNP database. Numerous sequence variations within the ZFP423 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=ZFP423, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cyclin Dependent Kinase 6," used interchangeably with the term "CDK6," refers to a member of the CMGC family of serine/threonine protein kinases. This kinase is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression and G1/S transition. The activity of this kinase first appears in mid-G1 phase, which is controlled by the regulatory subunits including D-type cyclins and members of INK4 family of CDK inhibitors. This kinase, as well as CDK4, has been shown to phosphorylate, and thus regulate the activity of, tumor suppressor protein Rb. Altered expression of this gene has been observed in multiple human cancers. A mutation in this gene resulting in reduced cell proliferation, and impaired cell motility and polarity, and has been identified in patients with primary microcephaly. CDK6 is also known as PLSTIRE, Serine/Threonine-Protein Kinase PLSTIRE, Cell Division Protein Kinase 6, EC 2.7.11.22, MCPH12 or CDKN6.

An exemplary sequence of a human CDK6 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1677500223 (NM_001259.8; SEQ ID NO:161; reverse complement, SEQ ID NO: 162). The sequence of mouse CDK6 mRNA can be found at, for example, GenBank Accession No. GI: 922304379 (NM_009873.3; SEQ ID NO:163; reverse complement, SEQ ID NO: 164). The sequence of rat CDK6 mRNA can be found at, for example, GenBank Accession No. GI: 1982560006 (NM_001191861.2; SEQ ID NO:165; reverse complement, SEQ ID NO: 166). The sequence of Macaca fascicularis CDK6 mRNA can be found at, for example, GenBank Accession No. GI: 982240553 (XM_015447745.1; SEQ ID NO: 167; reverse complement, SEQ ID NO: 168). The

sequence of Macaca mulatta CDK6 mRNA can be found at, for example, GenBank Accession No. GI: 386782158 (NM_001261307.1; SEQ ID NO: 169; reverse complement, SEQ ID NO: 170).

Additional examples of CDK6 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on CDK6 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CDK6.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CDK6, as used herein, also refers to variations of the CDK6 gene including variants provided in the SNP database. Numerous sequence variations within the CDK6 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CDK6, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Regulatory Associated Protein Of MTOR Complex 1," used interchangeably with the term "RPTOR," refers to a component of a signaling pathway that regulates cell growth in response to nutrient and insulin levels. The encoded protein forms a stoichiometric complex with the mTOR kinase, and also associates with eukaryotic initiation factor 4E-binding protein-1 and ribosomal protein S6 kinase. The protein positively regulates the downstream effector ribosomal protein S6 kinase, and negatively regulates the mTOR kinase. Mutations of RPTOR have been observed in cancers such as intestinal cancer, skin cancer, and stomach cancer. RPTOR is also known as Raptor, KIAA1303, KOG1, Mip1, Regulatory-Associated Protein Of MTOR, or P150 Target Of Rapamycin (TOR)-Scaffold Protein Containing WD-Repeats.

An exemplary sequence of a human RPTOR mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519244773 (NM_020761.3; SEQ ID NO:171; reverse complement, SEQ ID NO: 172). The sequence of mouse RPTOR mRNA can be found at, for example, GenBank Accession No. GI: 807045913 (NM_028898.3; SEQ ID NO:173; reverse complement, SEQ ID NO: 174). The sequence of rat RPTOR mRNA can be found at, for example, GenBank Accession No. GI: 260166602 (NM_001134499.2; SEQ ID NO:175; reverse complement, SEQ ID NO: 176). The sequence of Macaca fascicularis RPTOR mRNA can be found at, for example, GenBank Accession No. GI: 982307196 (XM_005585210.2; SEQ ID NO: 177; reverse complement, SEQ ID NO: 178). The sequence of Macaca mulatta RPTOR mRNA can be found at, for example, GenBank Accession No. GI: 1622881944 (XM_015120520.2; SEQ ID NO: 179; reverse complement, SEQ ID NO: 180).

Additional examples of RPTOR mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on RPTOR can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=RPTOR.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term RPTOR, as used herein, also refers to variations of the RPTOR gene including variants provided in the SNP database. Numerous sequence variations within the RPTOR gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=RPTOR, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Mechanistic Target Of Rapamycin Kinase," used interchangeably with the term "mTOR," refers to an atypical serine/threonine kinase of 289 kDa that belongs to the family of the phosphoinositide 3-kinase related kinase. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. Specifically, mTOR is the intracellular kinase linking nutrient availability with metabolic control, and its deregulation is a hallmark of diabetes and cancer. The mTOR kinase is encoded by a single gene in mammals, but it exerts its main cellular functions by forming mTORC1 and mTORC2 through assembly with specific adaptor proteins. mTORC1 controls protein synthesis, cell growth and proliferation, and mTORC2 is a regulator of the actin cytoskeleton, and promotes cell survival and cell cycle progression. mTOR is also known as RAFT1, Rapamycin And FKBP12 Target 1, Mammalian Target Of Rapamycin, FRAP1, FRAP2, FRAP, FK506-Binding Protein 12-Rapamycin Complex-Associated Protein 1, Serine/Threonine-Protein Kinase MTOR, Rapamycin Associated Protein FRAP2, FLJ44809, DJ576K7.1, FK506 Binding Protein 12-Rapamycin Associated Protein 1, FKBP12-Rapamycin Complex-Associated Protein, Rapamycin Target Protein, EC 2.7.11.1, or SKS.

An exemplary sequence of a human mTOR mRNA transcript can be found at, for example, GenBank Accession No. GI: 1653961062 (NM_004958.4; SEQ ID NO:181; reverse complement, SEQ ID NO: 182). The sequence of mouse mTOR mRNA can be found at, for example, GenBank Accession No. GI: 227330585 (NM_020009.2; SEQ ID NO:183; reverse complement, SEQ ID NO: 184). The sequence of rat mTOR mRNA can be found at, for example, GenBank Accession No. GI: 1935257123 (NM_019906.2; SEQ ID NO:185; reverse complement, SEQ ID NO: 186). The sequence of Macaca fascicularis mTOR mRNA can be found at, for example, GenBank Accession No. GI: 982230273 (XM_005544805.2; SEQ ID NO: 187; reverse complement, SEQ ID NO: 188). The sequence of Macaca mulatta mTOR mRNA can be found at, for example, GenBank Accession No. GI: 1622834993 (XM_015111100.2; SEQ ID NO: 189; reverse complement, SEQ ID NO: 190).

Additional examples of mTOR mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on mTOR can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=mTOR.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term mTOR, as used herein, also refers to variations of the mTOR gene including variants provided in the SNP database. Numerous sequence variations within the mTOR gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*,

www.ncbi.nlm.nih.gov/snp/?term=mTOR, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Forkhead Box P1," used interchangeably with the term "FOXP1," refers to a member of the subfamily P of the forkhead box (FOX) transcription factor family. Forkhead box transcription factors play important roles in the regulation of tissue- and cell type-specific gene transcription during both development and adulthood. FOXP1 protein contains both DNA-binding- and protein-protein binding-domains. Previous studies have investigated the biological roles of the transcription factor FOXP1 in brown/beige adipocyte differentiation and thermogenesis. Adipose-specific deletion of FOXP1 leads to an increase of brown adipose activity and browning program of white adipose tissues. The FOXP1 -deficient mice show an augmented energy expenditure and are protected from diet-induced obesity and insulin resistance. Consistently, overexpression of FOXP1 in adipocytes impairs adaptive thermogenesis and promotes diet-induced obesity. Thus, FOXP1 provides an important clue for its targeting and treatment of obesity. FOXP1 is also known as HSPC215, HFKH1B, 12CC4, QRF1, Fork Head-Related Protein Like B, Mac-1-Regulated Forkhead, Glutamine-Rich Factor 1, MFH or PAX5/FOXP1 Fusion Protein.

An exemplary sequence of a human FOXP1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1777535708 (NM_032682.6; SEQ ID NO:191; reverse complement, SEQ ID NO: 192). The sequence of mouse FOXP1 mRNA can be found at, for example, GenBank Accession No. GI: 309319789 (NM_053202.2; SEQ ID NO:193; reverse complement, SEQ ID NO: 194). The sequence of rat FOXP1 mRNA can be found at, for example, GenBank Accession No. GI: 1937889958 (NM_001034131.2 SEQ ID NO:195; reverse complement, SEQ ID NO: 196). The sequence of Macaca fascicularis FOXP1 mRNA can be found at, for example, GenBank Accession No. GI: 982232930 (XM_005547604.2; SEQ ID NO: 197; reverse complement, SEQ ID NO: 198). The sequence of Macaca mulatta FOXP1 mRNA can be found at, for example, GenBank Accession No. GI: 388453320 (NM_001266321.1; SEQ ID NO: 199; reverse complement, SEQ ID NO: 200).

Additional examples of FOXP1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on FOXP1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=FOXP1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term FOXP1, as used herein, also refers to variations of the FOXP1gene including variants provided in the SNP database. Numerous sequence variations within the FOXP1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=FOXP1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Phosphodiesterase 3B," used interchangeably with the term "PDE3B," refers to an isoform of the PDE3 family of cyclic nucleotide phosphodiesterases. Cyclic nucleotide

phosphodiesterases regulate intracellular signalling by hydrolysing cAMP and/or cGMP. Enzymes in the PDE3 family of phosphodiesterases are dual-specificity enzymes with high affinities for both cAMP and cGMP but much higher turnover rates for cAMP. PDE3B is relatively abundant in tissues that maintain energy homoeostasis. In adipocytes, PDE3B (phosphodiesterase 3B) is an important regulatory effector in signaling pathways controlled by insulin and cAMP-increasing hormones. Previous results from PDE3B-transgenic mice indicate that PDE3B plays an important role in modulation of energy metabolism. PDE3B is also known as HcGIP1, CGMP-Inhibited 3',5'-Cyclic Phosphodiesterase B, Cyclic GMP-Inhibited Phosphodiesterase B, EC 3.1.4.17, CGI-PDE B, CGIP1, or Cyclic Nucleotide Phosphodiesterase.

An exemplary sequence of a human PDE3B mRNA transcript can be found at, for example, GenBank Accession No. GI: 1889438535 (NM_001363570.2; SEQ ID NO:201; reverse complement, SEQ ID NO: 202). The sequence of mouse PDE3B mRNA can be found at, for example, GenBank Accession No. GI: 112983647 (NM_011055.2; SEQ ID NO:203; reverse complement, SEQ ID NO: 204). The sequence of rat PDE3B mRNA can be found at, for example, GenBank Accession No. GI: 1939401976 (NM_017229.2; SEQ ID NO:205; reverse complement, SEQ ID NO: 206). The sequence of Macaca fascicularis PDE3B mRNA can be found at, for example, GenBank Accession No. GI: 982294968 (XM_005578550.2; SEQ ID NO: 207; reverse complement, SEQ ID NO: 208). The sequence of Macaca mulatta PDE3B mRNA can be found at, for example, GenBank Accession No. GI: 1622864110 (XM_015114810.2; SEQ ID NO: 209; reverse complement, SEQ ID NO: 210).

Additional examples of PDE3B mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on PDE3B can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=PDE3B.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term PDE3B, as used herein, also refers to variations of the PDE3B gene including variants provided in the SNP database. Numerous sequence variations within the PDE3B gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=PDE3B, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Activin A Receptor Type 1C," used interchangeably with the term "ACVR1C," refers to a type I receptor for the TGFB family that mediates the activities of a diverse group of signaling molecules, including activin B, growth and differentiation factor 3 (GDF-3) and Nodal. Upon ligand binding, type I receptors phosphorylate cytoplasmic SMAD transcription factors, which then translocate to the nucleus and interact directly with DNA or in complex with other transcription factors. In rodents as well as humans, ALK7 expression is enriched in tissues that are important for the regulation of energy homeostasis, including adipose tissue, pancreatic islets, endocrine gut cells and the arcuate nucleus of the hypothalamus. In white adipose tissue, studies have

shown that ALK7 signaling facilitates fat accumulation under conditions of nutrient overload, by repressing the expression of adrenergic receptors, thereby reducing catecholamine sensitivity. Accordingly, mutant mice lacking ALK7 globally, or only in adipocytes, are resistant to diet-induced obesity. Recent studies have identified polymorphic variants in the human Acvr1c gene which affect body fat distribution and protect from type II diabetes, indicating that ALK7 has very similar functions in humans as in rodents. ACVR1C is also known as ALK7, ACVRLK7, Activin Receptor-Like Kinase 7, EC 2.7.11.30, ACTR-IC, Activin Receptor Type IC, or EC 2.7.11.

An exemplary sequence of a human ACVR1C mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519315475 (NM_145259.3; SEQ ID NO:211; reverse complement, SEQ ID NO: 212). The sequence of mouse ACVR1C mRNA can be found at, for example, GenBank Accession No. GI: 161333830 (NM_001111030.1; SEQ ID NO:213; reverse complement, SEQ ID NO: 214). The sequence of rat ACVR1C mRNA can be found at, for example, GenBank Accession No. GI: 1937875934 (NM_139090.2; SEQ ID NO:215; reverse complement, SEQ ID NO: 216). The sequence of Macaca fascicularis ACVR1C mRNA can be found at, for example, GenBank Accession No. GI: 982285785 (XM_005573224.2; SEQ ID NO: 217; reverse complement, SEQ ID NO: 218). The sequence of Macaca mulatta ACVR1C mRNA can be found at, for example, GenBank Accession No. GI: 388454445 (NM_001266690.1; SEQ ID NO: 219; reverse complement, SEQ ID NO: 220).

Additional examples of ACVR1C mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on ACVR1C can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=ACVR1C.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term ACVR1C, as used herein, also refers to variations of the ACVR1C gene including variants provided in the SNP database. Numerous sequence variations within the ACVR1C gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=ACVR1C, the entire contents of which is incorporated herein by reference as of the date of filing this application.

Specific exemplary target genes that mediate a skeletal muscle disorder include, but are not limited to, myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

As used herein, "myostatin," used interchangeably with the term "MSTN," refers to a secreted ligand of the TGF-beta (transforming growth factor-beta) superfamily of proteins. Ligands of

this family bind various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. This protein negatively regulates skeletal muscle cell proliferation and differentiation. Mutations in this gene are associated with increased skeletal muscle mass in humans and other mammals. Myostatin is also known as GDF8, Growth/Differentiation Factor 8, or MSLHP.

An exemplary sequence of a human myostatin mRNA transcript can be found at, for example, GenBank Accession No. GI: 1653961810 (NM_005259.3; SEQ ID NO:221; reverse complement, SEQ ID NO: 222). The sequence of mouse myostatin mRNA can be found at, for example, GenBank Accession No. GI: 922959927 (NM_010834.3; SEQ ID NO:223; reverse complement, SEQ ID NO: 224). The sequence of rat myostatin mRNA can be found at, for example, GenBank Accession No. GI: 9506906 (NM_019151.1; SEQ ID NO:225; reverse complement, SEQ ID NO: 226). The sequence of Macaca fascicularis myostatin mRNA can be found at, for example, GenBank Accession No. NM_001287623.1; SEQ ID NO: 227; reverse complement, SEQ ID NO: 228. The sequence of Macaca mulatta myostatin mRNA can be found at, for example, GenBank Accession No. GI: 121583757 (NM_001080119.1; SEQ ID NO: 229; reverse complement, SEQ ID NO: 230).

Additional examples of myostatin mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on myostatin can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=myostatin.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term myostatin, as used herein, also refers to variations of the myostatin gene including variants provided in the SNP database. Numerous sequence variations within the myostatin gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=myostatin, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cholinergic Receptor Nicotinic Alpha 1 Subunit," used interchangeably with the term "CHRNA1," refers to an alpha subunit of the muscle acetylcholine receptor (AChR). The muscle acetylcholine receptor consists of 5 subunits of 4 different types: 2 alpha subunits and 1 each of the beta, gamma, and delta subunits. This protein plays a role in acetlycholine binding/channel gating. After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane. CHRNA1 is associated with diseases associated such as Myasthenic Syndrome. CHRNA1 is also known as Cholinergic Receptor, Nicotinic, Alpha Polypeptide 1; Acetylcholine Receptor, Nicotinic, Alpha 1 (Muscle); ACHRA; CHRNA; Muscle Nicotinic Acetylcholine Receptor; CMS1A, CMS1B, CMS2A, FCCMS, SCCMS, or ACHRD.

An exemplary sequence of a human CHRNA1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1676317412 (NM_001039523.3; SEQ ID NO:231; reverse complement, SEQ ID NO: 232). The sequence of mouse CHRNA1 mRNA can be found at, for example, GenBank Accession No. GI: 425905338 (NM_007389.5; SEQ ID NO:233; reverse complement, SEQ ID NO: 234). The sequence of rat CHRNA1 mRNA can be found at, for example, GenBank Accession No. GI: 1937369362 (NM_024485.2; SEQ ID NO:235; reverse complement, SEQ ID NO: 236). The sequence of Macaca fascicularis CHRNA1 mRNA can be found at, for example, GenBank Accession No. GI: 982286285 (XM_015432377.1; SEQ ID NO: 237; reverse complement, SEQ ID NO: 238). The sequence of Macaca mulatta CHRNA1 mRNA can be found at, for example, GenBank Accession No. GI: 1622850381 (XM_001091711.4; SEQ ID NO: 239; reverse complement, SEQ ID NO: 240).

Additional examples of CHRNA1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the *Macaca* genome project web site. Further information on CHRNA1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CHRNA1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CHRNA1, as used herein, also refers to variations of the CHRNA1 gene including variants provided in the SNP database. Numerous sequence variations within the CHRNA1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CHRNA1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cholinergic Receptor Nicotinic Beta 1 Subunit," used interchangeably with the term "CHRNB1," refers to a beta subunit of the muscle acetylcholine receptor (AChR). The muscle acetylcholine receptor consists of 5 subunits of 4 different types: 2 alpha subunits and 1 each of the beta, gamma, and delta subunits. This protein plays a role in acetlycholine binding/channel gating. After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane. CHRNB1 is associated with diseases associated such as Myasthenic Syndrome. CHRNB1 is also known as Cholinergic Receptor, Nicotinic, Beta Polypeptide 1; Acetylcholine Receptor, Nicotinic, Beta 1 (Muscle); ACHRB; CHRNB; CMS1D, CMS2C, CMS2A, or SCCMS.

An exemplary sequence of a human CHRNB1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519313560 (NM_000747.3; SEQ ID NO:241; reverse complement, SEQ ID NO: 242). The sequence of mouse CHRNB1 mRNA can be found at, for example, GenBank Accession No. GI: 160358781 (NM_009601.4; SEQ ID NO:243; reverse complement, SEQ ID NO: 244). The sequence of rat CHRNB1 mRNA can be found at, for example, GenBank Accession No. GI: 2048631755 (NM_001395118.1; SEQ ID NO:245; reverse complement, SEQ ID NO: 246). The sequence of Macaca fascicularis CHRNB1 mRNA can be found at, for example, GenBank Accession No. GI: 982302904 (XM_005582753.2; SEQ ID NO: 247; reverse complement, SEQ ID NO: 248).

The sequence of Macaca mulatta CHRNB1 mRNA can be found at, for example, GenBank Accession No. GI: 1622877217 (XM_015118481.2; SEQ ID NO: 249; reverse complement, SEQ ID NO: 250).

Additional examples of CHRNB1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on CHRNB1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CHRNB1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CHRNB1, as used herein, also refers to variations of the CHRNB1 gene including variants provided in the SNP database. Numerous sequence variations within the CHRNB1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CHRNB1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cholinergic Receptor Nicotinic Delta Subunit," used interchangeably with the term "CHRND," refers to a delta subunit of the muscle acetylcholine receptor (AChR). The muscle acetylcholine receptor consists of 5 subunits of 4 different types: 2 alpha subunits and 1 each of the beta, gamma, and delta subunits. After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to opening of an ion-conducting channel across the plasma membrane. CHRND is associated with diseases associated such as Myasthenic Syndrome. CHRND is also known as ACHRD, Cholinergic Receptor, Nicotinic, Delta Polypeptide; Acetylcholine Receptor, Nicotinic, Delta (Muscle); CMS2A; CMS3A, CMS3B, CMS3C, FCCMS, or SCCMS.

An exemplary sequence of a human CHRND mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519243557 (NM_000751.3; SEQ ID NO:251; reverse complement, SEQ ID NO: 252). The sequence of mouse CHRND mRNA can be found at, for example, GenBank Accession No. GI: 426214082 (NM_021600.3; SEQ ID NO:253; reverse complement, SEQ ID NO: 254). The sequence of rat CHRND mRNA can be found at, for example, GenBank Accession No. GI: 9506486 (NM_019298.1; SEQ ID NO:255; reverse complement, SEQ ID NO: 256). The sequence of Macaca fascicularis CHRND mRNA can be found at, for example, GenBank Accession No. GI: 982288086 (XM_005574618.2; SEQ ID NO: 257; reverse complement, SEQ ID NO: 258). The sequence of Macaca mulatta CHRND mRNA can be found at, for example, GenBank Accession No. GI: 1622852529 (XM_028831231.1; SEQ ID NO: 259; reverse complement, SEQ ID NO: 260).

Additional examples of CHRND mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on CHRND can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CHRND.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CHRND, as used herein, also refers to variations of the CHRND gene including variants provided in the SNP database. Numerous sequence variations within the CHRND gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CHRND, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cholinergic Receptor Nicotinic Epsilon Subunit," used interchangeably with the term "CHRNE," refers to a subunit of the acetylcholine receptor. Acetylcholine receptors at mature mammalian neuromuscular junctions are pentameric protein complexes composed of four subunits in the ratio of two alpha subunits to one beta, one epsilon, and one delta subunit. The acetylcholine receptor changes subunit composition shortly after birth when the epsilon subunit replaces the gamma subunit seen in embryonic receptors. Mutations in the epsilon subunit are associated with congenital myasthenic syndrome. CHRNE is also known as Cholinergic Receptor, Nicotinic, Epsilon; Acetylcholine Receptor, Nicotinic, Epsilon; ACHRE; CMS1D, CMS1E, CMS2A, CMS4A, CMS4B, CMS4C, FCCMS, or SCCMS.

An exemplary sequence of a human CHRNE mRNA transcript can be found at, for example, GenBank Accession No. GI: 1433531118 (NM_000080.4; SEQ ID NO: 261; reverse complement, SEQ ID NO: 262). The sequence of mouse CHRNE mRNA can be found at, for example, GenBank Accession No. GI: 6752949 (NM_009603.1; SEQ ID NO: 263; reverse complement, SEQ ID NO: 264). The sequence of rat CHRNE mRNA can be found at, for example, GenBank Accession No. GI: 8393128 (NM_017194.1; SEQ ID NO: 265; reverse complement, SEQ ID NO: 266). The sequence of Macaca fascicularis CHRNE mRNA can be found at, for example, GenBank Accession No. GI: 982302635 (XM_015437499.1; SEQ ID NO: 267; reverse complement, SEQ ID NO: 268). The sequence of Macaca mulatta CHRNE mRNA can be found at, for example, GenBank Accession No. GI: 1622876897 (XM_015118354.2; SEQ ID NO: 269; reverse complement, SEQ ID NO: 270).

Additional examples of CHRNE mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on CHRNE can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CHRNE.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CHRNE, as used herein, also refers to variations of the CHRNE gene including variants provided in the SNP database. Numerous sequence variations within the CHRNE gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CHRNE, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Cholinergic Receptor Nicotinic Gamma Subunit," used interchangeably with the term "CHRNG," refers to a subunit of the acetylcholine receptor. The mammalian muscle-type acetylcholine receptor is a transmembrane pentameric glycoprotein with two alpha subunits, one beta,

one delta, and one epsilon (in adult skeletal muscle) or gamma (in fetal and denervated muscle) subunit. This gene, which encodes the gamma subunit, is expressed prior to the thirty-third week of gestation in humans. The gamma subunit of the acetylcholine receptor plays a role in neuromuscular organogenesis and ligand binding and disruption of gamma subunit expression prevents the correct localization of the receptor in cell membranes. Mutations in the subunit are associated with congenital myasthenic syndrome. CHRNG is also known as Cholinergic Receptor, Nicotinic, Gamma; Acetylcholine Receptor, Nicotinic, Gamma; or ACHRG.

An exemplary sequence of a human CHRNG mRNA transcript can be found at, for example, GenBank Accession No. GI: 1441481359 (NM_005199.5; SEQ ID NO: 271; reverse complement, SEQ ID NO: 272). The sequence of mouse CHRNG mRNA can be found at, for example, GenBank Accession No. GI: 119964695 (NM_009604.3; SEQ ID NO: 273; reverse complement, SEQ ID NO: 274). The sequence of rat CHRNG mRNA can be found at, for example, GenBank Accession No. GI: 9506488 (NM_019145.1; SEQ ID NO: 275; reverse complement, SEQ ID NO: 276). The sequence of Macaca fascicularis CHRNG mRNA can be found at, for example, GenBank Accession No. GI: 982288092 (XM_005574625.2; SEQ ID NO: 277; reverse complement, SEQ ID NO: 278). The sequence of Macaca mulatta CHRNG mRNA can be found at, for example, GenBank Accession No. GI: 1622852538 (XM_028831233.1; SEQ ID NO: 279; reverse complement, SEQ ID NO: 280).

Additional examples of CHRNG mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on CHRNG can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CHRNG.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CHRNG, as used herein, also refers to variations of the CHRNG gene including variants provided in the SNP database. Numerous sequence variations within the CHRNG gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CHRNG, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Collagen Type XIII Alpha 1 Chain," used interchangeably with the term "COL13A1," refers to a synaptic extracellular-matrix protein involved in the formation and maintenance of the neuromuscular synapse. COL13A1 encodes the collagen type XIII alpha1 chain (COL13A1), which is a single-pass type II transmembrane protein made of a short intracellular domain, a single transmembrane domain, and a triple-helical collagenous ectodomain. Studies have shown that patients with COL13A1 mutations underlie a myasthenic syndrome characterized by early onset muscle weakness with predominantly feeding and breathing difficulties often requiring ventilation and artificial feeding. COL13A1 is also known as COLXIIIA1, Collagen Alpha-1(XIII) Chain, or CMS19.

An exemplary sequence of a human COL13A1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1677498641 (NM_001130103.2; SEQ ID NO: 281; reverse complement, SEQ ID NO: 282). The sequence of mouse COL13A1 mRNA can be found at, for example, GenBank Accession No. GI: 755571593 (NM_007731.3; SEQ ID NO: 283; reverse complement, SEQ ID NO: 284). The sequence of rat COL13A1 mRNA can be found at, for example, GenBank Accession No. GI: 157821424 (NM_001109172.1; SEQ ID NO: 285; reverse complement, SEQ ID NO: 286). The sequence of Macaca fascicularis COL13A1 mRNA can be found at, for example, GenBank Accession No. GI: 982269148 (XM_015456252.1; SEQ ID NO: 287; reverse complement, SEQ ID NO: 288). The sequence of Macaca mulatta COL13A1 mRNA can be found at, for example, GenBank Accession No. GI: 1622966101 (XM_015147482.2; SEQ ID NO: 289; reverse complement, SEQ ID NO: 290).

Additional examples of COL13A1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on COL13A1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=COL13A1.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term COL13A1, as used herein, also refers to variations of the COL13A1 gene including variants provided in the SNP database. Numerous sequence variations within the COL13A1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=COL13A1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Docking Protein 7," used interchangeably with the term "DOK7," refers to a protein that is essential for neuromuscular synaptogenesis. The protein functions in aneural activation of muscle-specific receptor kinase, which is required for postsynaptic differentiation, and in the subsequent clustering of the acetylcholine receptor in myotubes. This protein can also induce autophosphorylation of muscle-specific receptor kinase. Mutations in this gene are a cause of congenital myasthenic syndrome. DOK7 is also known as C4orf25, Downstream Of Tyrosine Kinase 7, FLJ33718, FLJ39137, Chromosome 4 Open Reading Frame 25, CMS10, CMS1B, or FADS3.

An exemplary sequence of a human DOK7 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519242777 (NM_173660.5; SEQ ID NO: 291; reverse complement, SEQ ID NO: 292). The sequence of mouse DOK7 mRNA can be found at, for example, GenBank Accession No. GI: 1143077055 (NM_001348478.1; SEQ ID NO: 293; reverse complement, SEQ ID NO: 294). The sequence of rat DOK7 mRNA can be found at, for example, GenBank Accession No. GI: 194240570 (NM_001130062.1; SEQ ID NO: 295; reverse complement, SEQ ID NO: 296). The sequence of Macaca fascicularis DOK7 mRNA can be found at, for example, GenBank Accession No. GI: 982247946 (XM_015450057.1; SEQ ID NO: 297; reverse complement, SEQ ID NO: 298). The

sequence of Macaca mulatta DOK7 mRNA can be found at, for example, GenBank Accession No. GI: 1622938489 (XM_015137905.2; SEQ ID NO: 299; reverse complement, SEQ ID NO: 300).

Additional examples of DOK7 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on DOK7 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=DOK7.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term DOK7, as used herein, also refers to variations of the DOK7 gene including variants provided in the SNP database. Numerous sequence variations within the DOK7 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=DOK7, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "LDL Receptor Related Protein 4," used interchangeably with the term "LRP4," refers to a member of the low-density lipoprotein receptor-related protein family. LRP4 is a single-transmembrane protein that possesses a large extracellular domain with multiple LDLR repeats, EGF-like and β-propeller repeats; a transmembrane domain; and a short C-terminal region without an identifiable catalytic motif. Mice lacking LRP4 die at birth and do not form the NMJ, indicating a critical role in neuromuscular junction (NMJ) formation. LPR4 mutation or malfunction is implicated in disorders including congenital myasthenic syndrome, myasthenia gravis, and diseases of bone or kidney. LRP4 is also known as MEGF7, LRP-4, SOST2, CLSS, Low-Density Lipoprotein Receptor-Related Protein 4, Multiple Epidermal Growth Factor-Like Domains 7, LRP10, KIAA0816, or CMS17.

An exemplary sequence of a human LRP4 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519312025 (NM_002334.4; SEQ ID NO: 301; reverse complement, SEQ ID NO: 302). The sequence of mouse LRP4 mRNA can be found at, for example, GenBank Accession No. GI: 224994222 (NM_172668.3; SEQ ID NO: 303; reverse complement, SEQ ID NO: 304). The sequence of rat LRP4 mRNA can be found at, for example, GenBank Accession No. GI: 329112575 (NM_031322.3; SEQ ID NO: 305; reverse complement, SEQ ID NO: 306). The sequence of Macaca fascicularis LRP4 mRNA can be found at, for example, GenBank Accession No. GI: 982294148 (XM_005578015.2; SEQ ID NO: 307; reverse complement, SEQ ID NO: 308). The sequence of Macaca mulatta LRP4 mRNA can be found at, for example, GenBank Accession No. GI: 1622863351 (XM_015114355.2; SEQ ID NO: 309; reverse complement, SEQ ID NO: 310).

Additional examples of LRP4 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on LRP4 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=LRP4.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term LRP4, as used herein, also refers to variations of the LRP4 gene including variants provided in the SNP database. Numerous sequence variations within the LRP4 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=LRP4, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Muscle Associated Receptor Tyrosine Kinase," used interchangeably with the term "MUSK," refers to a muscle-specific tyrosine kinase receptor, which plays a central role in the formation and the maintenance of the neuromuscular junction (NMJ), the synapse between the motor neuron and the skeletal muscle. Recruitment of AGRIN by LRP4 to the MUSK signaling complex induces phosphorylation and activation of MUSK, the kinase of the complex. The activation of MUSK in myotubes regulates the formation of NMJs through the regulation of different processes including the specific expression of genes in subsynaptic nuclei, the reorganization of the actin cytoskeleton and the clustering of the acetylcholine receptors in the postsynaptic membrane. Mutations in this gene have been associated with congenital myasthenic syndrome. MUSK is also known as EC 2.7.10.1, FADS1, CMS9, FADS, Muscle, Skeletal Receptor Tyrosine-Protein Kinase, or Muscle-Specific Kinase Receptor.

An exemplary sequence of a human MUSK mRNA transcript can be found at, for example, GenBank Accession No. GI: 1609044119 (NM_005592.4; SEQ ID NO: 311; reverse complement, SEQ ID NO: 312). The sequence of mouse MUSK mRNA can be found at, for example, GenBank Accession No. GI: 260267047 (NM_001037127.2; SEQ ID NO: 313; reverse complement, SEQ ID NO: 314). The sequence of rat MUSK mRNA can be found at, for example, GenBank Accession No. GI: 1937920431 (NM_031061.2; SEQ ID NO: 315; reverse complement, SEQ ID NO: 316). The sequence of Macaca fascicularis MUSK mRNA can be found at, for example, GenBank Accession No. GI: 982300549 (XM_005581093.2; SEQ ID NO: 317; reverse complement, SEQ ID NO: 318). The sequence of Macaca mulatta MUSK mRNA can be found at, for example, GenBank Accession No. GI: 1622871800 (XM_015117113.2; SEQ ID NO: 319; reverse complement, SEQ ID NO: 320).

Additional examples of MUSK mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on MUSK can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=MUSK.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term MUSK, as used herein, also refers to variations of the MUSK gene including variants provided in the SNP database. Numerous sequence variations within the MUSK gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*,

www.ncbi.nlm.nih.gov/snp/?term=MUSK, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Receptor Associated Protein Of The Synapse," used interchangeably with the term "RAPSN," refers to a member of a family of proteins that are receptor associated proteins of the synapse. The encoded protein contains a conserved cAMP-dependent protein kinase phosphorylation site, and plays a critical role in clustering and anchoring nicotinic acetylcholine receptors at synaptic sites by linking the receptors to the underlying postsynaptic cytoskeleton, possibly by direct association with actin or spectrin. Mutations in this gene may play a role in postsynaptic congenital myasthenic syndromes. RAPSN is also known as RNF205, 43 KDa Receptor-Associated Protein Of The Synapse, RING Finger Protein 205, CMS1D, CMS1E, Acetylcholine Receptor-Associated 43 Kda Protein, RAPSYN, CMS11, CMS4C, FADS2, or FADS.

An exemplary sequence of a human RAPSN mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519241818 (NM_005055.5; SEQ ID NO: 321; reverse complement, SEQ ID NO: 322). The sequence of mouse RAPSN mRNA can be found at, for example, GenBank Accession No. GI: 224967080 (NM_009023.3; SEQ ID NO: 323; reverse complement, SEQ ID NO: 324). The sequence of rat RAPSN mRNA can be found at, for example, GenBank Accession No. GI: 157819696 (NM_001108584.1; SEQ ID NO: 325; reverse complement, SEQ ID NO: 326). The sequence of Macaca fascicularis RAPSN mRNA can be found at, for example, GenBank Accession No. GI: 982294016 (XM_015434747.1; SEQ ID NO: 327; reverse complement, SEQ ID NO: 328). The sequence of Macaca mulatta RAPSN mRNA can be found at, for example, GenBank Accession No. GI: 1622863236 (XM_015114296.2; SEQ ID NO: 329; reverse complement, SEQ ID NO: 330).

Additional examples of RAPSN mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on RAPSN can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=RAPSN.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term RAPSN, as used herein, also refers to variations of the RAPSN gene including variants provided in the SNP database. Numerous sequence variations within the RAPSN gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=RAPSN, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Sodium Voltage-Gated Channel Alpha Subunit 4," used interchangeably with the term "SCN4A," refers to a member of the voltage-gated sodium channel family. Voltage-gated sodium channels are transmembrane glycoprotein complexes composed of a large alpha subunit with 24 transmembrane domains and one or more regulatory beta subunits. They are responsible for the generation and propagation of action potentials in neurons and muscle. This gene encodes one member of the sodium channel alpha subunit gene family. It is expressed in skeletal muscle, and

mutations in this gene have been linked to congenital myasthenic syndrome, and several myotonia and periodic paralysis disorders. SCN4A is also known as SkM1, Nav1.4, HYPP, Sodium Channel Protein Skeletal Muscle Subunit Alpha, Voltage-Gated Sodium Channel Subunit Alpha Nav1.4, HYKPP, Skeletal Muscle Voltage-Dependent Sodium Channel Type IV Alpha Subunit, CTC-264K15.6, Na(V)1.4, HOKPP2, CMS16, or NAC1A.

An exemplary sequence of a human SCN4A mRNA transcript can be found at, for example, GenBank Accession No. GI: 93587341 (NM_000334.4; SEQ ID NO: 331; reverse complement, SEQ ID NO: 332). The sequence of mouse SCN4A mRNA can be found at, for example, GenBank Accession No. GI: 134948031 (NM_133199.2; SEQ ID NO: 333; reverse complement, SEQ ID NO: 334). The sequence of rat SCN4A mRNA can be found at, for example, GenBank Accession No. GI: 1937369400 (NM_013178.2; SEQ ID NO: 335; reverse complement, SEQ ID NO: 336). The sequence of Macaca fascicularis SCN4A mRNA can be found at, for example, GenBank Accession No. GI: 982306407 (XM_015438708.1; SEQ ID NO: 337; reverse complement, SEQ ID NO: 338).

The sequence of Macaca mulatta SCN4A mRNA can be found at, for example, GenBank Accession No. GI: 1622880585 (XM_015120096.2; SEQ ID NO: 339; reverse complement, SEQ ID NO: 340).

Additional examples of SCN4A mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on SCN4A can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=SCN4A.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term SCN4A, as used herein, also refers to variations of the SCN4A gene including variants provided in the SNP database. Numerous sequence variations within the SCN4A gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=SCN4A, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "Double Homeobox 4," used interchangeably with the term "DUX4," refers to a transcriptional activator of many genes. DUX4 is normally expressed during early embryonic development, and is then effectively silenced in all tissues except the testis and thymus. DUX4 has been implicated as being involved in cell death, oxidative stress, muscle differentiation and growth, epigenetic regulation, and a number of other signaling pathways in skeletal muscle. Inappropriate expression of DUX4 in muscle cells is the cause of facioscapulohumeral muscular dystrophy (FSHD), which is characterized by muscle weakness and wasting (atrophy) that worsens slowly over time. DUX4 is also known as Double Homeobox Protein 10, Double Homeobox Protein 4, Double Homeobox Protein 4/10, DUX4L, and DUX10.

An exemplary sequence of a human DUX4 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1774753171 (NM_001306068.3; SEQ ID NO: 341; reverse complement,

SEQ ID NO: 342). The sequence of mouse DUX4 mRNA can be found at, for example, GenBank Accession No. GI: 126432555 (NM_001081954.1; SEQ ID NO: 343; reverse complement, SEQ ID NO: 344). The sequence of rat DUX4 mRNA can be found at, for example, GenBank Accession No. GI: 1958689769 (XM_008771031.3; SEQ ID NO: 345; reverse complement, SEQ ID NO: 346). The sequence of Macaca mulatta DUX4 mRNA can be found at, for example, GenBank Accession No. GI: 1622942424 (XM_028848991.1; SEQ ID NO: 347; reverse complement, SEQ ID NO: 348).

Additional examples of DUX4 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on DUX4 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=DUX4.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term DUX4, as used herein, also refers to variations of the DUX4 gene including variants provided in the SNP database. Numerous sequence variations within the DUX4 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=DUX4, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "phospholamban," used interchangeably with the term "PLN," refers to a crucial regulator of cardiac contractility. PLN is a major substrate for the cAMP-dependent protein kinase in cardiac muscle. The encoded protein is an inhibitor of cardiac muscle sarcoplasmic reticulum Ca(2+)-ATPase in the unphosphorylated state, but inhibition is relieved upon phosphorylation of the protein. The subsequent activation of the Ca(2+) pump leads to enhanced muscle relaxation rates, thereby contributing to the inotropic response elicited in heart by beta-agonists. The encoded protein is a key regulator of cardiac diastolic function. Mutations in this gene are a cause of inherited human dilated cardiomyopathy with refractory congestive heart failure, and also familial hypertrophic cardiomyopathy. PLN is also known as CMD1P, PLB, Cardiac Phospholamban, or CMH.

An exemplary sequence of a human PLN mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519242997 (NM_002667.5; SEQ ID NO: 349; reverse complement, SEQ ID NO: 350). The sequence of mouse PLN mRNA can be found at, for example, GenBank Accession No. GI: 213512815 (NM_001141927.1; SEQ ID NO: 351; reverse complement, SEQ ID NO: 352). The sequence of rat PLN mRNA can be found at, for example, GenBank Accession No. GI: 399124783 (NM_022707.2; SEQ ID NO: 353; reverse complement, SEQ ID NO: 354). The sequence of Macaca mulatta PLN mRNA can be found at, for example, GenBank Accession No. GI: 1863319929 (NM_001190894.2; SEQ ID NO: 355; reverse complement, SEQ ID NO: 356).

Additional examples of PLN mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca

genome project web site. Further information on PLN can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=PLN.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term PLN, as used herein, also refers to variations of the PLN gene including variants provided in the SNP database. Numerous sequence variations within the PLN gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=PLN, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "calcium/calmodulin dependent protein kinase II delta," used interchangeably with the term "CAMK2D," refers to a member of the serine/threonine protein kinase family and the Ca(2+)/calmodulin-dependent protein kinase subfamily. CAMK2D is involved in the regulation of Ca(2+) homeostatis and excitation-contraction coupling in heart by targeting ion channels, transporters and accessory proteins involved in Ca(2+) influx into the myocyte, Ca(2+) release from the sarcoplasmic reticulum (SR), SR Ca(2+) uptake and Na(+) and K(+) channel transport. CAMK2D also targets transcription factors and signaling molecules to regulate heart function. In its activated form, CAMK2D is involved in the pathogenesis of dilated cardiomyopathy and heart failure. CAMK2D contributes to cardiac decompensation and heart failure by regulating SR Ca(2+) release via direct phosphorylation of RYR2 Ca(2+) channel. In the nucleus, CAMK2D phosphorylates the MEF2 repressor HDAC4, promoting its nuclear export and binding to 14-3-3 protein, and expression of MEF2 and genes involved in the hypertrophic program. CAMK2D is essential for left ventricular remodeling responses to myocardial infarction. In pathological myocardial remodeling, CAMK2D acts downstream of the beta adrenergic receptor signaling cascade to regulate key proteins involved in excitation-contraction coupling. CAMK2D regulates Ca(2+) influx to myocytes by binding and phosphorylating the L-type Ca(2+) channel subunit beta-2 CACNB2. In addition to Ca(2+) channels, CAMK2D can target and regulate the cardiac sarcolemmal Na(+) channel Nav1.5/SCN5A and the K+ channel Kv4.3/KCND3, which contribute to arrhythmogenesis in heart failure. CAMK2D phosphorylates phospholamban (PLN), an endogenous inhibitor of SERCA2A/ATP2A2, contributing to the enhancement of SR Ca(2+) uptake that may be important in frequency-dependent acceleration of relaxation and maintenance of contractile function during acidosis. CAMK2D may participate in the modulation of skeletal muscle function in response to exercise, by regulating SR Ca(2+) transport through phosphorylation of PLN and triadin, a ryanodine receptor-coupling factor. CAMK2D is also known as Calcium/Calmodulin-Dependent Protein Kinase Type II Delta Chain, CaM Kinase II Delta Subunit, CaM Kinase II Subunit Delta, CAMKD, EC 2.7.11.17, or EC 2.7.11.

An exemplary sequence of a human CAMK2D mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519243899 (NM_001321571.2; SEQ ID NO: 357; reverse complement, SEQ ID NO: 358). The sequence of mouse CAMK2D mRNA can be found at, for example, GenBank Accession No. GI: 654824235 (NM_001025439.2; SEQ ID NO: 359; reverse

complement, SEQ ID NO: 360). The sequence of rat CAMK2D mRNA can be found at, for example, GenBank Accession No. GI: 144922682 (NM_012519.2; SEQ ID NO: 361; reverse complement, SEQ ID NO: 362). The sequence of Macaca mulatta CAMK2D mRNA can be found at, for example, GenBank Accession No. GI: 1622941163 (XM_015139100.2; SEQ ID NO: 363; reverse complement, SEQ ID NO: 364).

Additional examples of CAMK2D mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, UCSC Genome Browser, and the Macaca genome project web site. Further information on CAMK2D can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term= CAMK2D.

The entire contents of each of the foregoing GenBank Accession numbers and the Gene database numbers are incorporated herein by reference as of the date of filing this application.

The term CAMK2D, as used herein, also refers to variations of the CAMK2D gene including variants provided in the SNP database. Numerous sequence variations within the CAMK2D gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, *e.g.*, www.ncbi.nlm.nih.gov/snp/?term=CAMK2D, the entire contents of which is incorporated herein by reference as of the date of filing this application.

In other embodiments, the antisense strand polynucleotides disclosed herein are substantially complementary to the target gene sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 1-4 for ADRB1, or a fragment of SEQ ID NOs: 1-4, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target ADRB1 sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 2-5, 7B, and 7C, and , or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-5, 7B, and 7C, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the disclosure includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is the same as a target ADRB1 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NOs: 5-8, or a fragment of any one of SEQ ID NOs: 5-8, such as about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target ADRB1 sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence

which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 2-5, 7B, and 7C, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-5, 7B, and 7C, such as about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target LEP sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 9-16, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 9-16, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target LEP sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 9-16, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 9-16, such as about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target PLN sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 19-22, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 19-22, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target PLN sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 19-22, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 19-22, such as about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target CAMK2D sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 23-26, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 23-26, such as about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target CAMK2D

sequence, and wherein the sense strand polynucleotide comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the antisense strand nucleotide sequences in any one of any one of Tables 23-26, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 23-26, such as about 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or about 99% complementary.

In some embodiments, the double-stranded region of a double-stranded iRNA agent is equal to or at least, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotide pairs in length.

In some embodiments, the antisense strand of a double-stranded iRNA agent is equal to or at least 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In some embodiments, the sense strand of a double-stranded iRNA agent is equal to or at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 15 to 30 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 19 to 25 nucleotides in length.

In one embodiment, the sense and antisense strands of the double-stranded iRNA agent are each independently 21 to 23 nucleotides in length.

In one embodiment, the sense strand of the iRNA agent 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.

In one aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense nucleic acid molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense RNA molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense RNA molecule may be about 15 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense RNA molecule may comprise a sequence that is at least about 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

In one embodiment, at least partial suppression of the expression of a target gene, is assessed by a reduction of the amount of target mRNA which can be isolated from or detected in a first cell or group of cells in which a target gene is transcribed and which has or have been treated such that the expression of a target gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition may be expressed in terms of:

$$\frac{\text{(mRNA in control cells) - (mRNA in treated cells)}}{\text{(mRNA in control cells)}} \bullet 100\%$$

In one embodiment, inhibition of expression is determined by the dual luciferase method wherein the RNAi agent is present at 10 nM.

The phrase "contacting a cell with an RNAi agent," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent includes contacting a cell *in vitro* with the RNAi agent or contacting a cell *in vivo* with the RNAi agent. The contacting may be done directly or indirectly. Thus, for example, the RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, or to the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. In some embodiments, the RNAi agent may contain or be coupled to a ligand, *e.g.,* one or more GalNAc derivatives as described below, that directs or otherwise stabilizes the RNAi agent at a site of interest, *e.g.,* the liver. In other embodiments, the RNAi agent may contain or be coupled to one or more C_{22} hydrocarbon chains and one or more GalNAc derivatives. In other embodiments, the RNAi agent contains or is coupled to one or more C_{22} hydrocarbon chains and does not contain or is not coupled to one or more GalNAc derivatives. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. For example, a cell may also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

In one embodiment, contacting a cell with an RNAi agent includes "introducing" or "delivering the RNAi agent into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of a RNAi agent can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. Introducing a RNAi agent into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, a RNAi agent can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, *e.g.,* a RNAi agent or a plasmid from which a RNAi agent is transcribed. LNPs are described in, for example, U.S. Patent Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, *e.g.,* a monkey, and a chimpanzee), or a non-primate (such as a a cow, a pig, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, or a mouse), or a

bird that expresses the target gene, either endogenously or heterologously. In one embodiment, the subject is a human, such as a human being treated or assessed for a disease, disorder, or condition that would benefit from reduction in target gene expression; a human at risk for a disease, disorder, or condition that would benefit from reduction in target gene expression; a human having a disease, disorder, or condition that would benefit from reduction in target gene expression; or human being treated for a disease, disorder, or condition that would benefit from reduction in target gene expression as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human. In one embodiment, the subject is an adult subject. In another embodiment, the subject is a pediatric subject.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more signs or symptoms associated with target gene expression or target gene protein production, *e.g.,* a target gene-associated disease, *e.g.,* a skeletal muscle disorder, a cardiac muscle disorder, or an adipose tissue disorder, or symptoms associated with unwanted target gene expression; diminishing the extent of unwanted target activation or stabilization; amelioration or palliation of unwanted target activation or stabilization. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of a target gene in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.,* protein or gene expression level. "Lower" in the context of the level of a target gene in a subject is a decrease to a level accepted as within the range of normal for an individual without such disorder. In certain embodiments, the expression of the target is normalized, *i.e.*, decreased towards or to a level accepted as within the range of normal for an individual without such disorder, *e.g.,* blood glucose level, blood uric acid level, blood lipid level, blood oxygen level, white blood cell count, kidney function, spleen function, liver function. For example, chronic hyperuricemia is defined as serum urate levels greater than 6.8 mg/dl (greater than 360 mmol/), the level above which the physiological saturation threshold is exceeded (Mandell, *Cleve. Clin. Med.* 75:S5-S8, 2008). As used here, "lower" in a subject can refer to lowering of gene expression or protein production in a cell in a subject does not require lowering of expression in all cells or tissues of a subject. For example, as used herein, lowering in a subject can include lowering of gene expression or protein production in a subject.

The term "lower" can also be used in association with normalizing a symptom of a disease or condition, *i.e.* decreasing the difference between a level in a subject suffering from a target gene-associated disease towards or to a level in a normal subject not suffering from a target gene-associated disease. As used herein, if a disease is associated with an elevated value for a symptom, "normal" is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, "normal" is considered to be the lower limit of normal.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder, or condition thereof, that would benefit from a reduction in expression of a target gene or production of a target protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.,* a symptom of a target gene-associated disease. The failure to develop a disease, disorder, or condition, or the reduction in the development of a symptom associated with such a disease, disorder, or condition (*e.g.,* by at least about 10% on a clinically accepted scale for that disease or disorder), or the exhibition of delayed symptoms delayed (*e.g.,* by days, weeks, months or years) is considered effective prevention.

As used herein, the term "target gene-associated disease," is a disease or disorder that would benefit from reduction in the expression or activity of the target gene. The term "target gene-associated disease," is a disease or disorder that is caused by, or associated with expression or protein production of the target gene. The term "target gene-associated disease" includes a disease, disorder or condition that would benefit from a decrease in expression or protein activity of the target gene. Additional information regarding specific target genes and disease that would benefit from reduction in expression of the target gene are descrived below.

In one embodiment, the target gene-associated disease is a cardiac muscle disease or disorder.

In one embodiment, the target gene-associated disease is a skeletal muscle disease or disorder.

In one embodiment, the target gene-associated disease is a adipose tissue disease or disorder.

Exemplary cardiac muscle disorders include obstructive hypertrophic cardiomyopathy (HOCM); familial hypertrophic cardiomyopathy (FHC); Heart failure with preserved ejection fraction (HFPEF); atrial fibrillation (AFIB); ventricular fibrillation (VFIB); angina; myocardial infarction (MI); heart failure or heart failure with reduced ejection fraction (HFREF); supraventricular tachycardia (SVT); and hypertrophic cardiomyopathy (HCM).

"Heart failure" ("HF") or "congestive heart failure" ("CHF") is a chronic condition in which the heart doesn't pump blood as well as it should. Heart failure occurs when the heart's capacity to pump blood cannot keep up with the body's need. Heart failure can occur if the heart cannot pump (systolic) or fill (diastolic) adequately. As the heart weakens, blood begins to back up and force liquid through the capillary walls. The term "congestive" refers to the resulting buildup of fluid in the ankles and feet, arms, lungs, and/or other organs.

One type of heart failure is "heart failure with preserved left ventricular function" ("HF-pEF") also known as "heart failure with preserved ejection fraction" ("HF-pEF") is a condition in which the heart contracts and pumps normally, but the ventricles are thicker and stiffer than normal. Because of this, the ventricles can't relax properly and fill up all the way. Because there's less blood in the ventricles, less blood is pumped out to the rest of the body when the heart contracts.

The most common cause of congestive heart failure is coronary artery disease. Risk factors for coronary artery disease include high levels of cholesterol and/or triglyceride, high blood pressure, poor diet, a sedentary lifestyle, diabetes, smoking, being overweight or obese, and stress. In addition to coronary artery disease, several other conditions can damage the heart muscles, including inherited

and genetic factors, some infections and autoimmune diseases and some treatments such as chemotherapy.

Symptoms of CHF include shortness of breath, fatigue, swollen legs, and rapid heartbeat.

Treatments can include eating less salt, limiting fluid intake, and taking prescription medications, *e.g.,* vasodilators, diuretics, aldosterone inhibitors, ACE inhibitors or ARB drugs, digitalis glycosides, anticoagulants or antiplatelets, beta-blockers, and tranquilizers, and surgical procedures, include for example, bypass surgery, heart valve replacement, implantation of a pacemaker, *e.g.,* biventricular pacing therapy or an implantable cardioverter defibrillator, ventricular assist devices (VAD therapy), and heart transplant.

"Hypertrophic cardiomyopathy" ("HCM") refers to impaired heart function associated with abnormally thick heart muscle in the absence of other heart disease; *e.g.*, valvular heart disease. "Hypertrophic obstructive cardiomyopathy" ("HOCM") is a subtype of HCM, where the wall (septum) between the two bottom chambers of the heart thickens. The walls of the pumping chamber can also become stiff. The thickened septum may cause a narrowing that can block or reduce the blood flow from the left ventricle to the aorta, which is a condition called "outflow tract obstruction."

Both HCM and HOCM may be caused by heart muscle gene mutation, which may be inherited. As such, multiple family members may be affected by HCM and HOCM. Phenotypic expression of the gene mutation may be variable.

Both HCM and HOCM may be caused by heart muscle gene mutation, which may be inherited. As such, multiple family members may be affected by HCM and HOCM. Phenotypic expression of the gene mutation may be variable. In other words, even with the same gene mutation, the severity of heart function impairment may vary between affected patients.

Symptoms associated with HCM may vary in severity and character as well, including, fatigue, chest pain, dyspnea, abnormal heart rhythm, heart failure, syncope, and sudden cardiac death.

Treatments include pacemakers, defibrillators, alcohol septal ablation, surgical myectomy, advanced heart failure therapy, beta blockers, calcium channel blockers, and anti-arrhythmics.

"Familial hypertrophic cardiomyopathy" is an autosomal dominant disease characterized mainly by left ventricular hypertrophy. Thickening usually occurs in the interventricular septum. In some, thickening of the interventricular septum impedes the flow of oxygen-rich blood from the heart, which may lead to an abnormal heart sound during a heartbeat (heart murmur) and other signs and symptoms of the condition. Other affected individuals do not have physical obstruction of blood flow, but the pumping of blood is less efficient, which can also lead to symptoms of the condition. Cardiac hypertrophy often begins in adolescence or young adulthood, although it can develop at any time throughout life.

The symptoms of familial hypertrophic cardiomyopathy are variable, even within the same family. Many affected individuals have no symptoms. Other people with familial hypertrophic cardiomyopathy may experience chest pain; shortness of breath, especially with physical exertion; a sensation of fluttering or pounding in the chest (palpitations); lightheadedness; dizziness; and fainting.

While most people with familial hypertrophic cardiomyopathy are symptom-free or have only mild symptoms, this condition can have serious consequences. It can cause abnormal heart rhythms (arrhythmias) that may be life threatening. People with familial hypertrophic cardiomyopathy have an increased risk of sudden death, even if they have no other symptoms of the condition. A small number of affected individuals develop potentially fatal heart failure, which may require heart transplantation.

Mutations in one of several genes can cause familial hypertrophic cardiomyopathy; the most commonly involved genes are MYH7, MYBPC3, TNNT2, and TNNI3. Other genes, including some that have not been identified, may also be involved in this condition.

Treatments include, beta blockers, calcium channel blockers, heart rhythm drugs such as amiodarone (Pacerone) or disopyramide (Norpace), and blood thinners such as warfarin (Coumadin, Jantoven), dabigatran (Pradaxa), rivaroxaban (Xarelto) or apixaban (Eliquis). Surgeries or other procedures include apical myectomy, septal myectomy, septal ablation, and implantable cardioverter-defibrillator (ICD).

"Atrial fibrillation" ("AFIB") is when the atria beat chaotically and irregularly - out of coordination with the ventricles. The result is a fast and irregular heart rhythm. The heart rate in atrial fibrillation may range from 100 to 175 beats a minute. The normal range for a heart rate is 60 to 100 beats a minute.

Episodes of atrial fibrillation may come and go, or may go away and may require treatment. Although atrial fibrillation itself usually isn't life-threatening, it is a serious medical condition that sometimes requires emergency treatment.

A major concern with atrial fibrillation is the potential to develop blood clots within the atria which may circulate to other organs and lead to blocked blood flow (ischemia).

Causes of AFIB include, abnormalities or damage to the heart's structure, high blood pressure, heart attack, coronary artery disease, abnormal heart valves, congenital heart defects, an overactive thyroid gland or other metabolic imbalance, exposure to stimulants, such as medications, caffeine, tobacco or alcohol, sick sinus syndrome — improper functioning of the heart's natural pacemaker, lung diseases, previous heart surgery, viral infections, stress due to surgery, pneumonia or other illnesses, and sleep apnea.

Symptoms include palpitations, which are sensations of a racing, uncomfortable, irregular heartbeat or a flip-flopping in the chest, weakness, reduced ability to exercise, fatigue, lightheadedness, dizziness, shortness of breath, and chest pain.

Treatments include, electrical cardioversion, anti-arrhythmics, digoxin, beta blockers, calcium channel blockers anticoagulants, catheter ablation, Maze procedure, atrioventricular (AV) node ablation, and left atrial appendage closure.

"Ventricular fibrillation" ("VFIB") is a type of abnormal heart rhythm (arrhythmia). During ventricular fibrillation, disorganized heart signals cause the ventricles to twitch (quiver) uselessly. As a result, the heart doesn't pump blood to the rest of the body.

Ventricular fibrillation is an emergency that requires immediate medical attention. It's the most frequent cause of sudden cardiac death.

Collapse and loss of consciousness is the most common symptom of ventricular fibrillation. Other symptoms include chest pain, very fast heartbeat (tachycardia), dizziness, nausea, and shortness of breath.

Risk factors include previous episode of ventricular fibrillation, previous heart attack,a congenital heart defect, heart muscle disease (cardiomyopathy), injuries that cause damage to the heart muscle, such as being struck by lightning, drug misuse, especially with cocaine or methamphetamine, and severe imbalance of potassium or magnesium.

Treatments include, cardiopulmonary resuscitation (CPR), defibrillation, anti-arrhythmics, an implantable cardioverter-defibrillator (ICD), cardiac ablation, coronary angioplasty and stent placement, and coronary bypass surgery.

A "myocardial infarction" or "MI" occurs when the flow of blood to the heart is blocked. The blockage is most often a buildup of fat, cholesterol and other substances, which form a plaque in the arteries that feed the heart (coronary arteries).

Symptoms include pressure, tightness, pain, or a squeezing or aching sensation in the chest or arms that may spread to the neck, jaw or back, nausea, indigestion, heartburn or abdominal pain, shortness of breath, cold sweat, fatigue, lightheadedness or sudden dizziness

Heart attack risk factors include age (*e.g.*, men age 45 or older and women age 55 or older are more likely to have a heart attack than are younger men and women, tobacco, high blood pressure. Over time, high blood pressure can damage arteries that lead to your heart. High blood pressure that occurs with other conditions, such as obesity, high cholesterol or diabetes, increases your risk even more, high cholesterol or triglyceride levels, obesity, diabetes, metabolic syndrome, family history of heart attacks, lack of physical activity, stress, illicit drug use, a history of preeclampsia, and an autoimmune condition.

Treatments include, aspirin, thrombolytics, antiplatelet agents, other blood-thinning medications, pain relievers, nitroglycerin, beta blockers, ACE inhibitors, statins, coronary angioplasty and stenting, and coronary artery bypass surgery.

"Supraventricular tachycardia" ("SVT") is as an abnormally fast or erratic heartbeat that affects the heart's atria. During an episode of SVT, the heart beats about 150 to 220 times per minute, but it can occasionally beat faster or slower.

The main symptom of supraventricular tachycardia (SVT) is a very fast heartbeat (100 beats a minute or more) that may last for a few minutes to a few days. The fast heartbeat may come and go suddenly, with stretches of normal heart rates in between.

Signs and symptoms of supraventricular tachycardia may include very fast (rapid) heartbeat, a fluttering or pounding in the chest (palpitations), a pounding sensation in the neck, weakness or feeling very tired (fatigue), chest pain, shortness of breath, lightheadedness or dizziness, sweating, and fainting (syncope) or near fainting. Some with SVT have no signs or symptoms at all.

For some, a supraventricular tachycardia episode is related to an obvious trigger, such as exercise, stress or lack of sleep. Some people may not have a noticeable trigger. Things that may cause an SVT episode include age, coronary artery disease, previous heart surgery, heart disease, heart failure, other heart problems, such as Wolff-Parkinson-White syndrome, chronic lung disease, consuming too much caffeine, drinking too much alcohol, drug use, particularly stimulants such as cocaine and methamphetamines, pregnancy, smoking, thyroid disease, tobacco, sleep apnea, diabetes, and certain medications, including asthma medications and over-the-counter cold and allergy drugs.

Treatments include, carotid sinus massage, vagal maneuvers, cardioversion, beta blockers, anti-arrhythmics, calcium channel blocker, catheter ablation, and pacemaker.

"Hypertrophic cardiomyopathy" ("HCM") is a disease in which the heart muscle becomes abnormally thick (hypertrophied). The thickened heart muscle can make it harder for the heart to pump blood.

"Angina" is a type of chest pain caused by reduced blood flow to the heart. Angina is a symptom of coronary artery disease.

Angina, also called angina pectoris, is often described as squeezing, pressure, heaviness, tightness or pain in your chest. Some with angina symptoms say angina feels like a vise squeezing their chest or a heavy weight lying on their chest. There may also be pain in the arms, neck, jaw, shoulder or back. Other symptoms that you may have with angina include dizziness, fatigue, nausea, shortness of breath, and sweating.

Risk factors include tobacco, diabetes, high blood pressure, high cholesterol or triglyceride levels, family history of heart disease, age (*e.g.,* men older than 45 and women older than 55 have a greater risk than do younger adults), lack of exercise, obesity, and stress.

Treatments include, lifestyle changes, nitrates, aspirin, clot-preventing drugs, beta blockers, statins, calcium channel blockers, blood pressure-lowering medications, angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs), ranolazine (Ranexa), angioplasty and stenting, coronary artery bypass surgery, and external counterpulsation (ECP).

Exemplary skeletal muscle disorders include Myostatin-related muscle hypertrophy, congenital myasthenic syndrome, and facioscapulohumeral muscular dystrophy (FSHD).

Myostatin-related muscle hypertrophy is a rare condition characterized by reduced body fat and increased muscle size. Affected individuals have up to twice the usual amount of muscle mass in their bodies. They also tend to have increased muscle strength. Myostatin-related muscle hypertrophy is caused by mutations in the MSTN gene. It follows an incomplete autosomal dominant pattern of inheritance.

Congenital myasthenic syndromes (CMS) are a heterogeneous group of early-onset genetic neuromuscular transmission disorders due to mutations in proteins involved in the organisation, maintenance, function, or modification of the motor endplate (endplate myopathies), *e.g.,* CHRNA1, CHRNB1, CHRBD, CHRNE, CHRNG, COL13A1, DOX7, LRP4, MUSK, RAPSN, or SCN4A. CMS are clinically characterised by abnormal fatigability, or transient or permanent weakness of extra-

ocular, facial, bulbar, truncal, respiratory, or limb muscles. Onset of endplate myopathy is intrauterine, congenital, in infancy, or childhood, and rarely in adolescence. Severity ranges from mild, phasic weakness, to disabling, permanent muscle weakness, respiratory insufficiency, and early death. All subtypes of CMS share the clinical features of fatigability and muscle weakness, but age of onset, presenting symptoms, and response to treatment vary depending on the molecular mechanism that results from the underlying genetic defect. The term CMS is misleading since not all CMS are congenital. See, Finsterer (2019) *Orphanet J Rare Dis.* 14: 57 for a review.

Facioscapulohumeral muscular dystrophy (FSHD) type 1 is an autosomal dominant condition caused by mutations in DUX4. FSHD typically presents before age 20 years with weakness of the facial muscles and the stabilizers of the scapula or the dorsiflexors of the foot. There is extreme clinical variability. In some cases, Congenital facial weakness may be present. In FSHD, the muscle weakness is slowly progressive and approximately 20% of affected individuals eventually require a wheelchair. Life expectancy is not shortened. The incidence is approximately 4 individuals affected per 100,000 people.

Exemplary adipose tissue disorders include a metabolic disorder, *e.g.* metabolic syndrome, a disorder of carbohydrates, *e.g.*, type II diabetes, pre-diabetes, a lipid metabolism disorder, *e.g.*, a hyperlipidemia, hypertension, a cardiovascular disease, a disorders of body weight.

As used herein, a "metabolic disorder" refers to any disease or disorder that disrupts normal metabolism, the process of converting food to energy on a cellular level. Metabolic diseases affect the ability of the cell to perform critical biochemical reactions that involve the processing or transport of proteins (amino acids), carbohydrates (sugars and starches), or lipids (fatty acids). Non-limiting examples of metabolic diseases include disorders of carbohydrates, *e.g.*, diabetes, type I diabetes, type II diabetes, galactosemia, hereditary fructose intolerance, fructose 1,6-diphosphatase deficiency, glycogen storage disorders, congenital disorders of glycosylation, insulin resistance, insulin insufficiency, hyperinsulinemia, impaired glucose tolerance (IGT), abnormal glycogen metabolism; disorders of amino acid metabolism, *e.g.*, maple syrup urine disease (MSUD), or homocystinuria; disorder of organic acid metabolism, *e.g.*,methylmalonic aciduria, 3-methylglutaconic aciduria -Barth syndrome, glutaric aciduria or 2-hydroxyglutaric aciduria – D and L forms; disorders of fatty acid beta-oxidation, *e.g.*, medium-chain acyl-CoA dehydrogenase deficiency (MCAD), long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD), very-long-chain acyl-CoA dehydrogenase deficiency (VLCAD); disorders of lipid metabolism, *e.g.*, GM1 Gangliosidosis, Tay-Sachs Disease, Sandhoff Disease, Fabry Disease, Gaucher Disease, Niemann-Pick Disease, Krabbe Disease, Mucolipidoses, or Mucopolysaccharidoses; mitochondrial disorders, *e.g.*, mitochondrial cardiomyopathies; Leigh disease; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS); myoclonic epilepsy with ragged-red fibers (MERRF); neuropathy, ataxia, and retinitis pigmentosa (NARP); Barth syndrome; peroxisomal disorders, *e.g.*, Zellweger Syndrome (cerebrohepatorenal syndrome), X-Linked Adrenoleukodystrophy, Refsum Disease, genetic obesity, Laron syndrome; Growth hormone insensitivity syndrome; Pituitary dwarfism II, adiponectin

deficency, CD36 deficiency; Platelet glycoprotein IV deficiency, Hyperlipoproteinemia, type I, Acatalasemia; Takahara disease, Glycogen storage disease type IV; Andersen disease, Acute alcohol sensitivity, Early childhood-onset progressive leukodystrophy, Secondary hyperammonemia, Glycogen storage disease of heart, 3-Methylcrotonylglycinuria; 3-Methylcrotonyl-CoA carboxylase deficiency, Leprechaunism; Donohue syndrome, Insulin-resistant diabetes mellitus with acanthosis nigricans; Type A insulin resistance, Pyruvate dehydrogenase complex deficiency, Pyruvate dehydrogenase E3-binding protein deficiency; andLacticacidemia due to PDX1 deficiency.

In one embodiment, a metabolic disorder is metabolic syndrome. The term "metabolic syndrome, as used herein, is disorder that includes a clustering of components that reflect overnutrition, sedentary lifestyles, genetic factors, increasing age, and resultant excess adiposity. Metabolic syndrome includes the clustering of abdominal obesity, insulin resistance, dyslipidemia, and elevated blood pressure and is associated with other comorbidities including the prothrombotic state, proinflammatory state, nonalcoholic fatty liver disease, and reproductive disorders. The prevalence of the metabolic syndrome has increased to epidemic proportions not only in the United States and the remainder of the urbanized world but also in developing nations. Metabolic syndrome is associated with an approximate doubling of cardiovascular disease risk and a 5-fold increased risk for incident type 2 diabetes mellitus.

Abdominal adiposity (*e.g.*, a large waist circumference (high waist-to-hip ratio)), high blood pressure, insulin resistance and dislipidemia are central to metabolic syndrome and its individual components (*e.g.*, central obesity, fasting blood glucose (FBG)/pre-diabetes/diabetes, hypercholesterolemia, hypertriglyceridemia, and hypertension).

In one embodiment, a metabolic disorder is a disorder of carbohydrates. In one embodiment, the disorder of carbohydrates is diabetes.

As used herein, the term "diabetes" refers to a group of metabolic disorders characterized by high blood sugar (glucose) levels which result from defects in insulin secretion or action, or both. There are two most common types of diabetes, namely type 1 diabetes and type 2 diabetes, which both result from the body's inability to regulate insulin. Insulin is a hormone released by the pancreas in response to increased levels of blood sugar (glucose) in the blood.

The term "type I diabetes," as used herein, refers to a chronic disease that occurs when the pancreas produces too little insulin to regulate blood sugar levels appropriately. Type I diabetes is also referred to as insulin-dependent diabetes mellitus, IDDM, and juvenile onset diabetes. People with type I diabetes (insulin-dependent diabetes) produce little or no insulin at all. Although about 6 percent of the United States population has some form of diabetes, only about 10 percent of all diabetics have type I disorder. Most people who have type I diabetes developed the disorder before age 30. Type 1 diabetes represents the result of a progressive autoimmune destruction of the pancreatic β-cells with subsequent insulin deficiency. More than 90 percent of the insulin-producing cells (beta cells) of the pancreas are permanently destroyed. The resulting insulin deficiency is severe, and to survive, a person with type I diabetes must regularly inject insulin.

In type II diabetes (also referred to as noninsulin-dependent diabetes mellitus, NDDM), the pancreas continues to manufacture insulin, sometimes even at higher than normal levels. However, the body develops resistance to its effects, resulting in a relative insulin deficiency. Type II diabetes may occur in children and adolescents but usually begins after age 30 and becomes progressively more common with age: about 15 percent of people over age 70 have type II diabetes. Obesity is a risk factor for type II diabetes, and 80 to 90 percent of the people with this disorder are obese.

In some embodiments, diabetes includes pre-diabetes. "Pre-diabetes" refers to one or more early diabetic conditions including impaired glucose utilization, abnormal or impaired fasting glucose levels, impaired glucose tolerance, impaired insulin sensitivity and insulin resistance. Prediabetes is a major risk factor for the development of type 2 diabetes mellitus, cardiovascular disease and mortality. Much focus has been given to developing therapeutic interventions that prevent the development of type 2 diabetes by effectively treating prediabetes.

Diabetes can be diagnosed by the administration of a glucose tolerance test. Clinically, diabetes is often divided into several basic categories. Primary examples of these categories include, autoimmune diabetes mellitus, non-insulin-dependent diabetes mellitus (type 1 NDDM), insulin-dependent diabetes mellitus (type 2 IDDM), non-autoimmune diabetes mellitus, non-insulin-dependent diabetes mellitus (type 2 NIDDM), and maturity-onset diabetes of the young (MODY). A further category, often referred to as secondary, refers to diabetes brought about by some identifiable condition which causes or allows a diabetic syndrome to develop. Examples of secondary categories include, diabetes caused by pancreatic disease, hormonal abnormalities, drug- or chemical-induced diabetes, diabetes caused by insulin receptor abnormalities, diabetes associated with genetic syndromes, and diabetes of other causes. (see *e.g.*, Harrison's (1996) 14th ed., New York, McGraw-Hill).

In one embodiment, a metabolic disorder is a lipid metabolism disorder. As used herein, a "lipid metabolism disorder" or "disorder of lipid metabolism" refers to any disorder associated with or caused by a disturbance in lipid metabolism. This term also includes any disorder, disease or condition that can lead to hyperlipidemia, or condition characterized by abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood. This term refers to an inherited disorder, such as familial hypertriglyceridemia, familial partial lipodystrophy type 1 (FPLD1), or an induced or acquired disorder, such as a disorder induced or acquired as a result of a disease, disorder or condition (*e.g.*, renal failure), a diet, or intake of certain drugs (*e.g.*, as a result of highly active antiretroviral therapy (HAART) used for treating, *e.g.*, AIDS or HIV).

Additional examples of disorders of lipid metabolism include, but are not limited to, atherosclerosis, dyslipidemia, hypertriglyceridemia (including drug-induced hypertriglyceridemia, diuretic-induced hypertriglyceridemia, alcohol-induced hypertriglyceridemia, β-adrenergic blocking agent-induced hypertriglyceridemia, estrogen-induced hypertriglyceridemia, glucocorticoid-induced hypertriglyceridemia, retinoid-induced hypertriglyceridemia, cimetidine-induced hypertriglyceridemia, and familial hypertriglyceridemia), acute pancreatitis associated with

hypertriglyceridemia, chylomicron syndrom, familial chylomicronemia, Apo-E deficiency or resistance, LPL deficiency or hypoactivity, hyperlipidemia (including familial combined hyperlipidemia), hypercholesterolemia, gout associated with hypercholesterolemia, xanthomatosis (subcutaneous cholesterol deposits), hyperlipidemia with heterogeneous LPL deficiency, hyperlipidemia with high LDL and heterogeneous LPL deficiency, fatty liver disease, or non-alcoholic stetohepatitis (NASH).

Cardiovascular diseases are also considered "metabolic disorders", as defined herein. These diseases may include coronary artery disease (also called ischemic heart disease), hypertension, inflammation associated with coronary artery disease, restenosis, peripheral vascular diseases, and stroke.

Disorders related to body weight are also considered "metabolic disorders", as defined herein. Such disorders may include obesity, hypo-metabolic states, hypothyroidism, uremia, and other conditions associated with weight gain (including rapid weight gain), weight loss, maintenance of weight loss, or risk of weight regain following weight loss.

Blood sugar disorders are further considered "metabolic disorders", as defined herein. Such disorders may include diabetes, hypertension, and polycystic ovarian syndrome related to insulin resistance. Other exemplary disorders of metabolic disorders may also include renal transplantation, nephrotic syndrome, Cushing's syndrome, acromegaly, systemic lupus erythematosus, dysglobulinemia, lipodystrophy, glycogenosis type I, and Addison's disease.

In one embodiment, an adipose-tissue-associated disorder is primary hypertension. "Primary hypertension" is a result of environmental or genetic causes (*e.g.*, a result of no obvious underlying medical cause).

In one embodiment, an adipose-tissue-associated disorder is secondary hypertension. "Secondary hypertension" has an identifiable underlying disorder which can be of multiple etiologies, including renal, vascular, and endocrine causes, *e.g.*, renal parenchymal disease (*e.g.*, polycystic kidneys, glomerular or interstitial disease), renal vascular disease (*e.g.*, renal artery stenosis, fibromuscular dysplasia), endocrine disorders (*e.g.*, adrenocorticosteroid or mineralocorticoid excess, pheochromocytoma, hyperthyroidism or hypothyroidism, growth hormone excess, hyperparathyroidism), coarctation of the aorta, or oral contraceptive use.

In one embodiment, an adipose-tissue-associated disorder is resistant hypertension. "Resistant hypertension" is blood pressure that remains above goal (*e.g.*, above 130 mm Hg systolic or above 90 diastolic) in spite of concurrent use of three antihypertensive agents of different classes, one of which is a thiazide diuretic. Subjects whose blood pressure is controlled with four or more medications are also considered to have resistant hypertension.

Additional diseases or conditions related to metabolic disorders that would be apparent to the skilled artisan and are within the scope of this disclosure.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a target gene-associated disease, is sufficient

to effect treatment of the disease (*e.g.,* by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of a RNAi agent that, when administered to a subject having a target gene-associated disorder, *e.g.,* gout or diabetes, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of a RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. A RNAi agent employed in the methods of the present disclosure may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials (including salts), compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.,* lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16)

pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations. Pharmaceutically acceptable carriers for pulmonary delivery are known in the art and will vary depending on the desired location for deposition of the agent, *e.g.*, upper or lower respiratory system, and the type of device to be used for delivery, *e.g.*, sprayer, nebulizer, dry powder inhaler.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, bronchial fluids, sputum, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, sputum, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs.

II. RNAi Agents of the Invention

Described herein are RNAi agents comprising one or more C22 hydrocarbon chains, *e.g.,* saturated or unsaturated, conjugated to one or more internal positions on at least one strand which inhibit the expression of a target gene in muscle tissue or an adipose tissue. In one embodiment, the RNAi agent includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a target gene in a cell, such as a cell within a subject, *e.g.,* a mammal, such as a human, *e.g.,* a subject having a target gene-associated disorder, *e.g.,* a muscle tissue disease or an adipose tissue disease, or a subject at risk of a target gene-associated disease, *e.g.,* a muscle tissue disease or an adipose tissue disease.

The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of a target RNA, *e.g.,* an mRNA formed in the expression of a target gene. The region of complementarity is about 15-30 nucleotides or less in length. Upon contact with a cell expressing the target gene, the RNAi agent inhibits the expression of the target gene (*e.g.,* a human gene, a primate gene, a non-primate gene) by at least 50% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting, flowcytometric techniques, or histology based method such as immunohistochemistry or *in situ* hybridization. In certain embodiments, inhibition of expression is by at least 50% as assayed by the Dual-Glo lucifierase assay in Example 1 where the siRNA is at a 10 nM concentration.

A dsRNA includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. For example, the target sequence can be derived from the sequence of an mRNA formed during the expression of a target gene. The other strand (the sense

strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. In certain embodiments, the duplex structure is 18 to 25 base pairs in length, *e.g.,* 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-25, 20-24,20-23, 20-22, 20-21, 21-25, 21-24, 21-23, 21-22, 22-25, 22-24, 22-23, 23-25, 23-24 or 24-25 base pairs in length, for example, 19-21 basepairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

Similarly, the region of complementarity to the target sequence is 15 to 30 nucleotides in length, *e.g.,* 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, for example 19-23 nucleotides in length or 21-23 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

In some embodiments, the dsRNA is 15 to 23 nucleotides in length, or 25 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well known in the art that dsRNAs longer than about 21-23 nucleotides can serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (*i.e.,* cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, *e.g.,* a duplex region of about 15 to 36 base pairs, *e.g.,* 15-36, 15-35, 15-34, 15-33, 15-32, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24,20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs, for example, 19-21 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of *e.g.,* 15-30 base pairs, that targets a desired RNA for cleavage, an

RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, a RNAi agent useful to target gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs *e.g.,* 1, 2, 3, or 4 nucleotides. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end or both ends of either an antisense or sense strand of a dsRNA. In certain embodiments, longer, extended overhangs are possible.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, *e.g.,* by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

iRNA compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligonucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

An iRNA can be produced, *e.g.,* in bulk, by a variety of methods. Exemplary methods include: organic synthesis and RNA cleavage, *e.g., in vitro* cleavage.

An iRNA can be made by separately synthesizing a single stranded RNA molecule, or each respective strand of a double-stranded RNA molecule, after which the component strands can then be annealed.

A large machine, *e.g.,* the OligoPilot II from Pharmacia Biotec AB (Uppsala Sweden), can be used to produce a large amount of a particular RNA strand for a given siRNA. The OligoPilotII reactor can efficiently couple a nucleotide using only a 1.5 molar excess of a phosphoramidite nucleotide. To make an RNA strand, ribonucleotides amidites are used. Standard cycles of monomer addition can be used to synthesize the 21 to 23 nucleotide strand for the siRNA. Typically, the two complementary strands are produced separately and then annealed, *e.g.,* after release from the solid support and deprotection.

Organic synthesis can be used to produce a discrete iRNA species. The complementary of the species to a target gene can be precisely specified. For example, the species may be complementary to a region that includes a polymorphism, *e.g.,* a single nucleotide polymorphism. Further the location of the polymorphism can be precisely defined. In some embodiments, the polymorphism is located in an internal region, *e.g.,* at least 4, 5, 7, or 9 nucleotides from one or both of the termini.

In one embodiment, dsRNA is cleaved *in vitro* into siRNAs, for example, using a Dicer or comparable RNAse III-based activity. For example, the dsRNA can be incubated in an *in vitro* extract from *Drosophila* or using purified components, *e.g.,* a purified RNAse or RISC complex (RNA-induced silencing complex). See, *e.g.,* Ketting *et al. Genes Dev* 2001 Oct 15;15(20):2654-9 and Hammond *Science* 2001 Aug 10;293(5532):1146-50.

dsRNA cleavage generally produces a plurality of siRNA species, each being a particular 21 to 23 nucleotide fragment of a source dsRNA molecule. For example, siRNAs that include sequences complementary to overlapping regions and adjacent regions of a source dsiRNA molecule may be present.

Regardless of the method of synthesis, the dsRNA preparation can be prepared in a solution (*e.g.,* an aqueous or organic solution) that is appropriate for formulation. For example, the dsRNA preparation can be precipitated and redissolved in pure double-distilled water, and lyophilized. The dried dsRNA can then be resuspended in a solution appropriate for the intended formulation process.

In one aspect, a dsRNA of the disclosure includes at least two nucleotide sequences, a sense sequence and an antisense sequence.

In one embodiment, the dsRNA of the disclosure targets the ADRB1 gene. The sense strand sequence for ADRB1 may be selected from the group of sequences provided in any one of Tables 2-5, 7B, and 7C, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 2-5, 7B, and 7C. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a target gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 2-5, 7B, and 7C, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 2-5, 7B, and 7C for ADRB1.

In one embodiment, the dsRNA of the disclosure targets the Leptin (LEP) gene. The sense strand sequence for LEP may be selected from the group of sequences provided in any one of Tables 9-16, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 9-16. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a target gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 9-16, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 9-16 for LEP.

In one embodiment, the dsRNA of the disclosure targets the PLN gene. The sense strand sequence for PLN may be selected from the group of sequences provided in any one of Tables 19-22, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected

from the group of sequences of any one of Tables 19-22. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a target gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 19-22, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 19-22 for PLN.

In one embodiment, the dsRNA of the disclosure targets the CAMK2D gene. The sense strand sequence for CAMK2D may be selected from the group of sequences provided in any one of Tables 23-26, and the corresponding nucleotide sequence of the antisense strand of the sense strand may be selected from the group of sequences of any one of Tables 23-26. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a target gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand (passenger strand) in any one of Tables 23-26, and the second oligonucleotide is described as the corresponding antisense strand (guide strand) of the sense strand in any one of Tables 23-26 for CAMK2D.

In one embodiment, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In another embodiment, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although the sequences provided herein are described as modified or conjugated sequences, the RNA of the RNAi agent of the disclosure *e.g.,* a dsRNA of the disclosure, may comprise any one of the sequences set forth in any one of Tables 2-5, 7B, 7C, 9-16, and 19-26 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. One or more lipophilic ligands or one or more GalNAc ligands can be included in any of the positions of the RNAi agents provided in the instant application.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, *e.g.,* 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir *et al.*, (2001) *EMBO J.*, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim *et al.* (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided herein, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences provided herein, and differing in their ability to inhibit the expression of a target gene by not more than 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence using the *in vitro* assay with Cos7 and a 10 nM

concentration of the RNA agent and the PCR assay as provided in the examples herein, are contemplated to be within the scope of the present disclosure.

In addition, the RNAs described herein identify a site(s) in a target gene transcript that is susceptible to RISC-mediated cleavage. As such, the present disclosure further features RNAi agents that target within this site(s). As used herein, a RNAi agent is said to target within a particular site of an RNA transcript if the RNAi agent promotes cleavage of the transcript anywhere within that particular site. Such a RNAi agent will generally include at least about 15 contiguous nucleotides, such as at least 19 nucleotides, from one of the sequences provided herein coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a target gene.

An RNAi agent as described herein can contain one or more mismatches to the target sequence. In one embodiment, an RNAi agent as described herein contains no more than 3 mismatches (***i.e.,*** 3, 2, 1, or 0 mismatches). In one embodiment, an RNAi agent as described herein contains no more than 2 mismatches. In one embodiment, an RNAi agent as described herein contains no more than 1 mismatch. In one embodiment, an RNAi agent as described herein contains 0 mismatches. In certain embodiments, if the antisense strand of the RNAi agent contains mismatches to the target sequence, the mismatch can optionally be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, in such embodiments, for a 23 nucleotide RNAi agent, the strand which is complementary to a region of a target gene generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a target gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a target gene is important, especially if the particular region of complementarity in a target gene is known to mutate.

III. C_{22} Hydrocarbon Chains

As described herein, conjugating a C_{22} hydrocarbon chain, *e.g.,* saturated or unsaturated, to one or more internal position(s) of the dsRNA agent increases lipophilicity of the dsRNA agent and provides optimal hydrophobicity for the enhanced *in vivo* delivery of dsRNA to muscle tissue, *e.g.,* skeletal muscle tissue or cardiac muscle tissue, or adipose tissue.

One way to characterize lipophilicity is by the octanol-water partition coefficient, $\log K_{ow}$, where K_{ow} is the ratio of a chemical's concentration in the octanol-phase to its concentration in the aqueous phase of a two-phase system at equilibrium. The octanol-water partition coefficient is a laboratory-measured property of a substance. However, it may also be predicted by using coefficients attributed to the structural components of a chemical which are calculated using first-principle or empirical methods (see, for example, Tetko et al., *J. Chem. Inf. Comput. Sci.* 41:1407-21 (2001), which is incorporated herein by reference in its entirety). It provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily milieu rather than water (*i.e.* its hydrophilic/lipophilic balance). In principle, a chemical substance is lipophilic in character when its

$logK_{ow}$ exceeds 0. Typically, the lipophilic moiety possesses a $logK_{ow}$ exceeding 1, exceeding 1.5, exceeding 2, exceeding 3, exceeding 4, exceeding 5, or exceeding 10. For instance, the $logK_{ow}$ of 6-amino hexanol, for instance, is predicted to be approximately 0.7. Using the same method, the $logK_{ow}$ of cholesteryl N-(hexan-6-ol) carbamate is predicted to be 10.7.

The lipophilicity of a molecule can change with respect to the functional group it carries. For instance, adding a hydroxyl group or amine group to the end of a C_{22} hydrocarbon chain can increase or decrease the partition coefficient (*e.g.*, $logK_{ow}$) value of the C_{22} hydrocarbon chain.

Alternatively, the hydrophobicity of the dsRNA agent, conjugated to one or more C_{22} hydrocarbon chains, can be measured by its protein binding characteristics. For instance, the unbound fraction in the plasma protein binding assay of the dsRNA agent can be determined to positively correlate to the relative hydrophobicity of the dsRNA agent, which can positively correlate to the silencing activity of the dsRNA agent.

In one embodiment, the plasma protein binding assay determined is an electrophoretic mobility shift assay (EMSA) using human serum albumin protein. The hydrophobicity of the dsRNA agent, measured by fraction of unbound dsRNA 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.

In certain embodiments, the one or more C_{22} hydrocarbon chains is an aliphatic, alicyclic, or polyalicyclic compound is an aliphatic, cyclic such as alicyclic, or polycyclic such as polyalicyclic compound. The hydrocarbon chain may comprise various substituents and/or one or more heteroatoms, such as an oxygen or nitrogen atom.

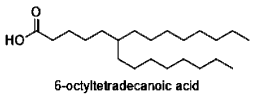
The one or more C_{22} hydrocarbon chains may be attached to the iRNA agent by any method known in the art, including via a functional grouping already present in the lipophilic moiety or introduced into the iRNA agent, such as a hydroxy group (*e.g.*, —CO—CH_2—OH). The functional groups already present in the C_{22} hydrocarbon chain or introduced into the dsRNA agent include, but are not limited to, hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

Conjugation of the dsRNA agent and the C_{22} hydrocarbon chain may occur, for example, through formation of an ether or a carboxylic or carbamoyl ester linkage between the hydroxy and an alkyl group R—, an alkanoyl group RCO— or a substituted carbamoyl group RNHCO—. The alkyl group R may be cyclic (*e.g.*, cyclohexyl) or acyclic (*e.g.*, straight-chained or branched; and saturated or unsaturated). Alkyl group R may be a butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl or octadecyl group, or the like.

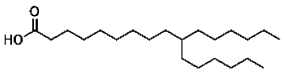
In some embodiments, the C_{22} hydrocarbon chain is conjugated to the dsRNA agent *via* a linker a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction (*e.g.*, a triazole from the azide-alkyne cycloaddition), or carbamate.

In one embodiment, the one or more C_{22} hydrocarbon chains is a C_{22} acid, *e.g.*, the C_{22} acid is selected from the group consisting of docosanoic acid, 6-octyltetradecanoic acid, 10-

hexylhexadecanoic acid, all-cis-7,10,13,16,19-docosapentaenoic acid, all-cis-4,7,10,13,16,19-docosahexaenoic acid, all-cis-13,16-docosadienoic acid, all-cis-7,10,13,16-docosatetraenoic acid, all-cis-4,7,10,13,16-docosapentaenoic acid, and cis-13-docosenoic acid.

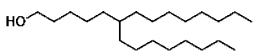


6-octyltetradecanoic acid

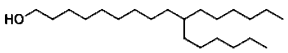


10-hexylhexadecanoic acid

In one embodiment, the one or more C_{22} hydrocarbon chains is a C_{22} alcohol, *e.g.* the C_{22} alcohol is selected from the group consisting of 1-docosanol, 6-octyltetradecan-1-ol, 10-hexylhexadecan-1-ol, cis-13-docosen-1-ol, docosan-9-ol, docosan-2-ol, docosan-10-ol, docosan-11-ol, and cis-4,7,10,13,16,19-docosahexanol.



6-octyltetradecan-1-ol



10-hexylhexadecan-1-ol

In one embodiment, the one or more C_{22} hydrocarbon chains is not cis-4,7,10,13,16,19-docosahexanoic acid. In one embodiment, the one or more C_{22} hydrocarbon chains is not cis-4,7,10,13,16,19-docosahexanol. In one embodiment, the one or more C_{22} hydrocarbon chains is not cis-4,7,10,13,16,19-docosahexanoic acid and is not cis-4,7,10,13,16,19-docosahexanol.

In one embodiment, the one or more C_{22} hydrocarbon chains is a C_{22} amide, *e.g.,* the C_{22} amide is selected from the group consisting of (E)-Docos-4-enamide, (E)-Docos-5-enamide, (Z)-Docos-9-enamide, (E)-Docos-11-enamide,12-Docosenamide, (Z)-Docos-13-enamide, (Z)-N-Hydroxy-13-docoseneamide, (E)-Docos-14-enamide, 6-cis-Docosenamide, 14-Docosenamide Docos-11-enamide, (4E,13E)-Docosa-4,13-dienamide, and (5E,13E)-Docosa-5,13-dienamide.

In certain embodiments, more than one C_{22} hydrocarbon chains can be incorporated into the double-strand iRNA agent, particularly when the C_{22} hydrocarbon chains has a low lipophilicity or hydrophobicity. In one embodiment, two or more C_{22} hydrocarbon chains are incorporated into the same strand of the double-strand iRNA agent. In one embodiment, each strand of the double-strand

iRNA agent has one or more C_{22} hydrocarbon chains incorporated. In one embodiment, two or more C_{22} hydrocarbon chains are incorporated into the same position (*i.e.*, the same nucleobase, same sugar moiety, or same internucleosidic linkage) of the double-stranded iRNA agent. This can be achieved by, *e.g.*, conjugating the two or more aturated or unsaturated C_{22} hydrocarbon chains via a carrier, and/or conjugating the two or more C_{22} hydrocarbon chains via a branched linker, and/or conjugating the two or more C_{22} hydrocarbon chains via one or more linkers, with one or more linkers linking the C_{22} hydrocarbon chains consecutively.

The one or more C_{22} hydrocarbon chains may be conjugated to the iRNA agent *via* a direct attachment to the ribosugar of the iRNA agent. Alternatively, the one or more C_{22} hydrocarbon chains may be conjugated to the double-strand iRNA agent *via* a linker or a carrier.

In certain embodiments, the one or more C_{22} hydrocarbon chains may be conjugated to the iRNA agent *via* one or more linkers (tethers).

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent *via* 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.

A. Linkers/Tethers

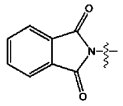
Linkers/Tethers are connected to the one or more C_{22} hydrocarbon chains at a "tethering attachment point (TAP)." Linkers/Tethers may include any C_1-C_{100} carbon-containing moiety, (*e.g.* C_1-C_{75}, C_1-C_{50}, C_1-C_{20}, C_1-C_{10}; C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, or C_{10}), and may have at least one nitrogen atom. In certain embodiments, the nitrogen atom forms part of a terminal amino or amido (NHC(O)-) group on the linker/tether, which may serve as a connection point for the lipophilic moiety. Non-limited examples of linkers/tethers (underlined) include TAP-$\underline{(CH_2)_nNH}$-; TAP-$\underline{C(O)(CH_2)_nNH}$-; TAP-$\underline{NR''''(CH_2)_nNH}$-, TAP-$\underline{C(O)-(CH_2)_n-C(O)}$-; TAP-$\underline{C(O)-(CH_2)_n-C(O)O}$-; TAP-$\underline{C(O)-O}$-; TAP-$\underline{C(O)-(CH_2)_n-NH-C(O)}$-; TAP-$\underline{C(O)-(CH_2)_n}$-; TAP-$\underline{C(O)-NH}$-; TAP-$\underline{C(O)}$-; TAP-$\underline{(CH_2)_n-C(O)}$-; TAP-$\underline{(CH_2)_n-C(O)O}$-; TAP-$\underline{(CH_2)_n}$-; or TAP-$\underline{(CH_2)_n-NH-C(O)}$-; in which n is 1-20 (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) and R'''' is C_1-C_6 alkyl. Preferably, n is 5, 6, or 11. In other embodiments, the nitrogen may form part of a terminal oxyamino group, *e.g.*, -ONH_2, or hydrazino group, -$NHNH_2$. The linker/tether may optionally be substituted, *e.g.*, with hydroxy, alkoxy, perhaloalkyl, and/or optionally inserted with one or more additional heteroatoms, *e.g.*, N, O, or S. Preferred tethered ligands may include, *e.g.*, TAP-$(CH_2)_n$NH(LIGAND); TAP-C(O)$(CH_2)_n$NH(LIGAND); TAP-NR''''$(CH_2)_n$NH(LIGAND); TAP-$(CH_2)_n$ONH(LIGAND); TAP-C(O)$(CH_2)_n$ONH(LIGAND); TAP-NR''''$(CH_2)_n$ONH(LIGAND); TAP-$(CH_2)_n$$NHNH_2$(LIGAND), TAP-C(O)$(CH_2)_n$$NHNH_2$(LIGAND); TAP-NR''''$(CH_2)_n$$NHNH_2$(LIGAND); TAP-C(O)-$(CH_2)_n$-C(O)(LIGAND); TAP-C(O)-$(CH_2)_n$-C(O)O(LIGAND); TAP-C(O)-O(LIGAND); TAP-C(O)-$(CH_2)_n$-NH-C(O)(LIGAND); TAP-C(O)-$(CH_2)_n$(LIGAND); TAP-C(O)-NH(LIGAND); TAP-C(O)(LIGAND); TAP-$(CH_2)_n$-C(O) (LIGAND);

TAP-$(CH_2)_n$-C(O)O(LIGAND); TAP-$(CH_2)_n$(LIGAND); or TAP-$(CH_2)_n$-NH-C(O)(LIGAND). In some embodiments, amino terminated linkers/tethers (*e.g.*, NH_2, ONH_2, NH_2NH_2) can form an imino bond (*i.e.*, C=N) with the ligand. In some embodiments, amino terminated linkers/tethers (*e.g.*, NH_2, ONH_2, NH_2NH_2) can acylated, *e.g.*, with $C(O)CF_3$.

In some embodiments, the linker/ tether can terminate with a mercapto group (*i.e.*, SH) or an olefin (*e.g.*, $CH=CH_2$). For example, the tether can be TAP-$(CH_2)_n$-SH, TAP-C(O)$(CH_2)_n$SH, TAP-$(CH_2)_n$-$(CH=CH_2)$, or TAP-C(O)$(CH_2)_n(CH=CH_2)$, in which n can be as described elsewhere. The tether may optionally be substituted, *e.g.*, with hydroxy, alkoxy, perhaloalkyl, and/or optionally inserted with one or more additional heteroatoms, *e.g.*, N, O, or S. The double bond can be *cis* or *trans* or *E* or *Z*.

In other embodiments, the linker/tether may include an electrophilic moiety, preferably at the terminal position of the linker/tether. Exemplary electrophilic moieties include, *e.g.*, an aldehyde, alkyl halide, mesylate, tosylate, nosylate, or brosylate, or an activated carboxylic acid ester, *e.g.* an NHS ester, or a pentafluorophenyl ester. Preferred linkers/tethers (underlined) include TAP-$(CH_2)_n$CHO; TAP-C(O)$(CH_2)_n$CHO; or TAP-NR''''$(CH_2)_n$CHO, in which n is 1-6 and R'''' is C_1-C_6 alkyl; or TAP-$(CH_2)_n$C(O)ONHS; TAP-C(O)$(CH_2)_n$C(O)ONHS; or TAP-NR''''$(CH_2)_n$C(O)ONHS, in which n is 1-6 and R'''' is C_1-C_6 alkyl; TAP-$(CH_2)_n$C(O)OC_6F_5; TAP-C(O)$(CH_2)_n$C(O) OC_6F_5; or TAP-NR''''$(CH_2)_n$C(O) OC_6F_5, in which n is 1-11 and R'''' is C_1-C_6 alkyl; or -$(CH_2)_n$$CH_2$LG; TAP-C(O)$(CH_2)_n$$CH_2$LG; or TAP-NR''''$(CH_2)_n$$CH_2$LG, in which n can be as described elsewhere and R'''' is C_1-C_6 alkyl (LG can be a leaving group, *e.g.*, halide, mesylate, tosylate, nosylate, brosylate). Tethering can be carried out by coupling a nucleophilic group of a ligand, *e.g.*, a thiol or amino group with an electrophilic group on the tether.

In other embodiments, it can be desirable for the monomer to include a phthalimido group (K)



at the terminal position of the linker/tether. K .

In other embodiments, other protected amino groups can be at the terminal position of the linker/tether, *e.g.*, alloc, monomethoxy trityl (MMT), trifluoroacetyl, Fmoc, or aryl sulfonyl (*e.g.*, the aryl portion can be *ortho*-nitrophenyl or *ortho, para*-dinitrophenyl).

Any of the linkers/tethers described herein may further include one or more additional linking groups, *e.g.*, -O-$(CH_2)_n$-, -$(CH_2)_n$-SS-, -$(CH_2)_n$-, or -(CH=CH)-.

B. Cleavable linkers/tethers

In some embodiments, at least one of the linkers/tethers can be a redox cleavable linker, an acid cleavable linker, an esterase cleavable linker, a phosphatase cleavable linker, or a peptidase cleavable linker.

In one embodiment, at least one of the linkers/tethers can be a reductively cleavable linker (*e.g.*, a disulfide group).

In one embodiment, at least one of the linkers/tethers can be an acid cleavable linker (*e.g.*, a hydrazone group, an ester group, an acetal group, or a ketal group).

In one embodiment, at least one of the linkers/tethers can be an esterase cleavable linker (*e.g.*, an ester group).

In one embodiment, at least one of the linkers/tethers can be a phosphatase cleavable linker (*e.g.*, a phosphate group).

In one embodiment, at least one of the linkers/tethers can be a peptidase cleavable linker (*e.g.*, a peptide bond).

Cleavable linking groups are susceptible to cleavage agents, *e.g.*, pH, redox potential or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, *e.g.*, oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, *e.g.*, those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some tethers will have a linkage group that is cleaved at a preferred pH, thereby releasing the iRNA agent from a ligand (*e.g.*, a targeting or cell-permeable ligand, such as cholesterol) inside the cell, or into the desired compartment of the cell.

A chemical junction (*e.g.*, a linking group) that links a ligand to an iRNA agent can include a disulfide bond. When the iRNA agent/ligand complex is taken up into the cell by endocytosis, the acidic environment of the endosome will cause the disulfide bond to be cleaved, thereby releasing the iRNA agent from the ligand (Quintana et al., *Pharm Res.* 19:1310-1316, 2002; Patri et al., *Curr. Opin. Curr. Biol.* 6:466-471, 2002). The ligand can be a targeting ligand or a second therapeutic agent that may complement the therapeutic effects of the iRNA agent.

A tether can include a linking group that is cleavable by a particular enzyme. The type of linking group incorporated into a tether can depend on the cell to be targeted by the iRNA agent. For example, an iRNA agent that targets an mRNA in liver cells can be conjugated to a tether that includes an ester group. Liver cells are rich in esterases, and therefore the tether will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Cleavage of the tether releases the iRNA agent from a ligand that is attached to the distal end of the tether, thereby potentially enhancing

silencing activity of the iRNA agent. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Tethers that contain peptide bonds can be conjugated to iRNA agents target to cell types rich in peptidases, such as liver cells and synoviocytes. For example, an iRNA agent targeted to synoviocytes, such as for the treatment of an inflammatory disease (*e.g.*, rheumatoid arthritis), can be conjugated to a tether containing a peptide bond.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue, *e.g.*, tissue the iRNA agent would be exposed to when administered to a subject. Thus one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, *e.g.*, blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It may be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least 2, 4, 10 or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

C. Redox Cleavable Linking Groups

One class of cleavable linking groups are redox cleavable linking groups that are cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (—S—S—). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In a preferred embodiment, candidate compounds are cleaved by at most 10% in the blood. In preferred embodiments, useful candidate compounds are degraded at least 2, 4, 10 or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

D. Phosphate-Based Cleavable Linking Groups

Phosphate-based linking groups are cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are —O—P(O)(ORk)-O—, —O—P(S)(ORk)-O—, —O—P(S)(SRk)-O—, —S—P(O)(ORk)-O—, —O—P(O)(ORk)-S—, —S—P(O)(ORk)-S—, —O—P(S)(ORk)-S—, —S—P(S)(ORk)-O—, —O—P(O)(Rk)-O—, —O—P(S)(Rk)-O—, —S—P(O)(Rk)-O—, —S—P(S)(Rk)-O—, —S—P(O)(Rk)-S—, —O—P(S)(Rk)-S—. Preferred embodiments are —O—P(O)(OH)—O—, —O—P(S)(OH)—O—, —O—P(S)(SH)—O—, —S—P(O)(OH)—O—, —O—P(O)(OH)—S—, —S—P(O)(OH)—S—, —O—P(S)(OH)—S—, —S—P(S)(OH)—O—, —O—P(O)(H)—O—, —O—P(S)(H)—O—, —S—P(O)(H)—O—, —S—P(S)(H)—O—, —S—P(O)(H)—S—, —O—P(S)(H)—S—. A preferred embodiment is —O—P(O)(OH)—O—. These candidates can be evaluated using methods analogous to those described above.

E. Acid Cleavable Linking Groups

Acid cleavable linking groups are linking groups that are cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (*e.g.*, about 6.0, 5.5, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, ketals, acetals, esters, and esters of amino acids. Acid cleavable groups can have the general formula —C=NN—, C(O)O, or —OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

F. Ester-Based Linking Groups

Ester-based linking groups are cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include but are not limited to esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula —C(O)O—, or —OC(O)—. These candidates can be evaluated using methods analogous to those described above.

G. Peptide-Based Cleaving Groups

Peptide-based linking groups are cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*e.g.*, dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (—C(O)NH—). The amide group can be formed between any alkylene,

alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide cleavable linking groups have the general formula —$NHCHR^1C(O)NHCHR^2C(O)$—, where R^1 and R^2 are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

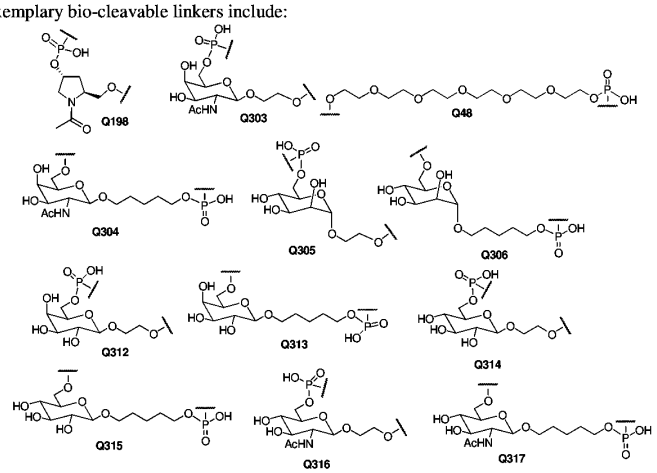
H. Biocleavable linkers/tethers

The linkers can also includes biocleavable linkers that are nucleotide and non-nucleotide linkers or combinations thereof that connect two parts of a molecule, for example, one or both strands of two individual siRNA molecules to generate a bis(siRNA). In some embodiments, mere electrostatic or stacking interaction between two individual siRNAs can represent a linker. The non-nucleotide linkers include tethers or linkers derived from monosaccharides, disaccharides, oligosaccharides, and derivatives thereof, aliphatic, alicyclic, hetercyclic, and combinations thereof.

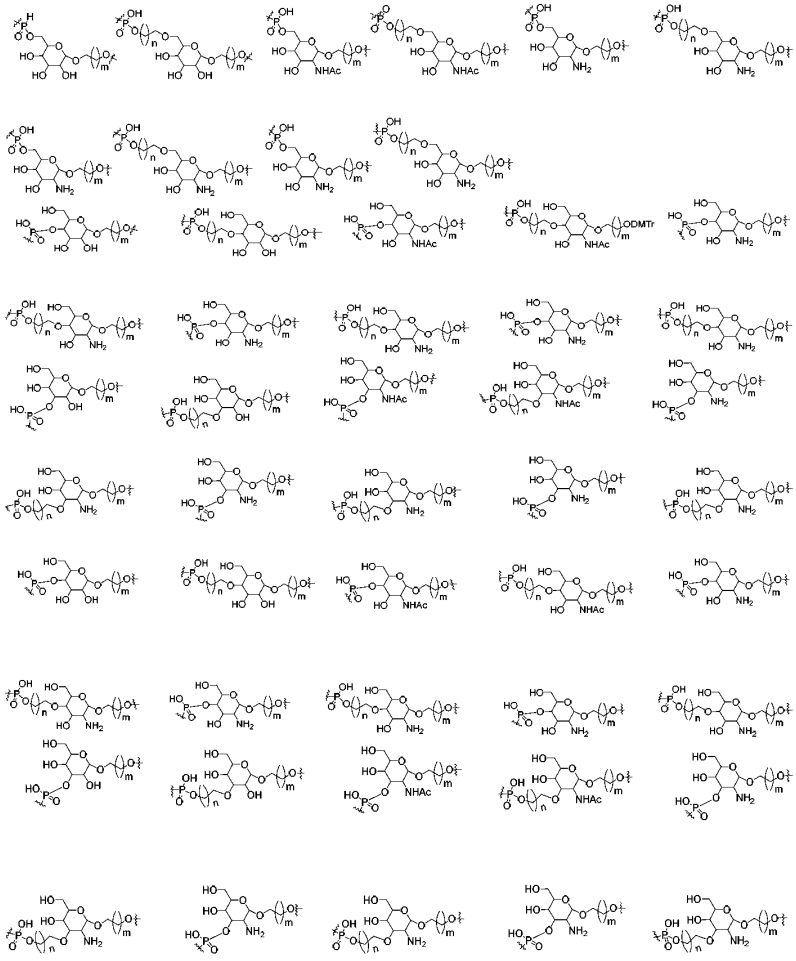
In some embodiments, at least one of the linkers (tethers) is a bio-clevable linker selected from the group consisting of DNA, RNA, disulfide, amide, functionalized monosaccharides or oligosaccharides of galactosamine, glucosamine, glucose, galactose, and mannose, and combinations thereof.

In one embodiment, the bio-cleavable carbohydrate linker may have 1 to 10 saccharide units, which have at least one anomeric linkage capable of connecting two siRNA units. When two or more saccharides are present, these units can be linked via 1-3, 1-4, or 1-6 sugar linkages, or via alkyl chains.

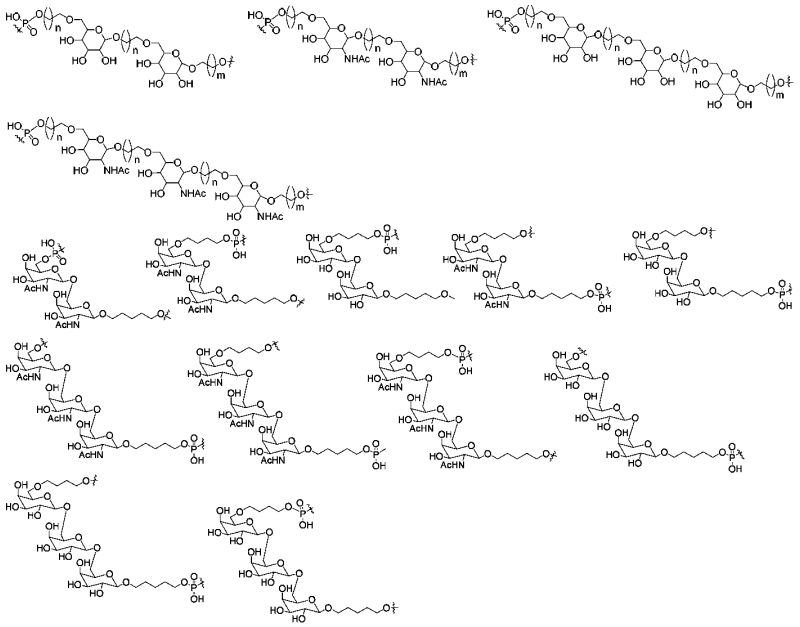
Exemplary bio-cleavable linkers include:



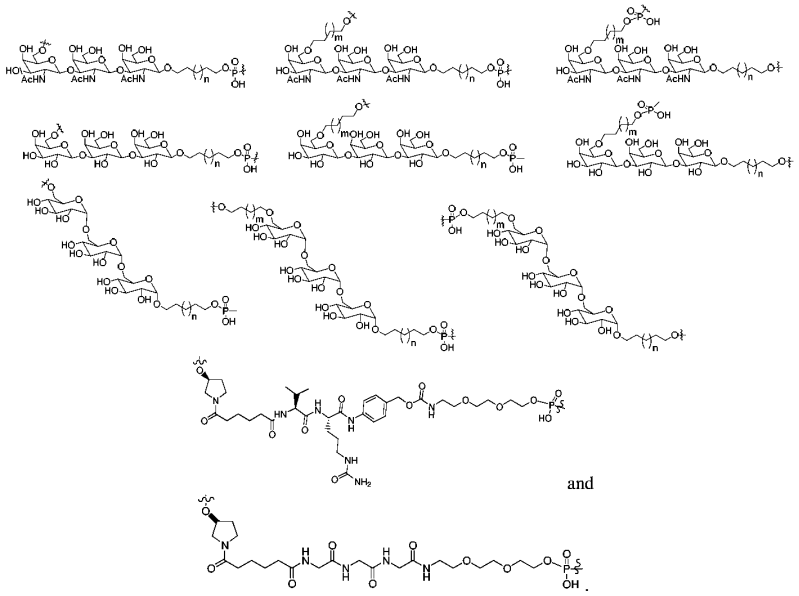
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and

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More discussion about the biocleavable linkers may be found in PCT application No. PCT/US18/14213, entitled "Endosomal Cleavable Linkers," filed on January 18, 2018, the entire contents of which are incorporated herein by reference.

1. Carriers

In certain embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the iRNA agent via a carrier that replaces one or more nucleotide(s).

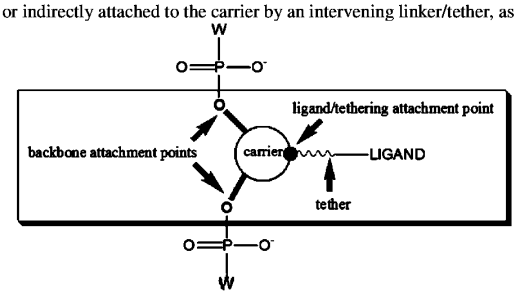
The carrier can be a cyclic group or an acyclic group. In one embodiment, the cyclic group is selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin. In one embodiment, the acyclic group is a moiety based on a serinol backbone or a diethanolamine backbone.

In some embodiments, the carrier replaces one or more nucleotide(s) in the internal position(s) of the dsRNA agent.

In other embodiments, the carrier replaces the nucleotides at the terminal end of the sense strand or antisense strand. In one embodiment, 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 embodiment, 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.

A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). The carrier can be a cyclic or acyclic moiety and include two "backbone attachment points" (*e.g.*, hydroxyl groups) and a ligand (*e.g.*, the lipophilic moiety). The one or more C_{22} hydrocarbon chains can be directly attached to the carrier or indirectly attached to the carrier by an intervening linker/tether, as described above.

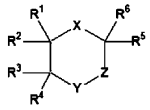


The ligand-conjugated monomer subunit may be the 5' or 3' terminal subunit of the iRNA molecule, *i.e.*, one of the two "W" groups may be a hydroxyl group, and the other "W" group may be a chain of two or more unmodified or modified ribonucleotides. Alternatively, the ligand-conjugated monomer subunit may occupy an internal position, and both "W" groups may be one or more unmodified or modified ribonucleotides. More than one ligand-conjugated monomer subunit may be present in an iRNA agent.

a. Sugar Replacement-Based Monomers, e.g., Ligand-Conjugated Monomers (Cyclic)

Cyclic sugar replacement-based monomers, *e.g.*, sugar replacement-based ligand-conjugated monomers, are also referred to herein as RRMS monomer compounds. The carriers may have the general formula (**LCM-2**) provided below (in that structure preferred backbone attachment points can be chosen from R^1 or R^2; R^3 or R^4; or R^9 and R^{10} if Y is CR^9R^{10} (two positions are chosen to give two backbone attachment points, *e.g.*, R^1 and R^4, or R^4 and R^9)). Preferred tethering attachment points include R^7; R^5 or R^6 when X is CH_2. The carriers are described below as an entity, which can be incorporated into a strand. Thus, it is understood that the structures also encompass the situations wherein one (in the case of a terminal position) or two (in the case of an internal position) of the attachment points, *e.g.*, R^1 or R^2; R^3 or R^4; or R^9 or R^{10} (when Y is CR^9R^{10}), is connected to the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone. *E.g.*, one of the above-named R groups can be $-CH_2-$, wherein one bond is connected to the carrier and one to a backbone atom, *e.g.*, a

linking oxygen or a central phosphorus atom.



(**LCM-2**)

wherein:

X is $N(CO)R^7$, NR^7 or CH_2;

Y is NR^8, O, S, CR^9R^{10};

Z is $CR^{11}R^{12}$ or absent;

Each of R^1, R^2, R^3, R^4, R^9, and R^{10} is, independently, H, OR^a, or $(CH_2)_nOR^b$, provided that at least two of R^1, R^2, R^3, R^4, R^9, and R^{10} are OR^a and/or $(CH_2)_nOR^b$;

Each of R^5, R^6, R^{11}, and R^{12} is, independently, a ligand, H, C_1-C_6 alkyl optionally substituted with 1-3 R^{13}, or $C(O)NHR^7$; or R^5 and R^{11} together are C_3-C_8 cycloalkyl optionally substituted with R^{14};

R^7 can be a ligand, *e.g.*, R^7 can be R^d , or R^7 can be a ligand tethered indirectly to the carrier, *e.g.*, through a tethering moiety, *e.g.*, C_1-C_{20} alkyl substituted with NR^cR^d; or C_1-C_{20} alkyl substituted with $NHC(O)R^d$;

R^8 is H or C_1-C_6 alkyl;

R^{13} is hydroxy, C_1-C_4 alkoxy, or halo;

R^{14} is NR^cR^7;

R^{15} is C_1-C_6 alkyl optionally substituted with cyano, or C_2-C_6 alkenyl;

R^{16} is C_1-C_{10} alkyl;

R^{17} is a liquid or solid phase support reagent;

L is $-C(O)(CH_2)_qC(O)-$, or $-C(O)(CH_2)_qS-$;

R^a is a protecting group, *e.g.*, CAr_3; (*e.g.*, a dimethoxytrityl group) or $Si(X^{5'})(X^{5''})(X^{5'''})$ in which $(X^{5'})$,$(X^{5''})$, and $(X^{5'''})$ are as described elsewhere.

R^b is $P(O)(O^-)H$, $P(OR^{15})N(R^{16})_2$ or $L\text{-}R^{17}$;

R^c is H or C_1-C_6 alkyl;

R^d is H or a ligand;

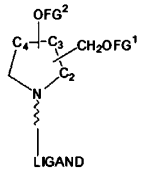
Each Ar is, independently, C_6-C_{10} aryl optionally substituted with C_1-C_4 alkoxy;

n is 1-4; and q is 0-4.

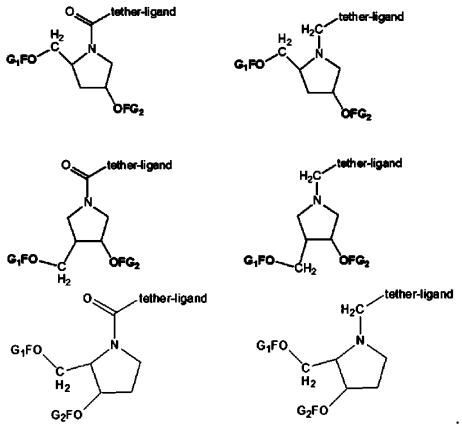
Exemplary carriers include those in which, *e.g.*, X is $N(CO)R^7$ or NR^7, Y is CR^9R^{10}, and Z is absent; or X is $N(CO)R^7$ or NR^7, Y is CR^9R^{10}, and Z is $CR^{11}R^{12}$; or X is $N(CO)R^7$ or NR^7, Y is NR^8, and Z is $CR^{11}R^{12}$; or X is $N(CO)R^7$ or NR^7, Y is O, and Z is $CR^{11}R^{12}$; or X is CH_2; Y is CR^9R^{10}; Z is $CR^{11}R^{12}$, and R^5 and R^{11} together form C_6 cycloalkyl (**H**, z = 2), or the indane ring system, *e.g.*, X is CH_2; Y is CR^9R^{10}; Z is $CR^{11}R^{12}$, and R^5 and R^{11} together form C_5 cycloalkyl (**H**, z = 1).

In certain embodiments, the carrier may be based on the pyrroline ring system or the 4-hydroxyproline ring system, *e.g.*, X is $N(CO)R^7$ or NR^7, Y is CR^9R^{10}, and Z is absent (**D**).

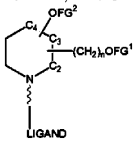
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D . OFG^1 is preferably attached to a primary carbon, *e.g.*, an exocyclic alkylene group, *e.g.*, a methylene group, connected to one of the carbons in the five-membered ring (-CH_2OFG^1 in **D**). OFG^2 is preferably attached directly to one of the carbons in the five-membered ring (-OFG^2 in **D**). For the pyrroline-based carriers, -CH_2OFG^1 may be attached to C-2 and OFG^2 may be attached to C-3; or -CH_2OFG^1 may be attached to C-3 and OFG^2 may be attached to C-4. In certain embodiments, CH_2OFG^1 and OFG^2 may be geminally substituted to one of the above-referenced carbons. For the 3-hydroxyproline-based carriers, -CH_2OFG^1 may be attached to C-2 and OFG^2 may be attached to C-4. The pyrroline- and 4-hydroxyproline-based monomers may therefore contain linkages (*e.g.*, carbon-carbon bonds) wherein bond rotation is restricted about that particular linkage, *e.g.* restriction resulting from the presence of a ring. Thus, CH_2OFG^1 and OFG^2 may be *cis* or *trans* with respect to one another in any of the pairings delineated above Accordingly, all *cis/trans* isomers are expressly included. 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 (*e.g.*, the centers bearing CH_2OFG^1 and OFG^2 can both have the R configuration; or both have the S configuration; or one center can have the R configuration and the other center can have the S configuration and *vice versa*). The tethering attachment point is preferably nitrogen. Preferred examples of carrier **D** include the following:



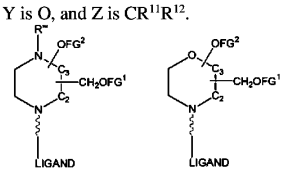
In certain embodiments, the carrier may be based on the piperidine ring system (**E**), *e.g.*, X is $N(CO)R^7$ or NR^7, Y is CR^9R^{10}, and Z is $CR^{11}R^{12}$.



E

OFG^1 is preferably attached to a primary carbon, *e.g.*, an exocyclic alkylene group, *e.g.*, a methylene group (n=1) or ethylene group (n=2), connected to one of the carbons in the six-membered ring [$-(CH_2)_nOFG^1$ in **E**]. OFG^2 is preferably attached directly to one of the carbons in the six-membered ring ($-OFG^2$ in **E**). $-(CH_2)_nOFG^1$ and OFG^2 may be disposed in a geminal manner on the ring, *i.e.*, both groups may be attached to the same carbon, *e.g.*, at C-2, C-3, or C-4. Alternatively, $-(CH_2)_nOFG^1$ and OFG^2 may be disposed in a vicinal manner on the ring, *i.e.*, both groups may be attached to adjacent ring carbon atoms, *e.g.*, $-(CH_2)_nOFG^1$ may be attached to C-2 and OFG^2 may be attached to C-3; $-(CH_2)_nOFG^1$ may be attached to C-3 and OFG^2 may be attached to C-2; $-(CH_2)_nOFG^1$ may be attached to C-3 and OFG^2 may be attached to C-4; or $-(CH_2)_nOFG^1$ may be attached to C-4 and OFG^2 may be attached to C-3. The piperidine-based monomers may therefore contain linkages (*e.g.*, carbon-carbon bonds) wherein bond rotation is restricted about that particular linkage, *e.g.* restriction resulting from the presence of a ring. Thus, $-(CH_2)_nOFG^1$ and OFG^2 may be *cis* or *trans* with respect to one another in any of the pairings delineated above. Accordingly, all *cis/trans* isomers are expressly included. 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 (*e.g.*, the centers bearing CH_2OFG^1 and OFG^2 can both have the R configuration; or both have the S configuration; or one center can have the R configuration and the other center can have the S configuration and *vice versa*). The tethering attachment point is preferably nitrogen.

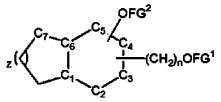
In certain embodiments, the carrier may be based on the piperazine ring system (**F**), *e.g.*, X is $N(CO)R^7$ or NR^7, Y is NR^8, and Z is $CR^{11}R^{12}$, or the morpholine ring system (**G**), *e.g.*, X is $N(CO)R^7$ or NR^7, Y is O, and Z is $CR^{11}R^{12}$.



OFG^1 is preferably attached to a primary carbon, *e.g.*, an exocyclic alkylene group, *e.g.*, a methylene group, connected to one of the carbons in the six-membered ring ($-CH_2OFG^1$ in **F** or **G**). OFG^2 is preferably attached directly to one of the carbons in the six-membered rings ($-OFG^2$ in **F** or

G). For both **F** and **G**, -CH_2OFG^1 may be attached to C-2 and OFG^2 may be attached to C-3; or *vice versa.* In certain embodiments, CH_2OFG^1 and OFG^2 may be geminally substituted to one of the above-referenced carbons.The piperazine- and morpholine-based monomers may therefore contain linkages (*e.g.*, carbon-carbon bonds) wherein bond rotation is restricted about that particular linkage, *e.g.* restriction resulting from the presence of a ring. Thus, CH_2OFG^1 and OFG^2 may be *cis* or *trans* with respect to one another in any of the pairings delineated above. Accordingly, all *cis/trans* isomers are expressly included. 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 (*e.g.*, the centers bearing CH_2OFG^1 and OFG^2 can both have the R configuration; or both have the S configuration; or one center can have the R configuration and the other center can have the S configuration and *vice versa*). R''' can be, *e.g.*, C_1-C_6 alkyl, preferably CH_3. The tethering attachment point is preferably nitrogen in both **F** and **G**.

In certain embodiments, the carrier may be based on the decalin ring system, *e.g.*, X is CH_2; Y is CR^9R^{10}; Z is $CR^{11}R^{12}$, and R^5 and R^{11} together form C_6 cycloalkyl (**H**, z = 2), or the indane ring system, *e.g.*, X is CH_2; Y is CR^9R^{10}; Z is $CR^{11}R^{12}$, and R^5 and R^{11} together form C_5 cycloalkyl (**H**, z = 1).

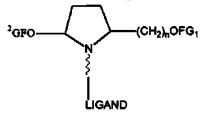


H

OFG^1 is preferably attached to a primary carbon, *e.g.*, an exocyclic methylene group (n=1) or ethylene group (n=2) connected to one of C-2, C-3, C-4, or C-5 [-$(CH_2)_nOFG^1$ in **H**]. OFG^2 is preferably attached directly to one of C-2, C-3, C-4, or C-5 (-OFG^2 in **H**). -$(CH_2)_nOFG^1$ and OFG^2 may be disposed in a geminal manner on the ring, *i.e.*, both groups may be attached to the same carbon, *e.g.*, at C-2, C-3, C-4, or C-5. Alternatively, -$(CH_2)_nOFG^1$ and OFG^2 may be disposed in a vicinal manner on the ring, *i.e.*, both groups may be attached to adjacent ring carbon atoms, *e.g.*, -$(CH_2)_nOFG^1$ may be attached to C-2 and OFG^2 may be attached to C-3; -$(CH_2)_nOFG^1$ may be attached to C-3 and OFG^2 may be attached to C-2; -$(CH_2)_nOFG^1$ may be attached to C-3 and OFG^2 may be attached to C-4; or -$(CH_2)_nOFG^1$ may be attached to C-4 and OFG^2 may be attached to C-3; -$(CH_2)_nOFG^1$ may be attached to C-4 and OFG^2 may be attached to C-5; or -$(CH_2)_nOFG^1$ may be attached to C-5 and OFG^2 may be attached to C-4. The decalin or indane-based monomers may therefore contain linkages (*e.g.*, carbon-carbon bonds) wherein bond rotation is restricted about that particular linkage, *e.g.* restriction resulting from the presence of a ring. Thus, -$(CH_2)_nOFG^1$ and OFG^2 may be *cis* or *trans* with respect to one another in any of the pairings delineated above. Accordingly, all *cis/trans* isomers are expressly included. 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 (*e.g.*, the centers bearing CH_2OFG^1 and OFG^2 can both have the R configuration; or both have the S configuration; or one center can have the R configuration and the other center can have the S configuration and *vice versa*). In a preferred embodiment, the substituents at C-1 and C-6 are *trans* with respect to one another. The tethering attachment point is preferably C-6 or C-7.

Other carriers may include those based on 3-hydroxyproline (**J**).



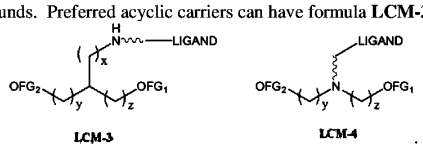
J

Thus, $-(CH_2)_nOFG^1$ and OFG^2 may be *cis* or *trans* with respect to one another. Accordingly, all *cis/trans* isomers are expressly included. 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 (*e.g.*, the centers bearing CH_2OFG^1 and OFG^2 can both have the R configuration; or both have the S configuration; or one center can have the R configuration and the other center can have the S configuration and *vice versa*). The tethering attachment point is preferably nitrogen.

Details about more representative cyclic, sugar replacement-based carriers can be found in U.S. Patent Nos. 7,745,608 and 8,017,762, which are herein incorporated by reference in their entireties.

b. Sugar Replacement-Based Monomers (Acyclic)

Acyclic sugar replacement-based monomers, *e.g.*, sugar replacement-based ligand-conjugated monomers, are also referred to herein as ribose replacement monomer subunit (RRMS) monomer compounds. Preferred acyclic carriers can have formula **LCM-3** or **LCM-4**:



LCM-3 **LCM-4**

In some embodiments, each of x, y, and z can be, independently of one another, 0, 1, 2, or 3. In formula **LCM-3**, when y and z are different, then the tertiary carbon can have either the R or S configuration. In preferred embodiments, x is zero and y and z are each 1 in formula **LCM-3** (*e.g.*, based on serinol), and y and z are each 1 in formula **LCM-3**. Each of formula **LCM-3** or **LCM-4** below can optionally be substituted, *e.g.*, with hydroxy, alkoxy, perhaloalkyl.

Details about more representative acyclic, sugar replacement-based carriers can be found in U.S. Patent Nos. 7,745,608 and 8,017,762, which are herein incorporated by reference in their entireties.

The one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand. Internal positions of a strand refers to the nucleotide on any position of the strand, except the terminal position from the 3' end and 5' end of the strand (*e.g.*, excluding 2 positions: position 1 counting from the 3' end and position 1 counting from the 5' end).

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand, which include all positions except the terminal two positions from each end of the strand (*e.g.*, excluding 4 positions: positions 1 and 2 counting from the 3' end and positions 1 and 2 counting from the 5' end). In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand, which include all positions except the terminal three positions from each end of the strand (*e.g.*, excluding 6 positions: positions 1, 2, and 3 counting from the 3' end and positions 1, 2, and 3 counting from the 5' end).

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand, except the cleavage site region of the sense strand, for instance, the one or more C_{22} hydrocarbon chains is not conjugated to positions 9-12 counting from the 5'-end of the sense strand, for example, the one or more C_{22} hydrocarbon chains is not conjugated to positions 9-11 counting from the 5'-end of the sense strand. Alternatively, the internal positions exclude positions 11-13 counting from the 3'-end of the sense strand.

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand, which exclude the cleavage site region of the antisense strand. For instance, the internal positions exclude positions 12-14 counting from the 5'-end of the antisense strand.

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more internal positions on at least one strand, which exclude positions 11-13 on the sense strand, counting from the 3'-end, and positions 12-14 on the antisense strand, counting from the 5'-end.

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'end of each strand.

In one embodiment, the one or more C_{22} hydrocarbon chains is conjugated to position 6 on the sense strand, counting from the 5'end of each strand.

In some embodiments, the one or more C_{22} hydrocarbon chains is conjugated to a nucleobase, sugar moiety, or internucleosidic phosphate linkage of the dsRNA agent.

IV. Additional Modifications for the RNAi Agents of the Invention

In one embodiment, the RNAi agent of the disclosure comprising one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand, does not comprise chemical modifications known in the art and described herein, in the remaining positions of the sense and anti-sense strands.

In some embodiments, the dsRNA agents of the invention comprising one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand, comprise at least one additional nucleic acid modification described herein. For example, at least one modification selected from the group consisting of modified internucleoside linkage, modified nucleobase, modified sugar, and any combinations thereof. Without limitations, such a modification can be present anywhere in the dsRNA agent of the invention. For example, the modification can be present in one of the RNA molecules.

Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); base modifications, *e.g.*, replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (*e.g.*, at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of RNAi agents useful in the embodiments described herein include, but are not limited to, RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified RNAi agent will have a phosphorus atom in its internucleoside backbone.

A. Nucleobase Modifications

The naturally occurring base portion of a nucleoside is typically a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. For those nucleosides that include a pentofuranosyl sugar, a phosphate group can be linked to the 2′, 3′ or 5′ hydroxyl moiety of the sugar. In forming oligonucleotides, those phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The naturally occurring linkage or backbone of RNA and of DNA is a 3′ to 5′ phosphodiester linkage.

In addition to "unmodified" or "natural" nucleobases such as the purine nucleobases adenine (A) and guanine (G), and the pyrimidine nucleobases thymine (T), cytosine (C) and uracil (U), many modified nucleobases or nucleobase mimetics known to those skilled in the art are amenable with the

compounds described herein. The unmodified or natural nucleobases can be modified or replaced to provide iRNAs having improved properties. For example, nuclease resistant oligonucleotides can be prepared with these bases or with synthetic and natural nucleobases (*e.g.*, inosine, xanthine, hypoxanthine, nubularine, isoguanisine, or tubercidine) and any one of the oligomer modifications described herein. Alternatively, substituted or modified analogs of any of the above bases and "universal bases" can be employed. When a natural base is replaced by a non-natural and/or universal base, the nucleotide is said to comprise a modified nucleobase and/or a nucleobase modification herein. Modified nucleobase and/or nucleobase modifications also include natural, non-natural and universal bases, which comprise conjugated moieties, *e.g.* a ligand described herein. Preferred conjugate moieties for conjugation with nucleobases include cationic amino groups which can be conjugated to the nucleobase via an appropriate alkyl, alkenyl or a linker with an amide linkage.

An oligomeric compound described herein can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Exemplary modified nucleobases include, but are not limited to, other synthetic and natural nucleobases such as inosine, xanthine, hypoxanthine, nubularine, isoguanisine, tubercidine, 2-(halo)adenine, 2-(alkyl)adenine, 2-(propyl)adenine, 2-(amino)adenine, 2-(aminoalkyll)adenine, 2-(aminopropyl)adenine, 2-(methylthio)-N^6-(isopentenyl)adenine, 6-(alkyl)adenine, 6-(methyl)adenine, 7-(deaza)adenine, 8-(alkenyl)adenine, 8-(alkyl)adenine, 8-(alkynyl)adenine, 8-(amino)adenine, 8-(halo)adenine, 8-(hydroxyl)adenine, 8-(thioalkyl)adenine, 8-(thiol)adenine, N^6-(isopentyl)adenine, N^6-(methyl)adenine, N^6, N^6-(dimethyl)adenine, 2-(alkyl)guanine,2-(propyl)guanine, 6-(alkyl)guanine, 6-(methyl)guanine, 7-(alkyl)guanine, 7-(methyl)guanine, 7-(deaza)guanine, 8-(alkyl)guanine, 8-(alkenyl)guanine, 8-(alkynyl)guanine, 8-(amino)guanine, 8-(halo)guanine, 8-(hydroxyl)guanine, 8-(thioalkyl)guanine, 8-(thiol)guanine, N-(methyl)guanine, 2-(thio)cytosine, 3-(deaza)-5-(aza)cytosine, 3-(alkyl)cytosine, 3-(methyl)cytosine, 5-(alkyl)cytosine, 5-(alkynyl)cytosine, 5-(halo)cytosine, 5-(methyl)cytosine, 5-(propynyl)cytosine, 5-(propynyl)cytosine, 5-(trifluoromethyl)cytosine, 6-(azo)cytosine, N^4-(acetyl)cytosine, 3-(3-amino-3-carboxypropyl)uracil, 2-(thio)uracil, 5-(methyl)-2-(thio)uracil, 5-(methylaminomethyl)-2-(thio)uracil, 4-(thio)uracil, 5-(methyl)-4-(thio)uracil, 5-(methylaminomethyl)-4-(thio)uracil, 5-(methyl)-2,4-(dithio)uracil, 5-(methylaminomethyl)-2,4-(dithio)uracil, 5-(2-aminopropyl)uracil, 5-(alkyl)uracil, 5-(alkynyl)uracil, 5-(allylamino)uracil, 5-(aminoallyl)uracil, 5-(aminoalkyl)uracil, 5-(guanidiniumalkyl)uracil, 5-(1,3-diazole-1-alkyl)uracil, 5-(cyanoalkyl)uracil, 5-(dialkylaminoalkyl)uracil, 5-(dimethylaminoalkyl)uracil, 5-(halo)uracil, 5-(methoxy)uracil, uracil-5-oxyacetic acid, 5-(methoxycarbonylmethyl)-2-(thio)uracil, 5-(methoxycarbonyl-methyl)uracil, 5-(propynyl)uracil, 5-(propynyl)uracil, 5-(trifluoromethyl)uracil, 6-(azo)uracil, dihydrouracil, N^3-(methyl)uracil, 5-uracil (*i.e.*, pseudouracil), 2-(thio)pseudouracil,4-(thio)pseudouracil,2,4-(dithio)psuedouracil,5-(alkyl)pseudouracil, 5-(methyl)pseudouracil, 5-(alkyl)-2-(thio)pseudouracil, 5-(methyl)-2-(thio)pseudouracil, 5-(alkyl)-4-(thio)pseudouracil, 5-(methyl)-4-(thio)pseudouracil, 5-

(alkyl)-2,4-(dithio)pseudouracil, 5-(methyl)-2,4-(dithio)pseudouracil, 1-substituted pseudouracil, 1-substituted 2(thio)-pseudouracil, 1-substituted 4-(thio)pseudouracil, 1-substituted 2,4-(dithio)pseudouracil, 1-(aminocarbonylethylenyl)-pseudouracil, 1-(aminocarbonylethylenyl)-2(thio)-pseudouracil, 1-(aminocarbonylethylenyl)-4-(thio)pseudouracil, 1-(aminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-pseudouracil, 1-(aminoalkylamino-carbonylethylenyl)-2(thio)-pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-4-(thio)pseudouracil, 1-(aminoalkylaminocarbonylethylenyl)-2,4-(dithio)pseudouracil, 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-substituted 1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-substituted 1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(aminoalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(aminoalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1,3-(diaza)-2-(oxo)-phenoxazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenoxazin-1-yl, 7-(guanidiniumalkyl-hydroxy)-1,3-(diaza)-2-(oxo)-phenthiazin-1-yl, 7-(guanidiniumalkylhydroxy)-1-(aza)-2-(thio)-3-(aza)-phenthiazin-1-yl, 1,3,5-(triaza)-2,6-(dioxa)-naphthalene, inosine, xanthine, hypoxanthine, nubularine, tubercidine, isoguanisine, inosinyl, 2-aza-inosinyl, 7-deaza-inosinyl, nitroimidazolyl, nitropyrazolyl, nitrobenzimidazolyl, nitroindazolyl, aminoindolyl, pyrrolopyrimidinyl, 3-(methyl)isocarbostyrilyl, 5-(methyl)isocarbostyrilyl, 3-(methyl)-7-(propynyl)isocarbostyrilyl, 7-(aza)indolyl, 6-(methyl)-7-(aza)indolyl, imidizopyridinyl, 9-(methyl)-imidizopyridinyl, pyrrolopyrizinyl, isocarbostyrilyl, 7-(propynyl)isocarbostyrilyl, propynyl-7-(aza)indolyl, 2,4,5-(trimethyl)phenyl, 4-(methyl)indolyl, 4,6-(dimethyl)indolyl, phenyl, napthalenyl, anthracenyl, phenanthracenyl, pyrenyl, stilbenyl, tetracenyl, pentacenyl, difluorotolyl, 4-(fluoro)-6-(methyl)benzimidazole, 4-(methyl)benzimidazole, 6-(azo)thymine, 2-pyridinone, 5-nitroindole, 3-nitropyrrole, 6-(aza)pyrimidine, 2-(amino)purine, 2,6-(diamino)purine, 5-substituted pyrimidines, N^2-substituted purines, N^6-substituted purines, O^6-substituted purines, substituted 1,2,4-triazoles, pyrrolo-pyrimidin-2-on-3-yl, 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, *para*-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, *ortho*-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-*ortho*-substituted-6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, *para*-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, *ortho*-(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, bis-*ortho*--(aminoalkylhydroxy)- 6-phenyl-pyrrolo-pyrimidin-2-on-3-yl, pyridopyrimidin-3-yl, 2-oxo-7-amino-pyridopyrimidin-3-yl, 2-oxo-pyridopyrimidine-3-yl, or any O-alkylated or N-alkylated derivatives thereof. Alternatively, substituted or modified analogs of any of the above bases and "universal bases" can be employed.

As used herein, a universal nucleobase is any nucleobase that can base pair with all of the four naturally occurring nucleobases without substantially affecting the melting behavior, recognition by intracellular enzymes or activity of the iRNA duplex. Some exemplary universal nucleobases include, but are not limited to, 2,4-difluorotoluene, nitropyrrolyl, nitroindolyl, 8-aza-7-deazaadenine,

4-fluoro-6-methylbenzimidazle, 4-methylbenzimidazle, 3-methyl isocarbostyrilyl, 5- methyl isocarbostyrilyl, 3-methyl-7-propynyl isocarbostyrilyl, 7-azaindolyl, 6-methyl-7-azaindolyl, imidizopyridinyl, 9-methyl-imidizopyridinyl, pyrrolopyrizinyl, isocarbostyrilyl, 7-propynyl isocarbostyrilyl, propynyl-7-azaindolyl, 2,4,5-trimethylphenyl, 4-methylinolyl, 4,6-dimethylindolyl, phenyl, napthalenyl, anthracenyl, phenanthracenyl, pyrenyl, stilbenyl, tetracenyl, pentacenyl, and structural derivatives thereof (see for example, Loakes, 2001, *Nucleic Acids Research*, 29, 2437-2447).

Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808; those disclosed in International Application No. PCT/US09/038425, filed March 26, 2009; those disclosed in the Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990; those disclosed by English *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613; those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijin, P.Ed. Wiley-VCH, 2008; and those disclosed by Sanghvi, Y.S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993. Contents of all of the above are herein incorporated by reference.

In certain embodiments, a modified nucleobase is a nucleobase that is fairly similar in structure to the parent nucleobase, such as for example a 7-deaza purine, a 5-methyl cytosine, or a G-clamp. In certain embodiments, nucleobase mimetic include more complicated structures, such as for example a tricyclic phenoxazine nucleobase mimetic. Methods for preparation of the above noted modified nucleobases are well known to those skilled in the art.

B. Sugar Modifications

DsRNA agent of the inventions provided herein can comprise one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more) monomer, including a nucleoside or nucleotide, having a modified sugar moiety. For example, the furanosyl sugar ring of a nucleoside can be modified in a number of ways including, but not limited to, addition of a substituent group, bridging of two non-geminal ring atoms to form a locked nucleic acid or bicyclic nucleic acid. In certain embodiments, oligomeric compounds comprise one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more) monomers that are LNA.

In some embodiments of a locked nucleic acid, the 2′ position of furnaosyl is connected to the 4' position by a linker selected independently from $-[C(R1)(R2)]_n-$, $-[C(R1)(R2)]_n-O-$, $-[C(R1)(R2)]_n-N(R1)-$, $-[C(R1)(R2)]_n-N(R1)-O-$, $—[C(R1R2)]_n-O-N(R1)—$, $-C(R1)=C(R2)-O-$, $-C(R1)=N-$, $-C(R1)=N-O-$, $—C(=NR1)-$, $—C(=NR1)-O-$, $—C(=O)—$, $—C(=O)O—$, $—C(=S)—$, $—C(=S)O—$, $—C(=S)S—$, $—O—$, $—Si(R1)2-$, $—S(=O)_x-$ and $—N(R1)-$;

wherein:

x is 0, 1, or 2;

n is 1, 2, 3, or 4;

each R1 and R2 is, independently, H, a protecting group, hydroxyl, C1-C12 alkyl, substituted C1-C12 alkyl, C2-C12 alkenyl, substituted C2-C12 alkenyl, C2-C12 alkynyl, substituted C2-C12 alkynyl, C5-C20 aryl, substituted C5-C20 aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C5-C7 alicyclic radical, substituted C5-C7 alicyclic radical, halogen, OJ1, NJ1J2, SJ1, N3, COOJ1, acyl (C(=O)—H), substituted acyl, CN, sulfonyl (S(=O)2-J1), or sulfoxyl (S(=O)-J1); and

each J1 and J2 is, independently, H, C1-C12 alkyl, substituted C1-C12 alkyl, C2-C12 alkenyl, substituted C2-C12 alkenyl, C2-C12 alkynyl, substituted C2-C12 alkynyl, C5-C20 aryl, substituted C5-C20 aryl, acyl (C(=O)—H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C1-C12 aminoalkyl, substituted C1-C12 aminoalkyl or a protecting group.

In some embodiments, each of the linkers of the LNA compounds is, independently, —[C(R1)(R2)]n-, —[C(R1)(R2)]n-O—, —C(R1R2)-N(R1)-O— or —C(R1R2)-O—N(R1)-. In another embodiment, each of said linkers is, independently, 4′-CH_2-2′, 4′-$(CH_2)_2$-2′, 4′-$(CH_2)_3$-2′, 4′-CH_2-O-2′, 4′-$(CH_2)_2$-O-2′, 4′-CH_2-O—N(R1)-2′ and 4′-CH_2-N(R1)-O-2′- wherein each R1 is, independently, H, a protecting group or C1-C12 alkyl.

Certain LNA's have been prepared and disclosed in the patent literature as well as in scientific literature (Singh et al., Chem. Commun., 1998, 4, 455-456; Koshkin et al., Tetrahedron, 1998, 54, 3607-3630; Wahlestedt et al., Proc. Natl. Acad. Sci. U.S.A., 2000, 97, 5633-5638; Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222; WO 94/14226; WO 2005/021570; Singh et al., J. Org. Chem., 1998, 63, 10035-10039; Examples of issued US patents and published applications that disclose LNA s include, for example, U.S. Pat. Nos. 7,053,207; 6,268,490; 6,770,748; 6,794,499; 7,034,133; and 6,525,191; and U.S. Pre-Grant Publication Nos. 2004-0171570; 2004-0219565; 2004-0014959; 2003-0207841; 2004-0143114; and 20030082807.

Also provided herein are LNAs in which the 2′-hydroxyl group of the ribosyl sugar ring is linked to the 4′ carbon atom of the sugar ring thereby forming a methyleneoxy (4′-CH_2-O-2′) linkage to form the bicyclic sugar moiety (reviewed in Elayadi et al., Curr. Opinion Invens. Drugs, 2001, 2, 558-561; Braasch et al., Chem. Biol., 2001, 8 1-7; and Orum et al., Curr. Opinion Mol. Ther., 2001, 3, 239-243; see also U.S. Pat. Nos. 6,268,490 and 6,670,461). The linkage can be a methylene (—CH_2-) group bridging the 2′ oxygen atom and the 4′ carbon atom, for which the term methyleneoxy (4′-CH_2-O-2′) LNA is used for the bicyclic moiety; in the case of an ethylene group in this position, the term ethyleneoxy (4′-CH_2CH_2-O-2′) LNA is used (Singh et al., Chem. Commun., 1998, 4, 455-456: Morita et al., Bioorganic Medicinal Chemistry, 2003, 11, 2211-2226). Methyleneoxy (4′-CH_2-O-2′) LNA and other bicyclic sugar analogs display very high duplex thermal stabilities with complementary DNA and RNA (Tm=+3 to +10° C.), stability towards 3′-exonucleolytic degradation and good solubility properties. Potent and nontoxic antisense oligonucleotides comprising BNAs have been described (Wahlestedt et al., Proc. Natl. Acad. Sci. U.S.A., 2000, 97, 5633-5638).

An isomer of methyleneoxy (4′-CH_2-O-2′) LNA that has also been discussed is alpha-L-methyleneoxy (4′-CH_2-O-2′) LNA which has been shown to have superior stability against a 3′-

exonuclease. The alpha-L-methyleneoxy (4′-CH_2-O-2′) LNA's were incorporated into antisense gapmers and chimeras that showed potent antisense activity (Frieden et al., Nucleic Acids Research, 2003, 21, 6365-6372).

The synthesis and preparation of the methyleneoxy (4′-CH_2-O-2′) LNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). BNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226.

Analogs of methyleneoxy (4′-CH_2-O-2′) LNA, phosphorothioate-methyleneoxy (4′-CH_2-O-2′) LNA and 2′-thio-LNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (Wengel et al., WO 99/14226). Furthermore, synthesis of 2′-amino-LNA, a novel comformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2′-Amino- and 2′-methylamino-LNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

Modified sugar moieties are well known and can be used to alter, typically increase, the affinity of the antisense compound for its target and/or increase nuclease resistance. A representative list of preferred modified sugars includes but is not limited to bicyclic modified sugars, including methyleneoxy (4′-CH_2-O-2′) LNA and ethyleneoxy (4′-$(CH_2)_2$-O-2′ bridge) ENA; substituted sugars, especially 2′-substituted sugars having a 2′-F, 2′-OCH_3 or a 2′-O$(CH_2)_2$-OCH_3 substituent group; and 4′-thio modified sugars. Sugars can also be replaced with sugar mimetic groups among others. Methods for the preparations of modified sugars are well known to those skilled in the art. Some representative patents and publications that teach the preparation of such modified sugars include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; 5,792,747; 5,700,920; 6,531,584; and 6,600,032; and WO 2005/121371.

Examples of "oxy"-2′ hydroxyl group modifications include alkoxy or aryloxy (OR, *e.g.*, R = H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar); polyethyleneglycols (PEG), $O(CH_2CH_2O)_nCH_2CH_2OR$, n =1-50; "locked" nucleic acids (LNA) in which the furanose portion of the nucleoside includes a bridge connecting two carbon atoms on the furanose ring, thereby forming a bicyclic ring system; O-AMINE or O-$(CH_2)_n$AMINE (n = 1-10, AMINE = NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, ethylene diamine or polyamino); and O-$CH_2CH_2(NCH_2CH_2NMe_2)_2$.

"Deoxy" modifications include hydrogen (*i.e.* deoxyribose sugars, which are of particular relevance to the single-strand overhangs); halo (*e.g.*, fluoro); amino (*e.g.* NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); $NH(CH_2CH_2NH)_nCH_2CH_2$-AMINE (AMINE = NH_2; alkylamino, dialkylamino, heterocyclyl,

arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino); -NHC(O)R (R = alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar); cyano; mercapto; alkyl-thio-alkyl; thioalkoxy; thioalkyl; alkyl; cycloalkyl; aryl; alkenyl and alkynyl, which can be optionally substituted with *e.g.*, an amino functionality.

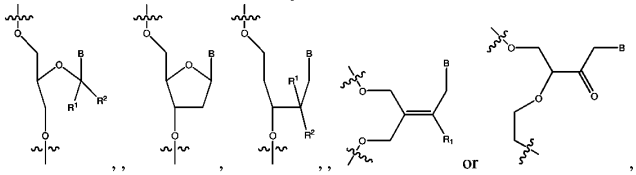
Other suitable 2'-modifications, *e.g.*, modified MOE, are described in U.S. Patent Application PublicationNo. 20130130378, contents of which are herein incorporated by reference.

A modification at the 2' position can be present in the arabinose configuration The term "arabinose configuration" refers to the placement of a substituent on the C2' of ribose in the same configuration as the 2'-OH is in the arabinose.

The sugar can comprise two different modifications at the same carbon in the sugar, *e.g.*, *gem* modification. The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, an oligomeric compound can include one or more monomers containing *e.g.*, arabinose, as the sugar. The monomer can have an alpha linkage at the 1' position on the sugar, *e.g.*, alpha-nucleosides. The monomer can also have the opposite configuration at the 4'-position, *e.g.*, C5' and H4' or substituents replacing them are interchanged with each other. When the C5' and H4' or substituents replacing them are interchanged with each other, the sugar is said to be modified at the 4' position.

DsRNA agent of the inventions disclosed herein can also include abasic sugars, *i.e.*, a sugar which lack a nucleobase at C-1′ or has other chemical groups in place of a nucleobase at C1'. See for example U.S. Pat. No. 5,998,203, content of which is herein incorporated in its entirety. These abasic sugars can also be further containing modifications at one or more of the constituent sugar atoms. DsRNA agent of the inventions can also contain one or more sugars that are the L isomer, *e.g.* L-nucleosides. Modification to the sugar group can also include replacement of the 4'-O with a sulfur, optionally substituted nitrogen or CH_2 group. In some embodiments, linkage between C1' and nucleobase is in α configuration.

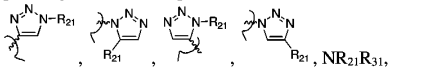
Sugar modifications can also include acyclic nucleotides, wherein a C-C bonds between ribose carbons (*e.g.*, C1'-C2', C2'-C3', C3'-C4', C4'-O4', C1'-O4') is absent and/or at least one of ribose carbons or oxygen (*e.g.*, C1', C2', C3', C4' or O4') are independently or in combination absent from the nucleotide. In some embodiments, acyclic nucleotide is



, , , , , or , wherein B is a modified or unmodified nucleobase, R_1 and R_2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar).

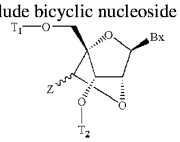
In some embodiments, sugar modifications are selected from the group consisting of 2'-H, 2'-*O*-Me (2'-*O*-methyl), 2'-*O*-MOE (2'-*O*-methoxyethyl), 2'-F, 2'-*O*-[2-(methylamino)-2-oxoethyl] (2'-*O*-NMA), 2'-*S*-methyl, 2'-O-CH_2-(4'-C) (LNA), 2'-O-CH_2CH_2-(4'-C) (ENA), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE) and *gem* 2'-OMe/2'F with 2'-O-Me in the arabinose configuration.

It is to be understood that when a particular nucleotide is linked through its 2'-position to the next nucleotide, the sugar modifications described herein can be placed at the 3'-position of the sugar for that particular nucleotide, *e.g.*, the nucleotide that is linked through its 2' -position. A modification at the 3' position can be present in the xylose configuration The term "xylose configuration" refers to the placement of a substituent on the C3' of ribose in the same configuration as the 3'-OH is in the xylose sugar.

The hydrogen attached to C4' and/or C1' can be replaced by a straight- or branched-optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, wherein backbone of the alkyl, alkenyl and alkynyl can contain one or more of O, S, S(O), SO_2, N(R'), C(O), N(R')C(O)O, OC(O)N(R'), CH(Z'), phosphorous containing linkage, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted heterocyclic or optionally substituted cycloalkyl, where R' is hydrogen, acyl or optionally substituted aliphatic, Z' is selected from the group consisting of OR_{11}, COR_{11}, CO_2R_{11},  , $NR_{21}R_{31}$, $CONR_{21}R_{31}$, $CON(H)NR_{21}R_{31}$, $ONR_{21}R_{31}$, $CON(H)N{=}CR_{41}R_{51}$, $N(R_{21})C(=NR_{31})NR_{21}R_{31}$, $N(R_{21})C(O)NR_{21}R_{31}$, $N(R_{21})C(S)NR_{21}R_{31}$, $OC(O)NR_{21}R_{31}$, $SC(O)NR_{21}R_{31}$, $N(R_{21})C(S)OR_{11}$, $N(R_{21})C(O)OR_{11}$, $N(R_{21})C(O)SR_{11}$, $N(R_{21})N{=}CR_{41}R_{51}$, $ON{=}CR_{41}R_{51}$, SO_2R_{11}, SOR_{11}, SR_{11}, and substituted or unsubstituted heterocyclic; R_{21} and R_{31} for each occurrence are independently hydrogen, acyl, unsubstituted or substituted aliphatic, aryl, heteroaryl, heterocyclic, OR_{11}, COR_{11}, CO_2R_{11}, or $NR_{11}R_{11}$'; or R_{21} and R_{31}, taken together with the atoms to which they are attached, form a heterocyclic ring; R_{41} and R_{51} for each occurrence are independently hydrogen, acyl, unsubstituted or substituted aliphatic, aryl, heteroaryl, heterocyclic, OR_{11}, COR_{11}, or CO_2R_{11}, or $NR_{11}R_{11}$'; and R_{11} and R_{11}' are independently hydrogen, aliphatic, substituted aliphatic, aryl, heteroaryl, or heterocyclic. In some embodiments, the hydrogen attached to the C4' of the 5' terminal nucleotide is replaced.

In some embodiments, C4' and C5' together form an optionally substituted heterocyclic, preferably comprising at least one -PX(Y)-, wherein X is H, OH, OM, SH, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted alkylthio, optionally substituted alkylamino or optionally substituted dialkylamino, where M is independently for each occurrence an alki metal or transition metal with an overall charge of +1; and Y is O, S, or NR', where R' is hydrogen, optionally substituted aliphatic. Preferably this modification is at the 5 terminal of the iRNA.

In certain embodiments, LNA's include bicyclic nucleoside having the formula:



wherein:

Bx is a heterocyclic base moiety;

T_1 is H or a hydroxyl protecting group;

T_2 is H, a hydroxyl protecting group or a reactive phosphorus group;

Z is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, or substituted amide.

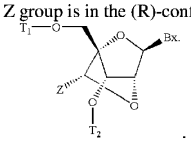
In some embodiments, each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 and CN, wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1.

In certain such embodiments, each of the substituted groups, is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, and NJ3C(=X)NJ1J2, wherein each J1, J2 and J3 is, independently, H, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl and X is O or NJ1.

In certain embodiments, the Z group is C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is independently OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1. In another embodiment, the Z group is C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is independently halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—), substituted alkoxy or azido.

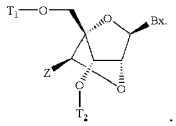
In certain embodiments, the Z group is —CH_2Xx, wherein Xx is OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1. In another embodiment, the Z group is —CH_2Xx, wherein Xx is halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—) or azido.

In certain such embodiments, the Z group is in the (R)-configuration:



In certain such embodiments, the Z group is in the (S)-configuration:

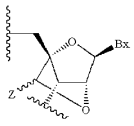
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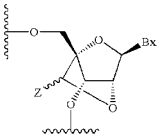
In certain embodiments, each T_1 and T_2 is a hydroxyl protecting group. A preferred list of hydroxyl protecting groups includes benzyl, benzoyl, 2,6-dichlorobenzyl, t-butyldimethylsilyl, t-butyldiphenylsilyl, mesylate, tosylate, dimethoxytrityl (DMT), 9-phenylxanthine-9-yl (Pixyl) and 9-(p-methoxyphenyl)xanthine-9-yl (MOX). In certain embodiments, T_1 is a hydroxyl protecting group selected from acetyl, benzyl, t-butyldimethylsilyl, t-butyldiphenylsilyl and dimethoxytrityl wherein a more preferred hydroxyl protecting group is T_1 is 4,4′-dimethoxytrityl.

In certain embodiments, T_2 is a reactive phosphorus group wherein preferred reactive phosphorus groups include diisopropylcyanoethoxy phosphoramidite and H-phosphonate. In certain embodiments T_1 is 4,4′-dimethoxytrityl and T_2 is diisopropylcyanoethoxy phosphoramidite.

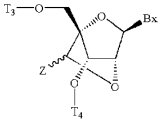
In certain embodiments, the compounds of the invention comprise at least one monomer of the formula:



or of the formula:



or of the formula:



wherein

Bx is a heterocyclic base moiety;

T_3 is H, a hydroxyl protecting group, a linked conjugate group or an internucleoside linking group attached to a nucleoside, a nucleotide, an oligonucleoside, an oligonucleotide, a monomeric subunit or an oligomeric compound;

T_4 is H, a hydroxyl protecting group, a linked conjugate group or an internucleoside linking group attached to a nucleoside, a nucleotide, an oligonucleoside, an oligonucleotide, a monomeric subunit or an oligomeric compound;

wherein at least one of T_3 and T_4 is an internucleoside linking group attached to a nucleoside, a nucleotide, an oligonucleoside, an oligonucleotide, a monomeric subunit or an oligomeric compound; and

Z is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, or substituted amide.

In some embodiments, each of the substituted groups, is, independently, mono or poly substituted with optionally protected substituent groups independently selected from halogen, oxo, hydroxyl, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 and CN, wherein each J1, J2 and J3 is, independently, H or C1-C6 alkyl, and X is O, S or NJ1.

In some embodiments, each of the substituted groups, is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, and NJ3C(=X)NJ1J2, wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O or NJ1.

In certain such embodiments, at least one Z is C_1-C_6 alkyl or substituted C_1-C_6 alkyl. In certain embodiments, each Z is, independently, C_1-C_6 alkyl or substituted C_1-C_6 alkyl. In certain embodiments, at least one Z is C_1-C_6 alkyl. In certain embodiments, each Z is, independently, C_1-C_6 alkyl. In certain embodiments, at least one Z is methyl. In certain embodiments, each Z is methyl. In certain embodiments, at least one Z is ethyl. In certain embodiments, each Z is ethyl. In certain embodiments, at least one Z is substituted C_1-C_6 alkyl. In certain embodiments, each Z is, independently, substituted C_1-C_6 alkyl. In certain embodiments, at least one Z is substituted methyl. In certain embodiments, each Z is substituted methyl. In certain embodiments, at least one Z is substituted ethyl. In certain embodiments, each Z is substituted ethyl.

In certain embodiments, at least one substituent group is C_1-C_6 alkoxy (*e.g.*, at least one Z is C_1-C_6 alkyl substituted with one or more C_1-C_6 alkoxy). In another embodiment, each substituent group is, independently, C_1-C_6 alkoxy (*e.g.*, each Z is, independently, C_1-C_6 alkyl substituted with one or more C_1-C_6 alkoxy).

In certain embodiments, at least one C_1-C_6 alkoxy substituent group is CH_3O— (*e.g.*, at least one Z is CH_3OCH_2-). In another embodiment, each C_1-C_6 alkoxy substituent group is CH_3O— (*e.g.*, each Z is CH_3OCH_2-).

In certain embodiments, at least one substituent group is halogen (*e.g.*, at least one Z is C_1-C_6 alkyl substituted with one or more halogen). In certain embodiments, each substituent group is, independently, halogen (*e.g.*, each Z is, independently, C_1-C_6 alkyl substituted with one or more halogen). In certain embodiments, at least one halogen substituent group is fluoro (*e.g.*, at least one Z is CH_2FCH_2-, CHF_2CH_2- or CF_3CH_2-). In certain embodiments, each halo substituent group is fluoro (*e.g.*, each Z is, independently, CH_2FCH_2-, CHF_2CH_2- or CF_3CH_2-).

In certain embodiments, at least one substituent group is hydroxyl (*e.g.*, at least one Z is C1-C6 alkyl substituted with one or more hydroxyl). In certain embodiments, each substituent group is, independently, hydroxyl (*e.g.*, each Z is, independently, C_1-C_6 alkyl substituted with one or more

hydroxyl). In certain embodiments, at least one Z is $HOCH_2$-. In another embodiment, each Z is $HOCH_2$-.

In certain embodiments, at least one Z is CH_3-, CH_3CH_2-, CH_2OCH_3-, CH_2F— or $HOCH_2$-. In certain embodiments, each Z is, independently, CH_3-, CH_3CH_2-, CH_2OCH_3-, CH_2F— or $HOCH_2$-.

In certain embodiments, at least one Z group is C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is, independently, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1. In another embodiment, at least one Z group is C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is, independently, halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—) or azido.

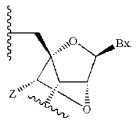
In certain embodiments, each Z group is, independently, C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is independently OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1. In another embodiment, each Z group is, independently, C_1-C_6 alkyl substituted with one or more Xx, wherein each Xx is independently halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—) or azido.

In certain embodiments, at least one Z group is —CH_2Xx, wherein Xx is OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1 In certain embodiments, at least one Z group is —CH_2Xx, wherein Xx is halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—) or azido.

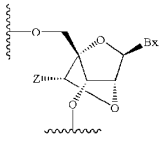
In certain embodiments, each Z group is, independently, —CH_2Xx, wherein each Xx is, independently, OJ1, NJ1J2, SJ1, N3, OC(=X)J1, OC(=X)NJ1J2, NJ3C(=X)NJ1J2 or CN; wherein each J1, J2 and J3 is, independently, H or C_1-C_6 alkyl, and X is O, S or NJ1. In another embodiment, each Z group is, independently, —CH_2Xx, wherein each Xx is, independently, halo (*e.g.*, fluoro), hydroxyl, alkoxy (*e.g.*, CH_3O—) or azido.

In certain embodiments, at least one Z is CH_3-. In another embodiment, each Z is, CH_3-.

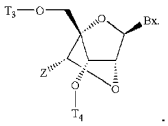
In certain embodiments, the Z group of at least one monomer is in the (R)— configuration represented by the formula:



or the formula:

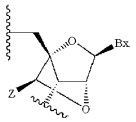


or the formula:

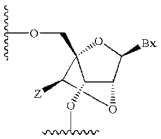


IN certain embodiments, the Z group of each monomer of the formula is in the (R)— configuration.

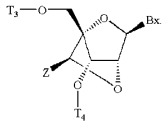
In certain embodiments, the Z group of at least one monomer is in the (S)— configuration represented by the formula:



or the formula:



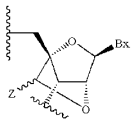
or the formula:



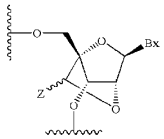
In certain embodiments, the Z group of each monomer of the formula is in the (S)— configuration.

In certain embodiments, T_3 is H or a hydroxyl protecting group. In certain embodiments, T_4 is H or a hydroxyl protecting group. In a further embodiment T_3 is an internucleoside linking group attached to a nucleoside, a nucleotide or a monomeric subunit. In certain embodiments, T_4 is an internucleoside linking group attached to a nucleoside, a nucleotide or a monomeric subunit. In certain embodiments, T_3 is an internucleoside linking group attached to an oligonucleoside or an oligonucleotide. In certain embodiments, T_4 is an internucleoside linking group attached to an oligonucleoside or an oligonucleotide. In certain embodiments, T_3 is an internucleoside linking group attached to an oligomeric compound. In certain embodiments, T_4 is an internucleoside linking group attached to an oligomeric compound. In certain embodiments, at least one of T_3 and T_4 comprises an internucleoside linking group selected from phosphodiester or phosphorothioate.

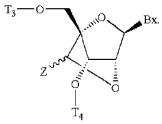
In certain embodiments, dsRNA agent of the invention comprise at least one region of at least two contiguous monomers of the formula:



or of the formula:



or of the formula:

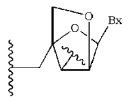


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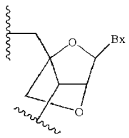
In certain such embodiments, LNAs include, but are not limited to, (A) α-L-Methyleneoxy (4′-CH_2-O-2′) LNA, (B) β-D-Methyleneoxy (4′-CH_2-O-2′) LNA, (C) Ethyleneoxy (4′-$(CH_2)_2$-O-2′) LNA, (D) Aminooxy (4′-CH_2-O—N(R)-2′) LNA and (E) Oxyamino (4′-CH_2-N(R)—O-2′) LNA, as depicted below:

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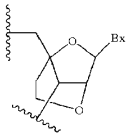
(A)



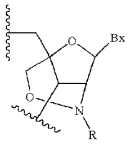
(B)



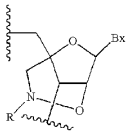
(C)



(D)

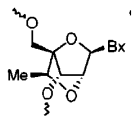


(E)



In certain embodiments, the dsRNA agent of the invention comprises at least two regions of at least two contiguous monomers of the above formula. In certain embodiments, the dsRNA agent of the invention comprises a gapped motif. In certain embodiments, the dsRNA agent of the invention comprises at least one region of from about 8 to about 14 contiguous β-D-2′-deoxyribofuranosyl nucleosides. In certain embodiments, the dsRNA agent of the invention comprises at least one region of from about 9 to about 12 contiguous β-D-2′-deoxyribofuranosyl nucleosides.

In certain embodiments, the dsRNA agent of the invention comprises at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more) comprises at least one (S)-cEt monomer of the formula:



S-cEt (C)

wherein Bx is heterocyclic base moiety.

In certain embodiments, monomers include sugar mimetics. In certain such embodiments, a mimetic is used in place of the sugar or sugar-internucleoside linkage combination, and the nucleobase is maintained for hybridization to a selected target. Representative examples of a sugar mimetics include, but are not limited to, cyclohexenyl or morpholino. Representative examples of a mimetic for a sugar-internucleoside linkage combination include, but are not limited to, peptide nucleic acids (PNA) and morpholino groups linked by uncharged achiral linkages. In some instances a mimetic is used in place of the nucleobase. Representative nucleobase mimetics are well known in the art and include, but are not limited to, tricyclic phenoxazine analogs and universal bases (Berger et al., Nuc Acid Res. 2000, 28:2911-14, incorporated herein by reference). Methods of synthesis of sugar, nucleoside and nucleobase mimetics are well known to those skilled in the art.

C. Intersugar Linkage Modifications

Described herein are linking groups that link monomers (including, but not limited to, modified and unmodified nucleosides and nucleotides) together, thereby forming an oligomeric compound, *e.g.*, an oligonucleotide. Such linking groups are also referred to as intersugar linkage. The two main classes of linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus containing linkages include, but are not limited to, phosphodiesters (P=O), phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates (P=S). Representative non-phosphorus containing linking groups include, but are not limited to, methylenemethylimino (—CH_2-$N(CH_3)$-O—CH2-), thiodiester (—O—C(O)—S—), thionocarbamate (—O—C(O)(NH)—S—); siloxane (—O—$Si(H)_2$-O—); and N,N′-dimethylhydrazine (—CH_2-$N(CH_3)$-$N(CH_3)$-). Modified linkages, compared to natural phosphodiester linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotides. In certain embodiments, linkages having a chiral atom can be prepared as racemic mixtures, as separate enantomers. Representative chiral linkages include, but are not limited to, alkylphosphonates and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known to those skilled in the art.

The phosphate group in the linking group can be modified by replacing one of the oxygens with a different substituent. One result of this modification can be increased resistance of the oligonucleotide to nucleolytic breakdown. Examples of modified phosphate groups include phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen

phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. In some embodiments, one of the non-bridging phosphate oxygen atoms in the linkage can be replaced by any of the following: S, Se, BR_3 (R is hydrogen, alkyl, aryl), C (*i.e.* an alkyl group, an aryl group, etc…), H, NR_2 (R is hydrogen, optionally substituted alkyl, aryl), or OR (R is optionally substituted alkyl or aryl). The phosphorous atom in an unmodified phosphate group is achiral. However, replacement of one of the non-bridging oxygens with one of the above atoms or groups of atoms renders the phosphorous atom chiral; in other words a phosphorous atom in a phosphate group modified in this way is a stereogenic center. The stereogenic phosphorous atom can possess either the "R" configuration (herein Rp) or the "S" configuration (herein Sp).

Phosphorodithioates have both non-bridging oxygens replaced by sulfur. The phosphorus center in the phosphorodithioates is achiral which precludes the formation of oligonucleotides diastereomers. Thus, while not wishing to be bound by theory, modifications to both non-bridging oxygens, which eliminate the chiral center, *e.g.* phosphorodithioate formation, can be desirable in that they cannot produce diastereomer mixtures. Thus, the non-bridging oxygens can be independently any one of O, S, Se, B, C, H, N, or OR (R is alkyl or aryl).

The phosphate linker can also be modified by replacement of bridging oxygen, (*i.e.* oxygen that links the phosphate to the sugar of the monomer), with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylenephosphonates). The replacement can occur at the either one of the linking oxygens or at both linking oxygens. When the bridging oxygen is the 3'-oxygen of a nucleoside, replacement with carbon is preferred. When the bridging oxygen is the 5'-oxygen of a nucleoside, replacement with nitrogen is preferred.

Modified phosphate linkages where at least one of the oxygen linked to the phosphate has been replaced or the phosphate group has been replaced by a non-phosphorous group, are also referred to as "non-phosphodiester intersugar linkage" or "non-phosphodiester linker."

In certain embodiments, the phosphate group can be replaced by non-phosphorus containing connectors, *e.g.* dephospho linkers. Dephospho linkers are also referred to as non-phosphodiester linkers herein. While not wishing to be bound by theory, it is believed that since the charged phosphodiester group is the reaction center in nucleolytic degradation, its replacement with neutral structural mimics should impart enhanced nuclease stability. Again, while not wishing to be bound by theory, it can be desirable, in some embodiment, to introduce alterations in which the charged phosphate group is replaced by a neutral moiety.

Examples of moieties which can replace the phosphate group include, but are not limited to, amides (for example amide-3 (3'-CH_2-C(=O)-N(H)-5') and amide-4 (3'-CH_2-N(H)-C(=O)-5')), hydroxylamino, siloxane (dialkylsiloxxane), carboxamide, carbonate, carboxymethyl, carbamate, carboxylate ester, thioether, ethylene oxide linker, sulfide,sulfonate, sulfonamide, sulfonate ester, thioformacetal (3'-S-CH_2-O-5'), formacetal (3 '-O-CH_2-O-5'), oxime, methyleneimino, methykenecarbonylamino, methylenemethylimino (MMI, 3'-CH_2-N(CH_3)-O-5'), methylenehydrazo, methylenedimethylhydrazo, methyleneoxymethylimino, ethers (C3'-O-C5'), thioethers (C3'-S-C5'),

thioacetamido (C3'-N(H)-C(=O)-CH_2-S-C5', C3'-O-P(O)-O-SS-C5', C3'-CH_2-NH-NH-C5', 3'-NHP(O)(OCH_3)-O-5' and 3'-NHP(O)(OCH_3)-O-5' and nonionic linkages containing mixed N, O, S and CH_2 component parts. See for example, Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook Eds. ACS Symposium Series 580; Chapters 3 and 4, (pp. 40-65). Preferred embodiments include methylenemethylimino (MMI),methylenecarbonylamino, amides,carbamate and ethylene oxide linker.

One skilled in the art is well aware that in certain instances replacement of a non-bridging oxygen can lead to enhanced cleavage of the intersugar linkage by the neighboring 2'-OH, thus in many instances, a modification of a non-bridging oxygen can necessitate modification of 2'-OH, *e.g.*, a modification that does not participate in cleavage of the neighboring intersugar linkage, *e.g.*, arabinose sugar, 2'-O-alkyl, 2'-F, LNA and ENA.

Preferred non-phosphodiester intersugar linkages include phosphorothioates, phosphorothioates with an at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% , 90% 95% or more enantiomeric excess of Sp isomer, phosphorothioates with an at least 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% , 90% 95% or more enantiomeric excess of Rp isomer, phosphorodithioates, phsophotriesters, aminoalkylphosphotrioesters, alkyl-phosphonaters (*e.g.*, methyl-phosphonate), selenophosphates, phosphoramidates (*e.g.*, N-alkylphosphoramidate), and boranophosphonates.

In some embodiments, the dsRNA agent of the invention comprises at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more and upto including all) modified or nonphosphodiester linkages. In some embodiments, the dsRNA agent of the invention comprises at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more and upto including all) phosphorothioate linkages.

The dsRNA agent of the inventions can also be constructed wherein the phosphate linker and the sugar are replaced by nuclease resistant nucleoside or nucleotide surrogates. While not wishing to be bound by theory, it is believed that the absence of a repetitively charged backbone diminishes binding to proteins that recognize polyanions (*e.g.* nucleases). Again, while not wishing to be bound by theory, it can be desirable in some embodiment, to introduce alterations in which the bases are tethered by a neutral surrogate backbone. Examples include the morpholino, cyclobutyl, pyrrolidine, peptide nucleic acid (PNA), aminoethylglycyl PNA (*aeg*PNA) and backnone-extended pyrrolidine PNA (*bep*PNA) nucleoside surrogates. A preferred surrogate is a PNA surrogate.

The dsRNA agent of the inventions described herein can contain one or more asymmetric centers and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (R) or (S), such as for sugar anomers, or as (D) or (L) such as for amino acids et al. Included in the dsRNA agent of the inventions provided herein are all such possible isomers, as well as their racemic and optically pure forms.

D. Terminal Modifications

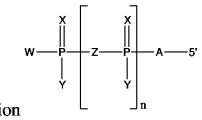
In some embodiments, the dsRNA agent further comprises a phosphate or phosphate mimic at

the 5'-end of the antisense strand. In one embodiment, the phosphate mimic is a 5'-vinyl phosphonate (VP).

In some embodiments, the 5'-end of the antisense strand of the dsRNA agent does not contain a 5'-vinyl phosphonate (VP).

Ends of the iRNA agent of the invention can be modified. Such modifications can be at one end or both ends. For example, the 3′ and/or 5′ ends of an iRNA can be conjugated to other functional molecular entities such as labeling moieties, *e.g.*, fluorophores (*e.g.*, pyrene, TAMRA, fluorescein, Cy3 or Cy5 dyes) or protecting groups (based *e.g.*, on sulfur, silicon, boron or ester). The functional molecular entities can be attached to the sugar through a phosphate group and/or a linker. The terminal atom of the linker can connect to or replace the linking atom of the phosphate group or the C-3′ or C-5′ O, N, S or C group of the sugar. Alternatively, the linker can connect to or replace the terminal atom of a nucleotide surrogate (*e.g.*, PNAs).

When a linker/phosphate-functional molecular entity-linker/phosphate array is interposed between two strands of a double stranded oligomeric compound, this array can substitute for a hairpin loop in a hairpin-type oligomeric compound.

Terminal modifications useful for modulating activity include modification of the 5' end of iRNAs with phosphate or phosphate analogs. In certain embodiments, the 5'end of an iRNA is phosphorylated or includes a phosphoryl analog. Exemplary 5'-phosphate modifications include those which are compatible with RISC mediated gene silencing. Modifications at the 5'-terminal end can also be useful in stimulating or inhibiting the immune system of a subject. In some embodiments, the 5'-end of the oligomeric compound comprises the modification 

, wherein W, X and Y are each independently selected from the group consisting of O, OR (R is hydrogen, alkyl, aryl), S, Se, BR_3 (R is hydrogen, alkyl, aryl), BH_3^-, C (*i.e.* an alkyl group, an aryl group, etc...), H, NR_2 (R is hydrogen, alkyl, aryl), or OR (R is hydrogen, alkyl or aryl); A and Z are each independently for each occurrence absent, O, S, CH_2, NR (R is hydrogen, alkyl, aryl), or optionally substituted alkylene, wherein backbone of the alkylene can comprise one or more of O, S, SS and NR (R is hydrogen, alkyl, aryl) internally and/or at the end; and n is 0-2. In some embodiments, n is 1 or 2. It is understood that A is replacing the oxygen linked to 5' carbon of sugar. When n is 0, W and Y together with the P to which they are attached can form an optionally substituted 5-8 membered heterocyclic, wherein W an Y are each independently O, S, NR' or alkylene. Preferably the heterocyclic is substituted with an aryl or heteroaryl. In some embodiments, one or both hydrogen on C5' of the 5'- terminal nucleotides are replaced with a halogen, *e.g.*, F.

Exemplary 5'-modifications include, but are not limited to, 5'-monophosphate ($(HO)_2(O)P$-O-5'); 5'-diphosphate ($(HO)_2(O)P$-O-P(HO)(O)-O-5'); 5'-triphosphate ($(HO)_2(O)P$-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-monothiophosphate (phosphorothioate; (HO)2(S)P-O-5'); 5'-monodithiophosphate

(phosphorodithioate; (HO)(HS)(S)P-O-5'), 5'-phosphorothiolate ((HO)2(O)P-S-5'); 5'-alpha-thiotriphosphate; 5'-beta-thiotriphosphate; 5'-gamma-thiotriphosphate; 5'-phosphoramidates ($(HO)_2(O)P$-NH-5', $(HO)(NH_2)(O)P$-O-5'). Other 5'-modification include 5'-alkylphosphonates (R(OH)(O)P-O-5', R=alkyl, *e.g.*, methyl, ethyl, isopropyl, propyl, etc…), 5'-alkyletherphosphonates (R(OH)(O)P-O-5', R=alkylether, *e.g.*, methoxymethyl (CH_2OMe), ethoxymethyl, etc…). Other exemplary 5'-modifications include where Z is optionally substituted alkyl at least once, *e.g.*, $((HO)_2(X)P$-O[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5', $((HO)_2(X)P$-O[-$(CH_2)_a$-P(X)(OH)-O]$_b$- 5', ((HO)2(X)P-[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5'; dialkyl terminal phosphates and phosphate mimics: HO[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5' , H_2N[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5', H[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5', Me_2N[-$(CH_2)_a$-O-P(X)(OH)-O]$_b$- 5', HO[-$(CH_2)_a$-P(X)(OH)-O]$_b$- 5' , H_2N[-$(CH_2)_a$-P(X)(OH)-O]$_b$- 5', H[-$(CH_2)_a$-P(X)(OH)-O]$_b$- 5', Me_2N[-$(CH_2)_a$-P(X)(OH)-O]$_b$- 5', wherein a and b are each independently 1-10. Other embodiments, include replacement of oxygen and/or sulfur with BH_3, BH_3^- and/or Se.

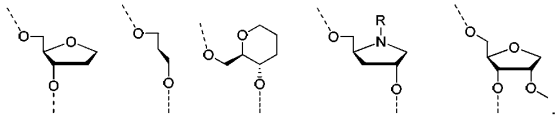
Terminal modifications can also be useful for monitoring distribution, and in such cases the preferred groups to be added include fluorophores, *e.g.*, fluorescein or an Alexa dye, *e.g.*, Alexa 488. Terminal modifications can also be useful for enhancing uptake, useful modifications for this include targeting ligands. Terminal modifications can also be useful for cross-linking an oligonucleotide to another moiety; modifications useful for this include mitomycin C, psoralen, and derivatives thereof.

E. Thermally Destabilizing Modifications

The compounds of the invention, such as iRNAs or dsRNA agents, can be optimized for RNA interference by increasing the propensity of the iRNA duplex to disassociate or melt (decreasing the free energy of duplex association) by introducing a thermally destabilizing modification in the sense strand at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand). This modification can increase the propensity of the duplex to disassociate or melt in the seed region of the antisense strand.

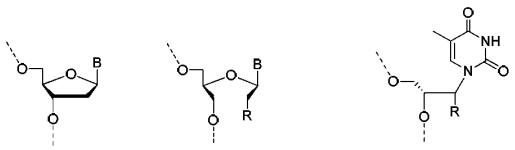
The thermally destabilizing modifications can include abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification or acyclic nucleotide, *e.g.*, unlocked nucleic acids (UNA) or glycerol nuceltic acid (GNA).

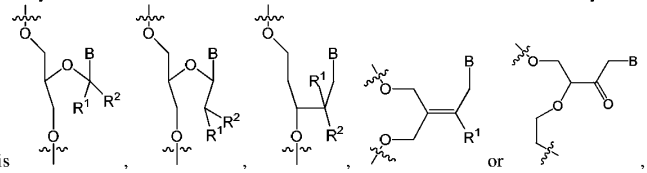
Exemplified abasic modifications are:



Exemplified sugar modifications are:

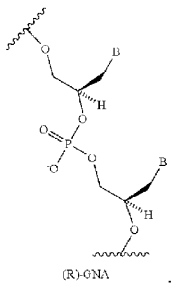
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The term "acyclic nucleotide" refers to any nucleotide having an acyclic ribose sugar, for example, where any of bonds between the ribose carbons (*e.g.*, C1'-C2', C2'-C3', C3'-C4', C4'-O4', or C1'-O4') is absent and/or at least one of ribose carbons or oxygen (*e.g.*, C1', C2', C3', C4' or O4') are independently or in combination absent from the nucleotide. In some embodiments, acyclic nucleotide is  wherein B is a modified or unmodified nucleobase, R^1 and R^2 independently are H, halogen, OR_3, or alkyl; and R_3 is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar). The term "UNA" refers to unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomers with bonds between C1'-C4' being removed (*i.e.* the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'-C3' bond (*i.e.* the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar is removed (see Mikhailov et. al., Tetrahedron Letters, 26 (17): 2059 (1985); and Fluiter et al., Mol. Biosyst., 10: 1039 (2009), which are hereby incorporated by reference in their entirety). The acyclic derivative provides greater backbone flexibility without affecting the Watson-Crick pairings. The acyclic nucleotide can be linked via 2'-5' or 3'-5' linkage.

The term 'GNA' refers to glycol nucleic acid which is a polymer similar to DNA or RNA but differing in the composition of its "backbone" in that is composed of repeating glycerol units linked by phosphodiester bonds:

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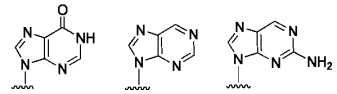
(R)-GNA

The thermally destabilizing modification can be mismatches (*i.e.*, noncomplementary base pairs) between the thermally destabilizing nucleotide and the opposing nucleotide in the opposite strand within the dsRNA duplex. Exemplary mismatch basepairs include G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, U:T, or a combination thereof. Other mismatch base pairings known in the art are also amenable to the present invention. A mismatch can occur between nucleotides that are either naturally occurring nucleotides or modified nucleotides, *i.e.*, the mismatch base pairing can occur between the nucleobases from respective nucleotides independent of the modifications on the ribose sugars of the nucleotides. In certain embodiments, the compounds of the invention, such as siRNA or iRNA agent, contains at least one nucleobase in the mismatch pairing that is a 2'-deoxy nucleobase; *e.g.*, the 2'-deoxy nucleobase is in the sense strand.

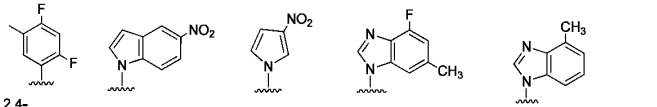
More examples of abasic nucleotide, acyclic nucleotide modifications (including UNA and GNA), and mismatch modifications have been described in detail in WO 2011/133876, which is herein incorporated by reference in its entirety.

The thermally destabilizing modifications may also include universal base with reduced or abolished capability to form hydrogen bonds with the opposing bases, and phosphate modifications.

Nucleobase modifications with impaired or completely abolished capability to form hydrogen bonds with bases in the opposite strand have been evaluated for destabilization of the central region of the dsRNA duplex as described in WO 2010/0011895, which is herein incorporated by reference in its entirety. Exemplary nucleobase modifications are:

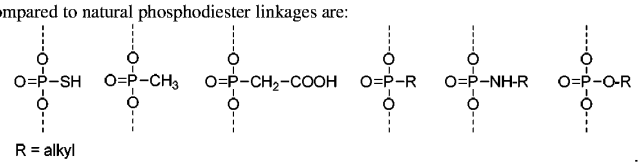


inosine nebularine 2-aminopurine



2,4-difluorotoluene 5-nitroindole 3-nitropyrrole 4-Fluoro-6-methylbenzimidazole 4-Methylbenzimidazole

Exemplary phosphate modifications known to decrease the thermal stability of dsRNA duplexes compared to natural phosphodiester linkages are:



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In some embodiments, compounds of the invention can comprise 2'-5' linkages (with 2'-H, 2'-OH and 2'-OMe and with P=O or P=S). For example, the 2'-5' linkages modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

In another embodiment, compounds of the invention can comprise L sugars (*e.g.*, L ribose, L-arabinose with 2'-H, 2'-OH and 2'-OMe). For example, these L sugar modifications can be used to promote nuclease resistance or to inhibit binding of the sense to the antisense strand, or can be used at the 5' end of the sense strand to avoid sense strand activation by RISC.

In one embodimennt the iRNA agent of the invention is conjugated to a ligand via a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In some embodoments, at least one strand of the iRNA agent of the invention disclosed herein is 5' phosphorylated or includes a phosphoryl analog at the 5' prime terminus. 5'-phosphate modifications include those which are compatible with RISC mediated gene silencing. Suitable modifications include: 5'-monophosphate ($(HO)_2(O)P-O-5'$); 5'-diphosphate ($(HO)_2(O)P-O-P(HO)(O)-O-5'$); 5'-triphosphate ($(HO)_2(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'$); 5'-guanosine cap (7-methylated or non-methylated) (7m-G-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-adenosine cap (Appp), and any modified or unmodified nucleotide cap structure (N-O-5'-(HO)(O)P-O-(HO)(O)P-O-P(HO)(O)-O-5'); 5'-monothiophosphate (phosphorothioate; $(HO)_2(S)P-O-5'$); 5'-monodithiophosphate (phosphorodithioate; (HO)(HS)(S)P-O-5'), 5'-phosphorothiolate ($(HO)_2(O)P-S-5'$); any additional combination of oxygen/sulfur replaced monophosphate, diphosphate and triphosphates (*e.g.* 5'-alpha-thiotriphosphate, 5'-gamma-thiotriphosphate, etc.), 5'-phosphoramidates ($(HO)_2(O)P-NH-5'$, (HO)(NH2)(O)P-O-5'), 5'-alkylphosphonates (R=alkyl=methyl, ethyl, isopropyl, propyl, etc., *e.g.* RP(OH)(O)-O-5'-, 5'-alkenylphosphonates (*i.e.* vinyl, substituted vinyl), $(OH)_2(O)P-5'-CH_2-$), 5'-alkyletherphosphonates (R=alkylether=methoxymethyl (MeOCH2-), ethoxymethyl, etc., *e.g.* RP(OH)(O)-O-5'-).

V. Modified RNAi agents of the Invention Comprising Motifs

In certain aspects of the disclosure, the double-stranded RNAi agents of the disclosure include agents with chemical modifications as disclosed, for example, in U.S. Patent Nos. 9,796,974 and 10,668,170, and U.S. Patent Publication Nos. 2014/288158, 2018/008724, 2019/038768, and 2020/353097, the entire contents of each of which are incorporated herein by reference. As shown therein and in PCT Publication No. WO 2013/074974 (the entire contents of which are incorporated by reference), one or more motifs of three identical modifications on three consecutive nucleotides may be introduced into a sense strand or antisense strand of an RNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The RNAi agent may be optionally modified with a (S)-glycol nucleic acid (GNA) modification, for instance on one or more residues of the antisense strand.

In one embodiment, the iRNA agent of the invention is a double ended bluntmer of 19 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7,8,9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end.

In one embodiment, the iRNA agent of the invention is a double ended bluntmer of 20 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8,9,10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end.

In one embodiment, the iRNA agent of the invention is a double ended bluntmer of 21 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9,10,11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end.

In one embodiment, the iRNA agent of the invention comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9,10,11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end, wherein one end of the iRNA is blunt, while the other end is comprises a 2 nt overhang. Preferably, the 2 nt overhang is at the 3'-end of the antisense.

In one embodiment, the iRNA agent of the invention comprises 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 first strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide,

comprises 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 target gene expression when said double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the iRNA agent of the invention comprises a sense and antisense strands, wherein said iRNA agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11,12,13 from the 5' end; wherein said 3' end of said first strand and said 5' end of said second strand form a blunt end and said second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and said second strand is sufficiently complemenatary to a target mRNA along at least 19 nt of said second strand length to reduce target gene expression when said iRNA agent is introduced into a mammalian cell, and wherein dicer cleavage of said iRNA preferentially results in an siRNA comprising said 3' end of said second strand, thereby reducing expression of the target gene in the mammal.

In one embodiment, the sense strand of the iRNA agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand. For instance, the sense strand can contain at least one motif of three 2'-F modifications on three consecutive nucleotides within 7-15 positions from the 5'end.

In one embodiment, the antisense strand of the iRNA agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand. For instance, the antisense strand can contain at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides within 9-15 positions from the 5'end.

For iRNA agent having a duplex region of 17-23 nt in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the antisense strand, the count starting from the 1st

nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the iRNA from the 5'-end.

In some embodiments, the iRNA agent comprises a sense strand and antisense strand each having 14 to 30 nucleotides, wherein the sense strand contains at least two motifs of three identical modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site within the strand and at least one of the motifs occurs at another portion of the strand that is separated from the motif at the cleavage site by at least one nucleotide. In one embodiment, the antisense strand also contains at least one motif of three identical modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site within the strand. The modification in the motif occurring at or near the cleavage site in the sense strand is different than the modification in the motif occurring at or near the cleavage site in the antisense strand.

In some embodiments, the iRNA agent comprises a sense strand and antisense strand each having 14 to 30 nucleotides, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site in the strand. In one embodiment, the antisense strand also contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In some embodiments, the iRNA agent comprises a sense strand and antisense strand each having 14 to 30 nucleotides, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9,10,11 from the 5'end, and wherein the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11,12,13 from the 5'end.

In one embodiment, the iRNA agent of the invention comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch can occur in the overhang region or the duplex region. The base pair can be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

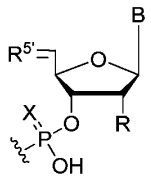
In one embodiment, the iRNA agent of the invention comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand can be chosen independently from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively,

at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

In another embodiment, the nucleotide at the 3'-end of the sense strand is deoxythimidine (dT). In another embodiment, the nucleotide at the 3'-end of the antisense strand is deoxythimidine (dT). In one embodiment, there is a short sequence of deoxythimidine nucleotides, for example, two dT nucleotides on the 3'-end of the sense or antisense strand.

In certain embodiments, the compositions and methods of the disclosure include a vinyl phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5'-vinyl phosphonate modified nucleotide of the disclosure has the structure:



wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or C_{1-20}alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

$R^{5'}$ is =C(H)-P(O)$(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphate structure includes the preceding structure, where R5' is =C(H)-OP(O)(OH)2 and the double bond between the C5' carbon and R5' is in the E or Z orientation (*e.g.*, E orientation).

In one aspect, the invention relates to a double-stranded RNA (dsRNA) agent for inhibiting the expression of a target gene having reduced off-target effects as described in U.S. Patent Nos. 10,233448, 10,612,024, and 10,612,027, and U.S. Patent Publication Nos. 2017/275626, 2019/241891, 2019/241893, and 2021/017519, the entire contents of each of which are incorporated herein by reference. As exemplified therein, a motif comprising, *e.g.,* a thermally destabilizing nucleotide, *e.g.,* i) a nucleotide that forms a mismatch pair with the opposing nucleotide in the antisense strand, ii) a nucleotide having an abasic modification, and/or iii) a nucleotide having a sugar

modification, and placed at a site opposite to the seed region (positions 2-8) may be introduced into the sense strand.

In one embodiment, the dsRNA agent of the invention does not contain any 2'-F modification.

In one embodiment, the sense strand and/or antisense strand of the dsRNA agent comprises one or more blocks of phosphorothioate or methylphosphonate internucleotide linkages. In one example, the sense strand comprises one block of two phosphorothioate or methylphosphonate internucleotide linkages. In one example, the antisense strand comprises two blocks of two phosphorothioate or methylphosphonate internucleotide linkages. For example, the two blocks of phosphorothioate or methylphosphonate internucleotide linkages are separated by 16-18 phosphate internucleotide linkages.

In one embodiment, each of the sense and antisense strands of the dsRNA agent has 15-30 nucleotides. In one example, the sense strand has 19-22 nucleotides, and the antisense strand has 19-25 nucleotides. In another example, the sense strand has 21 nucleotides, and the antisense strand has 23 nucleotides.

In one embodiment, the nucleotide at position 1 of the 5'-end of the antisense strand in the duplex is selected from the group consisting of A, dA, dU, U, and dT. In one embodiment, at least one of the first, second, and third base pair from the 5'-end of the antisense strand is an AU base pair.

In one embodiment, the antisense strand of the dsRNA agent of the invention is 100% complementary to a target RNA to hybridize thereto and inhibits its expression through RNA interference. In another embodiment, the antisense strand of the dsRNA agent of the invention is at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% complementary to a target RNA.

In one aspect, the invention relates to a dsRNA agent as defined herein capable of inhibiting the expression of a target gene. The dsRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The sense strand contains at least one thermally destabilizing nucleotide, wherein at least one of said thermally destabilizing nucleotide occurs at or near the site that is opposite to the seed region of the antisense strand (*i.e.* at position 2-8 of the 5'-end of the antisense strand). Each of the embodiments and aspects described in this specification relating to the dsRNA represented by formula (I) can also apply to the dsRNA containing the thermally destabilizing nucleotide.

The thermally destabilizing nucleotide can occur, for example, between positions 14-17 of the 5'-end of the sense strand when the sense strand is 21 nucleotides in length. The antisense strand contains at least two modified nucleic acids that are smaller than a sterically demanding 2'-OMe modification. Preferably, the two modified nucleic acids that are smaller than a sterically demanding 2'-OMe are separated by 11 nucleotides in length. For example, the two modified nucleic acids are at positions 2 and 14 of the 5'end of the antisense strand.

In some embodiment, the dsRNA agent as defined herein can comprise i) a phosphorus-containing group at the 5'-end of the sense strand or antisense strand; ii) with two phosphorothioate

internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications at positions 1 and 2 and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand); and iii) one or more C22 hydrocarbon chains.

In a particular embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14 to 16, 18, and 20 (counting from the 5' end); and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 9, 11 to 13, 15, 17, 19, 21, and 23, and 2'F modifications at positions 2, 4, 6 to 8, 10, 14, 16, 18, 20, and 22 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 21 and 22, and between nucleotide positions 22 and 23 (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 embodiment, t the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, 13, 15, 17, 19, and 21, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a desoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 7, 9, 11, 13, 15, 17, and 19 to 23, and 2'-F modifications at positions 2, 4 to 6, 8, 10, 12, 14, 16, and 18 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 6, 8, 10, 12, 14, and 16 to 21, and 2'-F modifications at positions 7, 9, 11, 13, and 15; and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2 to 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting from the 5' end); and

(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);

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 embodiment, t the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5, 7, 9, 11 to 13, 15, 17, 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-F modifications at positions 1, 3, 5, 7, 9 to 11, and 13, and 2'-OMe modifications at positions 2, 4, 6, 8, 12, and 14 to 21; and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3, 5 to 7, 9, 11 to 13, 15, 17 to 19, and 21 to 23, and 2'-F modifications at positions 2, 4, 8, 10, 14, 16, and 20 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, and 19 to 21, and 2'-F modifications at positions 3, 5, 7, 9 to 11, 13, 16, and 18; and

(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) a length of 25 nucleotides;

(ii) 2'-OMe modifications at positions 1, 4, 6, 7, 9, 11 to 13, 15, 17, and 19 to 23, 2'-F modifications at positions 2, 3, 5, 8, 10, 14, 16, and 18, and desoxy-nucleotides (*e.g.* dT) at positions 24 and 25 (counting from the 5' end); and

(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);

wherein the dsRNA agents have a four 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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 8, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 9, 14, and 16 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

(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) a length of 23 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 23, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(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);

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 embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 19 nucleotides;

(ii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and

(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) a length of 21 nucleotides;

(ii) 2'-OMe modifications at positions 1, 3 to 5, 7, 10 to 13, 15, and 17 to 21, and 2'-F modifications at positions 2, 6, 8, 9, 14, and 16 (counting from the 5' end); and

(iii) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, between nucleotide positions 2 and 3, between nucleotide positions 19 and 20, and between nucleotide positions 20 and 21 (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 one embodiment, the dsRNA agents of the present invention comprising one or more C22 hydrocarbon chains conjugated to one or more internal positions on at least one strand comprise:

(a) a sense strand having:

(i) a length of 18-23 nucleotides;

(ii) three consecutive 2'-F modifications at positions 7-15; and

(b) an antisense strand having:

(i) a length of 18-23 nucleotides;

(ii) at least 2'-F modifications anywhere on the strand; and

(iii) at least two phosphorothioate internucleotide linkages at the first five nucleotides (counting from the 5' end);

and either have two nucleotides overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand; or blunt end both ends of the duplex.

In one embodiment, the dsRNA agents of the present invention comprise:

(a) a sense strand having:

(i) a length of 18-23 nucleotides;

(ii) less than four 2'-F modifications;

(b) an antisense strand having:

(i) a length of 18-23 nucleotides;

(ii) at less than twelve 2'-F modfication; and

(iii) at least two phosphorothioate internucleotide linkages at the first five nucleotides (counting from the 5' end);

wherein the dsRNA agents have either have two nucleotides overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand; or blunt end both ends of the duplex.

In one embodiment, the dsRNA agents of the present invention comprise:

(a) a sense strand having:

(i) a length of 19-35 nucleotides;

(ii) less than four 2'-F modifications;

(b) an antisense strand having:

(i) a length of 19-35 nucleotides;

(ii) at less than twelve 2'-F modfication; and

(iii) at least two phosphorothioate internucleotide linkages at the first five nucleotides (counting from the 5' end);

wherein the duplex region is between 19 to 25 base pairs (preferably 19, 20, 21 or 22); and wherein the dsRNA agents have either have two nucleotides overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand; or blunt end both ends of the duplex.

In one embodiment, the dsRNA agents of the present invention comprise a sense strand and antisense strands 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); and wherein the dsRNA agents have less than 20% , less than 15% and less than 10% non-natural nucleotide.

Examples of non-natural nucleotide includes acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, , 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), or 2'-ara-F, and others.

In one embodiment, the dsRNA agents of the present invention comprise a sense strand and antisense strands 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 dsRNA agents have greater than 80% , greater than 85% and greater than 90% natural nucleotide, such as 2'-OH, 2'-deoxy and 2'-OMe are natural nucleotides.

In one embodiment, the dsRNA agents of the present invention comprise a sense strand and antisense strands 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); and wherein the dsRNA agents have 100% natural nucleotide, such as 2'-OH, 2'-deoxy and 2'-OMe are natural nucleotides.

Various publications described multimeric siRNA and can all be used with the iRNA of the invention. Such publications include WO2007/091269, US Patent No. 7858769, WO2010/141511, WO2007/117686, WO2009/014887 and WO2011/031520, which are hereby incorporated by reference in their entirety.

In some embodiments, 100%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35% or 30% of the iRNA agent of the invention is modified.

In some embodiments, each of the sense and antisense strands of the iRNA agent is independently modified with acyclic nucleotides, LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-fluoro, 2'-O-N-methylacetamido (2'-O-NMA), a 2'-O-dimethylaminoethoxyethyl (2'-O-DMAEOE), 2'-O-aminopropyl (2'-O-AP), or 2'-ara-F.

In some embodiments, each of the sense and antisense strands of the iRNA agent contains at least two different modifications.

In some embodiments, the dsRNA agent of the invention of the invention does not contain any 2'-F modification.

In some embodiments, the dsRNA agent of the invention contains one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve 2'-F modification(s). In one example, dsRNA agent of the invention contains nine or ten 2'-F modifications.

The iRNA agent of the invention may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand.

In one embodiment, the iRNA comprises the phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paried nucleotide next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

In some embodiments, the sense strand and/or antisense strand of the iRNA agent comprises one or more blocks of phosphorothioate or methylphosphonate internucleotide linkages. In one example, the sense strand comprises one block of two phosphorothioate or methylphosphonate internucleotide linkages. In one example, the antisense strand comprises two blocks of two

phosphorothioate or methylphosphonate internucleotide linkages. For example, the two blocks of phosphorothioate or methylphosphonate internucleotide linkages are separated by 16-18 phosphate internucleotide linkages.

In some embodiments, the antisense strand of the iRNA agent of the invention is 100% complementary to a target RNA to hybridize thereto and inhibits its expression through RNA interference. In another embodiment, the antisense strand of the iRNA agent of the invention is at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% complementary to a target RNA.

In one aspect, the invention relates to a iRNA agent capable of inhibiting the expression of a target gene. The iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The sense strand contains at least one thermally destabilizing nucleotide, wherein at at least one said thermally destabilizing nucleotide occurs at or near the site that is opposite to the seed region of the antisense strand (i.e .at position 2-8 of the 5'-end of the antisense strand), For example, the thermally destabilizing nucleotide occurs between positions 14-17 of the 5'-end of the sense strand when the sense strand is 21 nucleotides in length. The antisense strand contains at least two modified nucleic acids that are smaller than a sterically demanding 2'-OMe modification. Preferably, the two modified nucleic acids that is smaller than a sterically demanding 2'-OMe are separated by 11 nucleotides in length. For example, the two modified nucleic acids are at positions 2 and 14 of the 5'end of the antisense strand.

In some embodiments, the compound of the invention disclosed herein is a miRNA mimic. In one design, miRNA mimics are double stranded molecules (*e.g.*, with a duplex region of between about 16 and about 31 nucleotides in length) and contain one or more sequences that have identity with the mature strand of a given miRNA. Double-stranded miRNA mimics have designs similar to as described above for double-stranded iRNAs. In some embodiments, a miRNA mimic comprises a duplex region of between 16 and 31 nucleotides and one or more of the following chemical modification patterns: the sense strand contains 2'-O-methyl modifications of nucleotides 1 and 2 (counting from the 5' end of the sense oligonucleotide), and all of the Cs and Us; the antisense strand modifications can comprise 2' F modification of all of the Cs and Us, phosphorylation of the 5' end of the oligonucleotide, and stabilized internucleotide linkages associated with a 2 nucleotide 3 ' overhang.

VI. Synthesis of RNAi Agents of the Invention

The nucleic acids featured in the disclosure can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference.

An siRNA can be produced, *e.g.*, in bulk, by a variety of methods. Exemplary methods include: organic synthesis and RNA cleavage, *e.g.*, *in vitro* cleavage.

A. Organic Synthesis

An siRNA can be made by separately synthesizing a single stranded RNA molecule, or each respective strand of a double-stranded RNA molecule, after which the component strands can then be annealed.

A large machine, *e.g.*, the OligoPilot II from Pharmacia Biotec AB (Uppsala Sweden), can be used to produce a large amount of a particular RNA strand for a given siRNA. The OligoPilotII reactor can efficiently couple a nucleotide using only a 1.5 molar excess of a phosphoramidite nucleotide. To make an RNA strand, ribonucleotides amidites are used. Standard cycles of monomer addition can be used to synthesize the 21 to 23 nucleotide strand for the siRNA. Typically, the two complementary strands are produced separately and then annealed, *e.g.*, after release from the solid support and deprotection.

Organic synthesis can be used to produce a discrete siRNA species. The complementary of the species to a particular target gene can be precisely specified. For example, the species may be complementary to a region that includes a polymorphism, *e.g.*, a single nucleotide polymorphism. Further the location of the polymorphism can be precisely defined. In some embodiments, the polymorphism is located in an internal region, *e.g.*, at least 4, 5, 7, or 9 nucleotides from one or both of the termini.

B. dsiRNA Cleavage

siRNAs can also be made by cleaving a larger siRNA. The cleavage can be mediated *in vitro* or *in vivo*. For example, to produce iRNAs by cleavage *in vitro*, the following method can be used:

1. In vitro transcription.

dsiRNA is produced by transcribing a nucleic acid (DNA) segment in both directions. For example, the HiScribe™ RNAi transcription kit (New England Biolabs) provides a vector and a method for producing a dsiRNA for a nucleic acid segment that is cloned into the vector at a position flanked on either side by a T7 promoter. Separate templates are generated for T7 transcription of the two complementary strands for the dsiRNA. The templates are transcribed *in vitro* by addition of T7 RNA polymerase and dsiRNA is produced. Similar methods using PCR and/or other RNA polymerases (*e.g.*, T3 or SP6 polymerase) can also be dotoxins that may contaminate preparations of the recombinant enzymes. In one embodiment, RNA generated by this method is carefully purified to remove endotoxins that may contaminate preparations of the recombinant enzymes.

2. In Vitro Cleavage.

dsRNA is cleaved *in vitro* into siRNAs, for example, using a Dicer or comparable RNAse III-based activity. For example, the dsiRNA can be incubated in an *in vitro* extract from Drosophila or using purified components, *e.g.*, a purified RNAse or RISC complex (RNA-induced silencing complex). See, *e.g.*, Ketting *et al. Genes Dev* 2001 Oct 15;15(20):2654-9 and Hammond *Science* 2001 Aug 10;293(5532):1146-50.

dsiRNA cleavage generally produces a plurality of siRNA species, each being a particular 21 to 23 nt fragment of a source dsiRNA molecule. For example, siRNAs that include sequences complementary to overlapping regions and adjacent regions of a source dsiRNA molecule may be present.

Regardless of the method of synthesis, the siRNA preparation can be prepared in a solution (*e.g.*, an aqueous and/or organic solution) that is appropriate for formulation. For example, the siRNA preparation can be precipitated and redissolved in pure double-distilled water, and lyophilized. The dried siRNA can then be resuspended in a solution appropriate for the intended formulation process.

C. Making dsRNA agents conjugated to one or more C_{22} hydrocarbon chains

In some embodiments, the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a nucleobase, sugar moiety, or internucleosidic linkage.

Conjugation to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In some embodiments, the 2-, 6-, 7-, or 8-positions of a purine nucleobase are attached to a C_{22} hydrocarbon chain. Conjugation to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2-, 5-, and 6-positions of a pyrimidine nucleobase can be substituted with a C_{22} hydrocarbon chain. When one or more C_{22} hydrocarbon chains is conjugated to a nucleobase, the preferred position is one that does not interfere with hybridization, *i.e.*, does not interfere with the hydrogen bonding interactions needed for base pairing. In one embodiment, the one or more C_{22} hydrocarbon chains may be conjugated to a nucleobase via a linker containing an alkyl, alkenyl or amide linkage. .

Conjugation to sugar moieties of nucleosides can occur at any carbon atom. Exemplary carbon atoms of a sugar moiety that the one or more C_{22} hydrocarbon chains can be attached to include the 2', 3', and 5' carbon atoms. The one or more C_{22} hydrocarbon chains can also be attached to the 1' position, such as in an abasic residue. In one embodiment, the the one or more C_{22} hydrocarbon chains may be conjugated to a sugar moiety, via a 2'-O modification, with or without a linker.

Internucleosidic linkages can also bear the one or more C_{22} hydrocarbon chains. For phosphorus-containing linkages (*e.g.*, phosphodiester, phosphorothioate, phosphorodithiotate, phosphoroamidate, and the like), the the one or more C_{22} hydrocarbon chains can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleosidic linkages (*e.g.*, PNA), the the one or more C_{22} hydrocarbon chains can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

There are numerous methods for preparing conjugates of oligonuclotides. Generally, an oligonucleotide is attached to a conjugate moiety by contacting a reactive group (*e.g.*, OH, SH, amine, carboxyl, aldehyde, and the like) on the oligonucleotide with a reactive group on the conjugate moiety. In some embodiments, one reactive group is electrophilic and the other is nucleophilic.

For example, an electrophilic group can be a carbonyl-containing functionality and a nucleophilic group can be an amine or thiol. Methods for conjugation of nucleic acids and related oligomeric compounds with and without linking groups are well described in the literature such as, for example, in Manoharan in Antisense Research and Applications, Crooke and LeBleu, eds., CRC Press, Boca Raton, Fla., 1993, Chapter 17, which is incorporated herein by reference in its entirety.

In one embodiment, a first (complementary) RNA strand and a second (sense) RNA strand can be synthesized separately, wherein one of the RNA strands comprises a pendant C_{22} hydrocarbon chain, and the first and second RNA strands can be mixed to form a dsRNA. The step of synthesizing the RNA strand preferably involves solid-phase synthesis, wherein individual nucleotides are joined end to end through the formation of internucleotide 3′-5′ phosphodiester bonds in consecutive synthesis cycles.

In one embodiment, the C_{22} hydrocarbon chain having a phosphoramidite group is coupled to the 3'-end or 5′-end of either the first (complementary) or second (sense) RNA strand in the last synthesis cycle. In the solid-phase synthesis of an RNA, the nucleotides are initially in the form of nucleoside phosphoramidites. In each synthesis cycle, a further nucleoside phosphoramidite is linked to the -OH group of the previously incorporated nucleotide. If the the one or more C_{22} hydrocarbon chains has a phosphoramidite group, it can be coupled in a manner similar to a nucleoside phosphoramidite to the free OH end of the RNA synthesized previously in the solid-phase synthesis. The synthesis can take place in an automated and standardized manner using a conventional RNA synthesizer. Synthesis of the molecule having the phosphoramidite group may include phosphitylation of a free hydroxyl to generate the phosphoramidite group.

Synthesis procedures of the one or more C_{22} hydrocarbon chain-conjugated phosphoramidites are exemplified in Example 1.

In general, the oligonucleotides can be synthesized using protocols known in the art, for example, as described in Caruthers et al., Methods in Enzymology (1992) 211:3-19; WO 99/54459; Wincott et al., Nucl. Acids Res. (1995) 23:2677-2684; Wincott et al., Methods Mol. Bio., (1997) 74:59; Brennan et al., Biotechnol. Bioeng. (1998) 61:33-45; and U.S. Pat. No. 6,001,311; each of which is hereby incorporated by reference in its entirety. In general, the synthesis of oligonucleotides involves conventional nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5′-end, and phosphoramidites at the 3′-end. In a non-limiting example, small scale syntheses are conducted on a Expedite 8909 RNA synthesizer sold by Applied Biosystems, Inc. (Weiterstadt, Germany), using ribonucleoside phosphoramidites sold by ChemGenes Corporation (Ashland, Mass.). Alternatively, syntheses can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.), or by methods such as those described in Usman et al., J. Am. Chem. Soc. (1987) 109:7845; Scaringe, et al., Nucl. Acids Res. (1990) 18:5433; Wincott, et al., Nucl. Acids Res. (1990) 23:2677-2684; and Wincott, et al., Methods Mol. Bio. (1997) 74:59, each of which is hereby incorporated by reference in its entirety.

The nucleic acid molecules of the present invention may be synthesized separately and joined together post-synthetically, for example, by ligation (Moore et al., Science (1992) 256:9923; WO 93/23569; Shabarova et al., Nucl. Acids Res. (1991) 19:4247; Bellon et al., Nucleosides & Nucleotides (1997) 16:951; Bellon et al., Bioconjugate Chem. (1997) 8:204; or by hybridization following synthesis and/or deprotection. The nucleic acid molecules can be purified by gel electrophoresis using conventional methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott et al., supra, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

VII. Ligands

In certain embodiments, the dsRNA agent of the invention is further modified by covalent attachment of one or more conjugate groups. In general, conjugate groups modify one or more properties of the attached dsRNA agent of the invention including but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and clearance. Conjugate groups are routinely used in the chemical arts and are linked directly or via an optional linking moiety or linking group to a parent compound such as an oligomeric compound. A preferred list of conjugate groups includes without limitation, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, thioethers, polyethers, cholesterols, thiocholesterols, cholic acid moieties, folate, lipids, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins and dyes.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a receptor which mediates delivery to a specific muscle or adipose tissue. These targeting ligands can be conjugated by otself or in combination with the one or more C_{22} hydrocarbon chains to enable specific systemic delivery.

Exemplary targeting ligands that targets the receptor mediated delivery to a muscle or adipose tissue are peptide ligands such as Angiopep-2, lipoprotein receptor related protein (LRP) ligand, bEnd.3 cell binding ligand; transferrin receptor (TfR) ligand (which can utilize iron transport system in brain and cargo transport into the brain parenchyma); manose receptor ligand (which targets olfactory ensheathing cells, glial cells), glucose transporter protein, and LDL receptor ligand.

Preferred conjugate groups amenable to the present invention include lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553); cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053); a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765); a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533); an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 111; Kabanov et al., FEBS Lett., 1990, 259, 327; Svinarchuk et al., Biochimie, 1993, 75, 49); a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium-1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651; Shea et al., Nucl. Acids Res.,

1990, 18, 3777); a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969); adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651); a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229); or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923).

Generally, a wide variety of entities, *e.g.*, ligands, can be coupled to the oligomeric compounds described herein. Ligands can include naturally occurring molecules, or recombinant or synthetic molecules. Exemplary ligands include, but are not limited to, polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG, *e.g.*, PEG-2K, PEG-5K, PEG-10K, PEG-12K, PEG-15K, PEG-20K, PEG-40K), MPEG, $[MPEG]_2$, polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, polyphosphazine, polyethylenimine, cationic groups, spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, mucin, glycosylated polyaminoacids, transferrin, bisphosphonate, polyglutamate, polyaspartate, aptamer, asialofetuin, hyaluronan, procollagen, immunoglobulins (*e.g.*, antibodies), insulin, transferrin, albumin, sugar-albumin conjugates, intercalating agents (*e.g.*, acridines), cross-linkers (*e.g.* psoralen, mitomycin C), porphyrins (*e.g.*, TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.*, EDTA), lipophilic molecules (e.g, steroids, bile acids, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), peptides (*e.g.*, an alpha helical peptide, amphipathic peptide, RGD peptide, cell permeation peptide, endosomolytic/fusogenic peptide), alkylating agents, phosphate, amino, mercapto, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, naproxen, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, AP, antibodies, hormones and hormone receptors, lectins, carbohydrates, multivalent carbohydrates, vitamins (*e.g.*, vitamin A, vitamin E, vitamin K, vitamin B, *e.g.*, folic acid, B12, riboflavin, biotin and pyridoxal), vitamin cofactors, lipopolysaccharide, an activator of p38 MAP kinase, an activator of NF-κB, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, myoservin, tumor necrosis factor alpha (TNFalpha), interleukin-1 beta, gamma interferon, natural or recombinant low density lipoprotein (LDL), natural or recombinant high-density lipoprotein (HDL), and a cell-permeation agent (*e.g.*, a.helical cell-permeation agent).

Peptide and peptidomimetic ligands include those having naturally occurring or modified peptides, *e.g.*, D or L peptides; α, β, or γ peptides; N-methyl peptides; azapeptides; peptides having one or more amide, *i.e.*, peptide, linkages replaced with one or more urea, thiourea, carbamate, or sulfonyl urea linkages; or cyclic peptides. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The peptide or peptidomimetic ligand can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

Exemplary amphipathic peptides include, but are not limited to, cecropins, lycotoxins, paradaxins, buforin, CPF, bombinin-like peptide (BLP), cathelicidins, ceratotoxins, *S. clava* peptides, hagfish intestinal antimicrobial peptides (HFIAPs), magainines, brevinins-2, dermaseptins, melittins, pleurocidin, H_2A peptides, Xenopus peptides, esculentinis-1, and caerins.

As used herein, the term "endosomolytic ligand" refers to molecules having endosomolytic properties. Endosomolytic ligands promote the lysis of and/or transport of the composition of the invention, or its components, from the cellular compartments such as the endosome, lysosome, endoplasmic reticulum (ER), Golgi apparatus, microtubule, peroxisome, or other vesicular bodies within the cell, to the cytoplasm of the cell. Some exemplary endosomolytic ligands include, but are not limited to, imidazoles, poly or oligoimidazoles, linear or branched polyethyleneimines (PEIs), linear and brached polyamines, *e.g.* spermine, cationic linear and branched polyamines, polycarboxylates, polycations, masked oligo or poly cations or anions, acetals, polyacetals, ketals/polyketals, orthoesters, linear or branched polymers with masked or unmasked cationic or anionic charges, dendrimers with masked or unmasked cationic or anionic charges, polyanionic peptides, polyanionic peptidomimetics, pH-sensitive peptides, natural and synthetic fusogenic lipids, natural and synthetic cationic lipids.

Exemplary endosomolytic/fusogenic peptides include, but are not limited to, AALEALAEALEALAEALEALAEAAAAGGC (GALA); AALAEALAEALAEALAEALAEALAAAAGGC (EALA); ALEALAEALEALAEA; GLFEAIEGFIENGWEGMIWDYG (INF-7); GLFGAIAGFIENGWEGMIDGWYG (Inf HA-2); GLFEAIEGFIENGWEGMIDGWYGCGLFEAIEGFIENGWEGMID GWYGC (diINF-7); GLFEAIEGFIENGWEGMIDGGCGLFEAIEGFIENGWEGMIDGGC (diINF-3); GLFGALAEALAEALAEHLAEALAEALEALAAGGSC (GLF); GLFEAIEGFIENGWEGLAEALAEALEALAAGGSC (GALA-INF3); GLF EAI EGFI ENGW EGnI DG K GLF EAI EGFI ENGW EGnI DG (INF-5, n is norleucine); LFEALLELLESLWELLLEA (JTS-1); GLFKALLKLLKSLWKLLLKA (ppTG1); GLFRALLRLLRSLWRLLLRA (ppTG20); WEAKLAKALAKALAKHLAKALAKALKACEA (KALA); GLFFEAIAEFIEGGWEGLIEGC (HA); GIGAVLKVLTTGLPALISWIKRKRQQ (Melittin); H_5WYG; and CHK_6HC.

Without wishing to be bound by theory, fusogenic lipids fuse with and consequently destabilize a membrane. Fusogenic lipids usually have small head groups and unsaturated acyl chains. Exemplary fusogenic lipids include, but are not limited to, 1,2-dileoyl-sn-3-

phosphoethanolamine (DOPE), phosphatidylethanolamine (POPE), palmitoyloleoylphosphatidylcholine (POPC), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-ol (Di-Lin), N-methyl(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)methanamine (DLin-k-DMA) and N-methyl-2-(2,2-di((9Z,12Z)-octadeca-9,12-dienyl)-1,3-dioxolan-4-yl)ethanamine (also refered to as XTC herein).

Synthetic polymers with endosomolytic activity amenable to the present invention are described in U.S. Pat. App. Pub. Nos. 2009/0048410; 2009/0023890; 2008/0287630; 2008/0287628; 2008/0281044; 2008/0281041; 2008/0269450; 2007/0105804; 20070036865; and 2004/0198687, contents of which are hereby incorporated by reference in their entirety.

Exemplary cell permeation peptides include, but are not limited to, RQIKIWFQNRRMKWKK (penetratin); GRKKRRQRRRPPQC (Tat fragment 48-60); GALFLGWLGAAGSTMGAWSQPKKKRKV (signal sequence based peptide); LLIILRRRIRKQAHAHSK (PVEC); GWTLNSAGYLLKINLKALAALAKKIL (transportan); KLALKLALKALKAALKLA (amphiphilic model peptide); RRRRRRRRR (Arg9); KFFKFFKFFK (Bacterial cell wall permeating peptide); LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES (LL-37); SWLSKTAKKLENSAKKRISEGIAIAIQGGPR (cecropin P1); ACYCRIPACIAGERRYGTCIYQGRLWAFCC (α-defensin); DHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK (β-defensin); RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFPGKR-NH2 (PR-39); ILPWKWPWWPWRR-NH2 (indolicidin); AAVALLPAVLLALLAP (RFGF); AALLPVLLAAP (RFGF analogue); and RKCRIVVIRVCR (bactenecin).

Exemplary cationic groups include, but are not limited to, protonated amino groups, derived from *e.g.*, O-AMINE (AMINE = NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, polyamino); aminoalkoxy, *e.g.*, $O(CH_2)_nAMINE$, (*e.g.*, AMINE = NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino, ethylene diamine, polyamino); amino (*e.g.* NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); and $NH(CH_2CH_2NH)_nCH_2CH_2$-AMINE (AMINE = NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, or diheteroaryl amino).

As used herein the term "targeting ligand" refers to any molecule that provides an enhanced affinity for a selected target, *e.g.*, a cell, cell type, tissue, organ, region of the body, or a compartment, *e.g.*, a cellular, tissue or organ compartment. Some exemplary targeting ligands include, but are not limited to, antibodies, antigens, folates, receptor ligands, carbohydrates, aptamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL and HDL ligands.

Carbohydrate based targeting ligands include, but are not limited to, D-galactose, multivalent galactose, N-acetyl-D-galactosamine (GalNAc), multivalent GalNAc, *e.g.* $GalNAc_2$ and $GalNAc_3$ (GalNAc and multivalent GalNAc are collectively referred to herein as GalNAc conjugates); D-

mannose, multivalent mannose, multivalent lactose, N-acetyl-glucosamine, Glucose, multivalent Glucose, multivalent fucose, glycosylated polyaminoacids and lectins. The term multivalent indicates that more than one monosaccharide unit is present. Such monosaccharide subunits can be linked to each other through glycosidic linkages or linked to a scaffold molecule.

A number of folate and folate analogs amenable to the present invention as ligands are described in U.S. Pat. Nos. 2,816,110; 5,552,545; 6,335,434 and 7,128,893, contents of which are herein incorporated in their entireties by reference.

As used herein, the terms "PK modulating ligand" and "PK modulator" refers to molecules which can modulate the pharmacokinetics of the composition of the invention. Some exemplary PK modulator include, but are not limited to, lipophilic molecules, bile acids, sterols, phospholipid analogues, peptides, protein binding agents, vitamins, fatty acids, phenoxazine, aspirin, naproxen, ibuprofen, suprofen, ketoprofen, (S)-(+)-pranoprofen, carprofen, PEGs, biotin, and transthyretia-binding ligands (*e.g.*, tetraiidothyroacetic acid, 2, 4, 6-triiodophenol and flufenamic acid). Oligomeric compounds that comprise a number of phosphorothioate intersugar linkages are also known to bind to serum protein, thus short oligomeric compounds, *e.g.* oligonucleotides of comprising from about 5 to 30 nucleotides (*e.g.*, 5 to 25 nucleotides, preferably 5 to 20 nucleotides, *e.g.*, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides), and that comprise a plurality of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). The PK modulating oligonucleotide can comprise at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more phosphorothioate and/or phosphorodithioate linkages. In some embodiments, all internucleotide linkages in PK modulating oligonucleotide are phosphorothioate and/or phosphorodithioates linkages. In addition, aptamers that bind serum components (*e.g.* serum proteins) are also amenable to the present invention as PK modulating ligands. Binding to serum components (*e.g.* serum proteins) can be predicted from albumin binding assays, scuh as those described in Oravcova, et al., Journal of Chromatography B (1996), 677: 1-27.

When two or more ligands are present, the ligands can all have same properties, all have different properties or some ligands have the same properties while others have different properties. For example, a ligand can have targeting properties, have endosomolytic activity or have PK modulating properties. In a preferred embodiment, all the ligands have different properties.

The ligand or tethered ligand can be present on a monomer when said monomer is incorporated into a component of the dsRNA agent of the invention (*e.g.*, a dsRNA agent of the invention or linker). In some embodiments, the ligand can be incorporated via coupling to a "precursor" monomer after said "precursor" monomer has been incorporated into a component of the dsRNA agent of the invention (*e.g.*, a dsRNA agent of the invention or linker). For example, a monomer having, *e.g.*, an amino-terminated tether (*i.e.*, having no associated ligand), *e.g.*, monomer-linker-NH_2 can be incorporated into into a component of the compounds of the invention (*e.g.*, a dsRNA agent of the invention or linker). In a subsequent operation, *i.e.*, after incorporation of the precursor monomer into a component of the compounds of the invention (*e.g.*, a dsRNA agent of the

invention or linker), a ligand having an electrophilic group, *e.g.*, a pentafluorophenyl ester or aldehyde group, can subsequently be attached to the precursor monomer by coupling the electrophilic group of the ligand with the terminal nucleophilic group of the precursor monomer's tether.

In another example, a monomer having a chemical group suitable for taking part in Click Chemistry reaction can be incorporated *e.g.*, an azide or alkyne terminated tether/linker. In a subsequent operation, *i.e.*, after incorporation of the precursor monomer into the strand, a ligand having complementary chemical group, *e.g.* an alkyne or azide can be attached to the precursor monomer by coupling the alkyne and the azide together.

In some embodiments, ligand can be conjugated to nucleobases, sugar moieties, or internucleosidic linkages of the dsRNA agent of the invention. Conjugation to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In some embodiments, the 2-, 6-, 7-, or 8-positions of a purine nucleobase are attached to a conjugate moiety. Conjugation to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2-, 5-, and 6-positions of a pyrimidine nucleobase can be substituted with a conjugate moiety. When a ligand is conjugated to a nucleobase, the preferred position is one that does not interfere with hybridization, *i.e.*, does not interfere with the hydrogen bonding interactions needed for base pairing.

Conjugation to sugar moieties of nucleosides can occur at any carbon atom. Example carbon atoms of a sugar moiety that can be attached to a conjugate moiety include the 2', 3', and 5' carbon atoms. The 1' position can also be attached to a conjugate moiety, such as in an abasic residue. Internucleosidic linkages can also bear conjugate moieties. For phosphorus-containing linkages (*e.g.*, phosphodiester, phosphorothioate, phosphorodithiotate, phosphoroamidate, and the like), the conjugate moiety can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleosidic linkages (*e.g.*, PNA), the conjugate moiety can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

There are numerous methods for preparing conjugates of oligonuclotides. Generally, an oligonucleotide is attached to a conjugate moiety by contacting a reactive group (*e.g.*, OH, SH, amine, carboxyl, aldehyde, and the like) on the oligonucleotide with a reactive group on the conjugate moiety. In some embodiments, one reactive group is electrophilic and the other is nucleophilic.

For example, an electrophilic group can be a carbonyl-containing functionality and a nucleophilic group can be an amine or thiol. Methods for conjugation of nucleic acids and related oligomeric compounds with and without linking groups are well described in the literature such as, for example, in Manoharan in Antisense Research and Applications, Crooke and LeBleu, eds., CRC Press, Boca Raton, Fla., 1993, Chapter 17, which is incorporated herein by reference in its entirety.

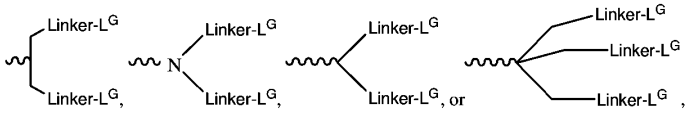
The ligand can be attached to the dsRNA agent of the inventions via a linker or a carrier monomer, *e.g.*, a ligand carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A

"backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier monomer into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of an oligonucleotide. A "tethering attachment point" (TAP) in refers to an atom of the carrier monomer, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The selected moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the carrier monomer. Thus, the carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent atom.

Representative U.S. patents that teach the preparation of conjugates of nucleic acids include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218, 105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578, 717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118, 802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578, 718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762, 779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904, 582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082, 830; 5,112,963; 5,149,782; 5,214,136; 5,245,022; 5,254, 469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317, 098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510, 475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574, 142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599, 923; 5,599,928; 5,672,662; 5,688,941; 5,714,166; 6,153, 737; 6,172,208; 6,300,319; 6,335,434; 6,335,437; 6,395, 437; 6,444,806; 6,486,308; 6,525,031; 6,528,631; 6,559, 279; contents of which are herein incorporated in their entireties by reference.

In some embodiments, the dsRNA agent further comprises a targeting ligand that targets a liver tissue. In some embodiments, the targeting ligand is a carbohydrate-based ligand. In one embodiment, the targeting ligand is a GalNAc conjugate.

In certain embodiments, the dsRNA agent of the invention further comprises a ligand having a structure shown below:



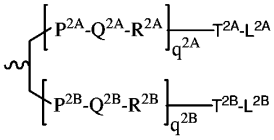
wherein:

L^G is independently for each occurrence a ligand, *e.g.*, carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, polysaccharide; and

Z', Z", Z"' and Z"" are each independently for each occurrence O or S.

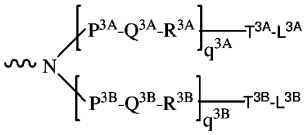
In certain embodiments, the dsRNA agent of the invention comprises a ligand of Formula (II), (III), (IV) or (V):

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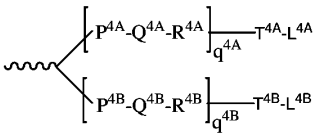
Formula (II)

,



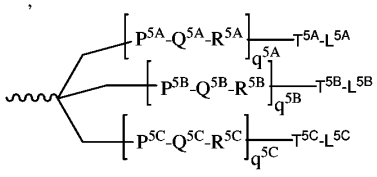
Formula (III)

,



Formula (IV)

, or



Formula (V)

,

wherein:

q^{2A}, q^{2B}, q^{3A}, q^{3B}, $q4^{A}$, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

Q and Q' are independently for each occurrence is absent, $-(P^{7}\text{-}Q^{7}\text{-}R^{7})_{p}\text{-}T^{7}$- or $-T^{7}\text{-}Q^{7}\text{-}T^{7'}\text{-}B\text{-}T^{8'}\text{-}Q^{8}\text{-}T^{8}$;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, P^{7}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C}, T^{7}, $T^{7'}$, T^{8} and $T^{8'}$ are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH or CH_2O;

B is $-CH_2\text{-}N(B^{L})\text{-}CH_2$-;

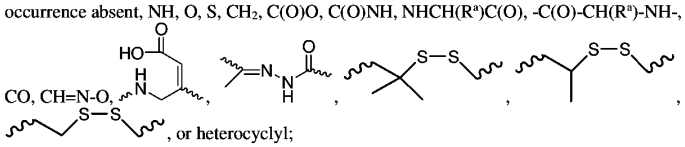
B^{L} is $-T^{B}\text{-}Q^{B}\text{-}T^{B'}\text{-}R^{x}$;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C}, Q^{7}, Q^{8} and Q^{B} are independently for each occurrence absent, alkylene, substituted alkylene and wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^{N})$, C(R')=C(R'), C≡C or C(O);

T^{B} and $T^{B'}$ are each independently for each occurrence absent, CO, NH, O, S, OC(O), OC(O)O, NHC(O), NHC(O)NH, NHC(O)O, CH_2, CH_2NH or CH_2O;

R^{x} is a lipophile (*e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine), a vitamin (*e.g.*, folate, vitamin A, vitamin E, biotin, pyridoxal), a peptide, a carbohydrate (*e.g.*, monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, polysaccharide), an endosomolytic component, a steroid (*e.g.*, uvaol, hecigenin, diosgenin), a terpene (*e.g.*, triterpene, *e.g.*, sarsasapogenin, Friedelin, epifriedelanol derivatized lithocholic acid), or a cationic lipid;

R^1, R^2, R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C}, R^7 are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-NH-,



, or heterocyclyl;

L^1, L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} are each independently for each occurrence a carbohydrate, *e.g.*, monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide;

R' and R" are each independently H, C_1-C_6 alkyl, OH, SH, or $N(R^N)_2$;

R^N is independently for each occurrence H, methyl, ethyl, propyl, isopropyl, butyl or benzyl;

R^a is H or amino acid side chain;

Z', Z", Z"' and Z"" are each independently for each occurrence O or S;

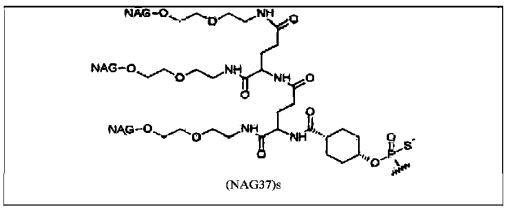
p represent independently for each occurrence 0-20.

As discussed above, because the ligand can be conjugated to the iRNA agent via a linker or carrier, and because the linker or carrier can contain a branched linker, the iRNA agent can then contain multiple ligands via the same or different backbone attachment points to the carrier, or via the branched linker(s). For instance, the branchpoint of the branched linker may be a bivalent, trivalent, tetravalent, pentavalent ,or hexavalent atom, or a group presenting such multiple valencies. In certain embodiments, the branchpoint is -N, -N(Q)-C, -O-C, -S-C, -SS-C, -C(O)N(Q)-C, -OC(O)N(Q)-C, -N(Q)C(O)-C, or -N(Q)C(O)O-C; wherein Q is independently for each occurrence H or optionally substituted alkyl. In other embodiment, the branchpoint is glycerol or glycerol derivative.

Suitable ligands for use in the compositions of the invention are described in U.S. Patent Nos. 8,106,022, 8,450,467, 8,882,895, 9,352,048, 9,370,581, 9,370,582, 9,867,882, 10,806,791, and 11,110,174, and U.S. Patent Publication Nos. 2009/239814, 200/9247608, 2012/136042, 2013/178512, 2014/179761, 2015/011615, 2015/119444, 2015/119445, 2016/051691, 2016/375137, 2018/326070, 2019/099493, 2019/184018, and 2020/297853, the entire contents of each of which are incorporated herein by reference.

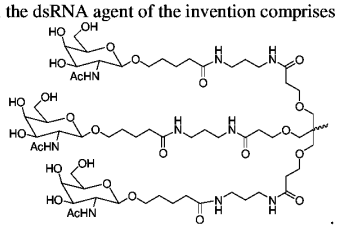
In some embodiments, a suitable ligand is a ligand disclosed in WO 2019/055633, the entire contents of which are incorporated herein by reference. In one embodiment the ligand comprises the structure below:

M



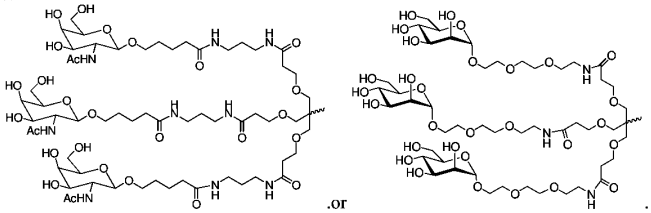
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In certain embodiments, the dsRNA agent of the invention comprises a ligand of structure:



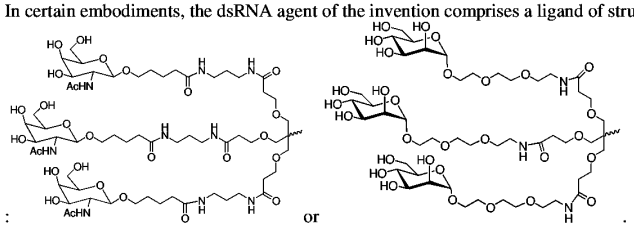
.

In certain embodiments, the dsRNA agent of the invention is conjugated with a ligand of structure:



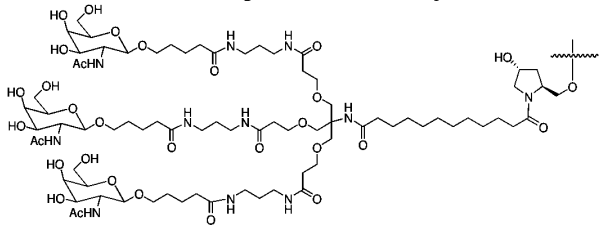
.or .

In certain embodiments, the dsRNA agent of the invention comprises a ligand of structure:



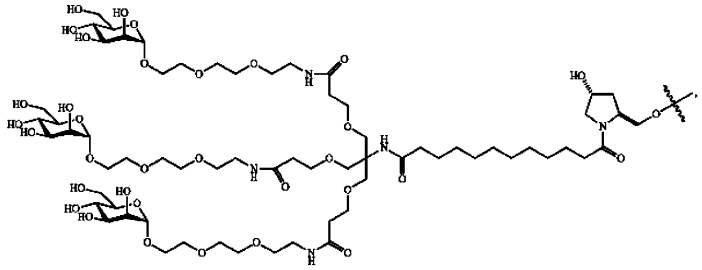
: or .

In certain embodiments, the dsRNA agent of the invention comprises a monomer of structure:

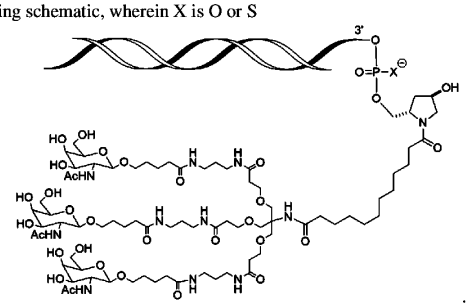


or

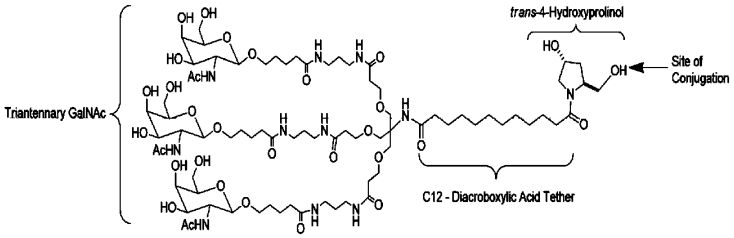
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In some embodiments, the RNAi agent is attached to the carbohydrate conjugate via a linker as shown in the following schematic, wherein X is O or S



In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



Synthesis of above described ligands and monomers is described, for example, in US Patent No. 8,106,022, content of which is incorporated herein by reference in its entirety.

VIII. Delivery of an RNAi Agent of the Disclosure

The delivery of a RNAi agent of the disclosure to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having a target gene-associated disorder, can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an RNAi agent of the disclosure either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an RNAi agent, *e.g.,* a dsRNA, to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the RNAi agent. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (*in vitro* or *in vivo*) can be adapted for use with a RNAi agent of the disclosure (see *e.g.,* Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to consider in order to deliver an RNAi agent include, for example, biological stability of the delivered agent, prevention of non-specific effects, and accumulation of the delivered agent in the target tissue. The non-specific effects of an RNAi agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the RNAi agent to be administered. Several studies have shown successful knockdown of gene products when an RNAi agent is administered locally. For example, pulmonary delivery, *e.g.,* inhalation, of a dsRNA, *e.g.,* SOD1, has been shown to effectively knockdown gene and protein expression in lung tissue and that there is excellent uptake of the dsRNA by the bronchioles and alveoli of the lung. Intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, MJ. *et al.*, (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, SJ. *et al.* (2003) *Mol. Vis.* 9:210-216) were also both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J. *et al.* (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, WJ. *et al.*, (2006) *Mol. Ther.* 14:343-350; Li, S. *et al.*, (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the lungs by intranasal administration (Howard, KA. *et al.*, (2006) *Mol. Ther.* 14:476-484; Zhang, X. *et al.*, (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V. *et al.*, (2005) *Nat. Med.* 11:50-55). For administering a RNAi agent systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases *in vivo*. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the RNAi agent to the target tissue and avoid undesirable off-target effects (*e.g.,* without wishing to be bound by theory, use of GNAs as described herein has been identified to destabilize the seed region of a dsRNA, resulting in enhanced preference of such dsRNAs for on-target effectiveness,

relative to off-target effects, as such off-target effects are significantly weakened by such seed region destabilization). RNAi agents can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, a RNAi agent directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J. *et al.*, (2004) *Nature* 432:173-178). Conjugation of an RNAi agent to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, JO. *et al.*, (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the RNAi agent can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of molecule RNAi agent (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an RNAi agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an RNAi agent, or induced to form a vesicle or micelle (see *e.g.,* Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases an RNAi agent. The formation of vesicles or micelles further prevents degradation of the RNAi agent when administered systemically. Methods for making and administering cationic- RNAi agent complexes are well within the abilities of one skilled in the art (see *e.g.,* Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of RNAi agents include DOTAP (Sorensen, DR., *et al* (2003), supra; Verma, UN. *et al.*, (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.,* (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, a RNAi agent forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of RNAi agents and cyclodextrins can be found in U.S. Patent No. 7, 427, 605, which is herein incorporated by reference in its entirety.

Certain aspects of the instant disclosure relate to a method of reducing the expression of a target gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a skeletal muscle. In one embodiment, the cell is a cardiac muscle cell. In one embodiment, the cell is an adipocyte.

In certain embodiments, the RNAi agent is taken up on one or more tissue or cell types present in organs, *e.g.,* liver, skeletal muscle, cardiac muscle, adipose tissue.

Another aspect of the disclosure relates to a method of reducing the expression and/or activity of a target gene in a subject, comprising administering to the subject the double-stranded RNAi agent of the disclosure.

Another aspect of the disclosure relates to a method of treating a subject having a target gene-associated disorder or at risk of having or at risk of developing a target gene-associated disorder, comprising administering to the subject a therapeutically effective amount of the double-stranded RNAi agent of the disclosure, thereby treating the subject.

In one embodiment, the double-stranded RNAi agent is administered subcutaneously.

In one embodiment, the double-stranded RNAi agent is administered intramuscularly.

In one embodiment, the double-stranded RNAi agent is administered by intravenously.

In one embodiment, the double-stranded RNAi agent is administered by pulmonary sytem administration, *e.g.*, intranasal administration, or oral inhalative administration.

For ease of exposition the formulations, compositions and methods in this section are discussed largely with regard to modified siRNA compounds. It may be understood, however, that these formulations, compositions and methods can be practiced with other siRNA compounds, *e.g.*, unmodified siRNA compounds, and such practice is within the disclosure. A composition that includes a RNAi agent can be delivered to a subject by a variety of routes. Exemplary routes include pulmonary system, intravenous, subcutaneous, oral, topical, rectal, anal, vaginal, nasal, and ocular.

The RNAi agents of the disclosure can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of RNAi agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The pharmaceutical compositions of the present disclosure may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be intratracheal, intranasal, topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral, parenteral, or pulmonary, *e.g.*, by inhalation or insufflation of powders or aerosols, including by nebulizer. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal, or intramuscular injection administration.

The route and site of administration may be chosen to enhance targeting. For example, to target muscle cells, intramuscular injection into the muscles of interest would be a logical choice. Lung cells might be targeted by administering the RNAi agent in powder or aerosol form. The vascular endothelial cells could be targeted by coating a balloon catheter with the RNAi agent and mechanically introducing the RNA.

Compositions for pulmonary system delivery may include aqueous solutions, *e.g.*, for intranasal or oral inhalative administration, suitable carriers composed of, *e.g.*, lipids (liposomes, niosomes, microemulsions, lipidic micelles, solid lipid nanoparticles) or polymers (polymer micelles, dendrimers, polymeric nanoparticles, nonogels, nanocapsules), adjuvant, *e.g.*, for oral inhalative administration. Aqueous compositions may be sterile and may optionally contain buffers, diluents, absorbtion enhancers and other suitable additives. Such administration permits both systemic and local delivery of the double stranded RNAi agents of the invention.

Intranasal administration may include instilling or insufflating a double stranded RNAi agent into the nasal cavity with syringes or droppers by applying a few drops at a time or via atomization. Suitable dosage forms for intranasal administration include drops, powders, nebulized mists, and sprays. Nasal delivery devices include, but not limited to, vapor inhaler, nasal dropper, spray bottle, metered dose spray pump, gas driven spray atomizer, nebulizer, mechanical powder sprayer, breath actuated inhaler, and insufflator. Devices for delivery deeper into the respiratory system, *e.g.*, into the lung, include nebulizer, pressured metered-dose inhaler, dry powder inhaler, and thermal vaporization aerosol device. Devices for delivery by inhalation are available from commercial suppliers.Devices can be fixed or variable dose, single or multidose, disposable or reusable depending on, for example, the disease or disorder to be prevented or treated, the volume of the agent to be delivered, the frequency of delivery of the agent, and other considerations in the art.

Oral inhalative administration may include use of device, *e.g.*, a passive breath driven or active power driven single/-multiple dose dry powder inhaler (DPI), to deliver a double stranded RNAi agent to the pulmonary system. Suitable dosage forms for oral inhalative administration include powders and solutions. Suitable devices for oral inhalative administration include nebulizers, metered-dose inhalers, and dry powder inhalers. Dry powder inhalers are of the most popular devices used to deliver drugs, especially proteins to the lungs. Exemplary commercially available dry powder inhalers include Spinhaler (Fisons Pharmaceuticals, Rochester, NY) and Rotahaler (GSK, RTP, NC). Several types of nebulizers are available, namely jet nebulizers, ultrasonic nebulizers, vibrating mesh nebulizers. Jet nebulizers are driven by compressed air. Ultrasonic nebulizers use a piezoelectric transducer in order to create droplets from an open liquid reservoir. Vibrating mesh nebulizers use perforated membranes actuated by an annular piezoelement to vibrate in resonant bending mode. The holes in the membrane have a large cross-section size on the liquid supply side and a narrow cross-section size on the side from where the droplets emerge. Depending on the therapeutic application, the hole sizes and number of holes can be adjusted. Selection of a suitable device depends on parameters, such as nature of the drug and its formulation, the site of action, and pathophysiology of the lung. Aqueous suspensions and solutions are nebulized effectively. Aerosols based on mechanically generated vibration mesh technologies also have been used successfully to deliver proteins to lungs.

The amount of RNAi agent for pulmonary system administration may vary from one target gene to another target gene and the appropriate amount that has to be applied may have to be

determined individually for each target gene. Typically, this amount ranges from 10 µg to 2 mg, or 50 µg to 1500 µg, or 100 µg to 1000 µg.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves, and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening or flavoring agents can be added. Compositions suitable for oral administration of the agents of the invention are further described in PCT Application No. PCT/US20/33156, the entire contents of which are incorporated herein by reference.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

In one embodiment, the administration of the siRNA compound, *e.g.,* a double-stranded siRNA compound, is parenteral, *e.g.,* intravenous (*e.g.,* as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary system, intranasal, urethral, or ocular. Administration can be provided by the subject or by another person, *e.g.,* a health care provider. The medication can be provided in measured doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

A. *Vector encoded RNAi agents of the Disclosure*

RNAi agents targeting the target gene can be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.,* Couture, A, *et al.*, *TIG.* (1996), 12:5-10; WO 00/22113, WO 00/22114, and US 6,054,299). Expression can be sustained (months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292).

The individual strand or strands of a RNAi agent can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.,* by transfection or infection) into a target

cell. Alternatively, each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

RNAi agent expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, such as those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of a RNAi agent as described herein. Delivery of RNAi agent expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.,* vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of a RNAi agent will generally require regulatory elements, *e.g.,* promoters, enhancers, *etc.*, to ensure the expression of the RNAi agent in target cells. Other aspects to consider for vectors and constructs are known in the art.

IX. Pharmaceutical Compositons

The present disclosure also includes pharmaceutical compositions and formulations which include the RNAi agents of the disclosure. In one embodiment, provided herein are pharmaceutical compositions containing an RNAi agent, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the RNAi agent are useful for treating a subject who would benefit from inhibiting or reducing the expression of a target gene, *e.g.,* a subject having a target gene –associated disorder. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration *via* parenteral delivery, *e.g.,* by intravenous (IV), intramuscular (IM), or for subcutaneous (subQ) delivery.

In some embodiments, the pharmaceutical compositions of the invention are pyrogen free or non-pyrogenic.

In one embodiment, the delivery vehicle can deliver an iRNA compound, *e.g.*, a double-stranded iRNA compound, or ssiRNA compound, (*e.g.*, a precursor thereof, *e.g.*, a larger siRNA compound which can be processed into a ssiRNA compound, or a DNA which encodes an siRNA

compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof) to a cell by a topical route of administration. The delivery vehicle can be microscopic vesicles. In one example the microscopic vesicles are liposomes. In some embodiments the liposomes are cationic liposomes. In another example the microscopic vesicles are micelles.In one aspect, the invention features a pharmaceutical composition including an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, (*e.g.*, a precursor thereof, *e.g.*, a larger siRNA compound which can be processed into a ssiRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof) in an injectable dosage form. In one embodiment, the injectable dosage form of the pharmaceutical composition includes sterile aqueous solutions or dispersions and sterile powders. In some embodiments the sterile solution can include a diluent such as water; saline solution; fixed oils, polyethylene glycols, glycerin, or propylene glycol.

In one aspect, the invention features a pharmaceutical composition including an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, (*e.g.*, a precursor thereof, *e.g.*, a larger siRNA compound which can be processed into a ssiRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof) in oral dosage form. In one embodiment, the oral dosage form is selected from the group consisting of tablets, capsules and gel capsules. In another embodiment, the pharmaceutical composition includes an enteric material that substantially prevents dissolution of the tablets, capsules or gel capsules in a mammalian stomach. In some embodiments the enteric material is a coating. The coating can be acetate phthalate, propylene glycol, sorbitan monoleate, cellulose acetate trimellitate, hydroxy propyl methyl cellulose phthalate or cellulose acetate phthalate. In one embodiment, the oral dosage form of the pharmaceutical composition includes a penetration enhancer, *e.g.*, a penetration enhancer described herein.

In another embodiment, the oral dosage form of the pharmaceutical composition includes an excipient. In one example the excipient is polyethyleneglycol. In another example the excipient is precirol.

In another embodiment, the oral dosage form of the pharmaceutical composition includes a plasticizer. The plasticizer can be diethyl phthalate, triacetin dibutyl sebacate, dibutyl phthalate or triethyl citrate.

X. Methods of the Invention

Another aspect of the invention relates to a method of reducing the expression of a target gene in a cell, comprising contacting the cell with a dsRNA agent of the invention. The methods include contacting the cell with a dsRNA of the disclosure and maintaining the cell for a time sufficient to obtain degradation of the mRNA transcripts of a target gene, thereby inhibiting expression of the target gene in the cell.

Reduction in gene expression can be assessed by any methods known in the art. For example, a reduction in the expression of a target may be determined by determining the mRNA expression level of the target gene using methods routine to one of ordinary skill in the art, *e.g.*, northern blotting, qRT-PCR; by determining the protein level of a target protein using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques or histology based methods, such as IHC and ISH.

In the methods of the disclosure the cell may be contacted *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject. Contacting a cell in vivo with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of in vitro and in vivo methods of contacting a cell are also possible.

The cell may be an extra-heptic cell, such as askeletal muscle cell, a cardiac muscle cell, or an adipocyte.

A cell suitable for treatment using the methods of the disclosure may be any cell that expresses a target gene. A cell suitable for use in the methods of the disclosure may be a mammalian cell, *e.g.,* a primate cell (such as a human cell or a non-human primate cell, *e.g.,* a monkey cell or a chimpanzee cell), a non-primate cell (such as a rat cell, or a mouse cell. In one embodiment, the cell is a human cell, *e.g.,* a human liver cell or a human kidney cell.

Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In some embodiments, the targeting ligand is a carbohydrate moiety, *e.g.,* a GalNAc ligand, or any other ligand that directs the RNAi agent to a site of interest. In certain embodiments, the RNAi agent does not include a targeting ligand.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing" and other similar terms, and includes any level of inhibition. In certain embodiments, a level of inhibition, *e.g.,* for an RNAi agent of the instant disclosure, can be assessed in cell culture conditions, *e.g.,* wherein cells in cell culture are transfected *via* Lipofectamine™-mediated transfection at a concentration in the vicinity of a cell of 10 nM or less, 1 nM or less, *etc.* Knockdown of a given RNAi agent can be determined *via* comparison of pre-treated levels in cell culture versus post-treated levels in cell culture, optionally also comparing against cells treated in parallel with a scrambled or other form of control RNAi agent. Knockdown in cell culture of, *e.g.,* 50% or more, can thereby be identified as indicative of "inhibiting" or "reducing", "downregulating" or "suppressing", *etc.* having occurred. It is expressly contemplated that assessment of targeted mRNA or encoded protein levels (and therefore an extent of "inhibiting", *etc.* caused by a RNAi agent of the disclosure) can also be assessed in *in vivo* systems for the RNAi agents of the instant disclosure, under properly controlled conditions as described in the art.

The phrase "inhibiting expression of a target gene" or "inhibiting expression of a target," as used herein, includes inhibition of expression of any target gene (such as, *e.g.,* a mouse target gene, a rat target gene, a monkey target gene, or a human target gene) as well as variants or mutants of a

target gene that encode a target protein. Thus, the target gene may be a wild-type target gene, a mutant target gene , or a transgenic target gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a target gene" includes any level of inhibition of a target gene, *e.g.*, at least partial suppression of the expression of a target gene, such as an inhibition by at least 20%. In certain embodiments, inhibition is by at least 30%, at least 40%, at least 50%, at least about 60%, at least 70%, at least about 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%; or to below the level of detection of the assay method. In certain method, inhibition is measured at a 10 nM concentration of the siRNA using the luciferase assay provided in Example 1.

The expression of a target gene may be assessed based on the level of any variable associated with target gene expression, *e.g.*, target mRNA level or target protein level.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the disclosure, expression of a target gene is inhibited by at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In certain embodiments, the methods include a clinically relevant inhibition of expression of a target gene, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of a target gene.

Inhibition of the expression of a target gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a target gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with a RNAi agent of the disclosure, or by administering a RNAi agent of the disclosure to a subject in which the cells are or were present) such that the expression of a target gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with a RNAi agent or not treated with a RNAi agent targeted to the genome of interest). The degree of inhibition may be expressed in terms of:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

In other embodiments, inhibition of the expression of a target gene may be assessed in terms of a reduction of a parameter that is functionally linked to a target gene expression, *e.g.*, target protein

expression. Target gene silencing may be determined in any cell expressing a target gene, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a target protein may be manifested by a reduction in the level of the target protein that is expressed by a cell or group of cells (*e.g.,* the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of genome suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of a target gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the disclosure. For example, the control cell or group of cells may be derived from an individual subject (*e.g.,* a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of target gene mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing RNA expression. In one embodiment, the level of expression of target gene in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the target gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, *in situ* hybridization, microarray analysis, and or histology based methods such as IHC and ISH. Circulating target mRNA may be detected using methods the described in WO2012/177906, the entire contents of which are hereby incorporated herein by reference.

In some embodiments, the level of expression of target gene is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific target nucleic acid or protein, or fragment thereof. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of RNA levels involves contacting the isolated RNA with a nucleic acid molecule (probe) that can hybridize to target RNA. In one embodiment, the RNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated RNA on an agarose gel and transferring the RNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the RNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known RNA detection methods for use in determining the level of target mRNA.

An alternative method for determining the level of expression of target in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.,* by RT-PCR (the experimental embodiment set forth in Mullis, 1987, US Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.*, US Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the disclosure, the level of expression of target is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System), by a Dual-Glo® Luciferase assay, or by other art-recognized method for measurement of target expression or mRNA level.

The expression level of target mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See US Patent Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of target expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of RNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of this PCR method is described and exemplified in the Examples presented herein. Such methods can also be used for the detection of target nucleic acids.

The level of target protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like. Such assays can also be used for the detection of proteins indicative of the presence or replication of target proteins.

In some embodiments, the efficacy of the methods of the disclosure in the treatment of a target gene-related disease is assessed by a decrease in target mRNA level (*e.g*, by assessment of a blood target gene level, or otherwise).

In some embodiments, the efficacy of the methods of the disclosure in the treatment of a target gene-related disease is assessed by a decrease in target mRNA level (*e.g*, by assessment of a liver or kidney sample for target level, by biopsy, or otherwise).

In some embodiments of the methods of the disclosure, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of target may be assessed using measurements of the level or change in the level of target mRNA or target protein in a sample derived from a specific site within the subject, *e.g.,* liver or kidney cells. In certain embodiments, the methods include a clinically relevant inhibition of expression of target, *e.g.* as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of target gene.

As used herein, the terms detecting or determining a level of an analyte are understood to mean performing the steps to determine if a material, *e.g.,* protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an analyte level that is below the level of detection for the method used.

The *in vivo* methods of the disclosure may include administering to a subject a composition containing a RNAi agent, where the RNAi agent includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the taregt gene of the subject to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection. In certain embodiments, the compositions are administered orally. In certain embodiments, the compositions are administered by pulmonary delivery, *e.g.*, oral inhalation or intranasal delivery.

In some embodiments, the administration is *via* a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.,* a desired inhibition of the target gene, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In certain embodiments, the depot injection is a subcutaneous injection.

In one embodiment, the double-stranded RNAi agent is administered by pulmonary sytem administration, *e.g.*, intranasal administration or oral inhalative administration. Pulmonary system administration may be via a syringe, a dropper, atomization, or use of device, *e.g.*, a passive breath driven or active power driven single/-multiple dose dry powder inhaler (DPI) device.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present disclosure also provides methods for inhibiting the expression of a target gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a target gene in a cell of the mammal and maintaining the mammal

for a time sufficient to obtain degradation of the RNA transcript of the target gene, thereby inhibiting expression of the target gene in the cell. Reduction in genome expression can be assessed by any methods known it the art and by methods, *e.g.* qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, *e.g.* ELISA, described herein.

The present disclosure further provides methods of treatment of a subject in need thereof. The treatment methods of the disclosure include administering an RNAi agent of the disclosure to a subject, *e.g.,* a subject that would benefit from inhibition of target gene expression, in a therapeutically effective amount of a RNAi agent targeting a target gene or a pharmaceutical composition comprising a RNAi agent targeting a target gene.

Target genes (described above), target gene-associated disorders, and subjects that would benefit from a reduction or inhibition of target gene expression, *e..g.,* those having a target gene-associated disease, subjects at risk of developing a target gene-associate disease, are described below.

An RNAi agent of the disclosure may be administered as a "free RNAi agent." A free RNAi agent is administered in the absence of a pharmaceutical composition. The naked RNAi agent may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the RNAi agent can be adjusted such that it is suitable for administering to a subject. In certain embodiments, the free RNAi agent may be formulated in water or normal saline.

Alternatively, an RNAi agent of the disclosure may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

In one aspect, the present invention provides a method of treating a subject having a skeletal muscle disorder, a cardiac muscle disorder, or an adipose tissue disorder, comprising administering to the subject a therapeutically effective amount of a dsRNA agent of the invention, thereby treating the subject.

Exemplary cardiac muscle disorders include obstructive hypertrophic cardiomyopathy (HOCM); familial hypertrophic cardiomyopathy (FHC); Heart failure with preserved ejection fraction (HFPEF); atrial fibrillation (AFIB); ventricular fibrillation (VFIB); angina; myocardial infarction (MI); heart failure or heart failure with reduced ejection fraction (HFREF); supraventricular tachycardia (SVT); and hypertrophic cardiomyopathy (HCM).

Exemplary skeletal muscle disorders include Myostatin-related muscle hypertrophy, congenital myasthenic syndrome, and facioscapulohumeral muscular dystrophy (FSHD).

Exemplary adipose tissue disorders include a metabolic disorder, *e.g.* metabolic syndrome, a disorder of carbohydrates, *e.g.*, type II diabetes, pre-diabetes, a lipid metabolism disorder, *e.g.*, a hyperlipidemia, hypertension, a cardiovascular disease, a disorders of body weight.

The dsRNA agent of the invention can be delivered to a subject by a variety of routes, depending on the type of genes targeted and the type of disorders to be treated. In some

embodiments, the dsRNA agent is administered extra-hepatically, such as intravenous, intramuscular, or subcutaneous administration.

The disclosure further provides methods for the use of a RNAi agent or a pharmaceutical composition thereof, *e.g.*, for treating a subject that would benefit from reduction or inhibition of target gene expression, *e.g.*, a subject having a target-gene-associated disorder, in combination with other pharmaceuticals or other therapeutic methods, *e.g.*, with known pharmaceuticals or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

Examples of the additional therapeutic agents which can be used with an RNAi agent of the invention include, but are not limited to, diabetes mellitus-treating agents, diabetic complication-treating agents, cardiovascular diseases-treating agents, anti-hyperlipemic agents, hypotensive or antihypertensive agents, anti-obesity agents, nonalcoholic steatohepatitis (NASH)-treating agents, chemotherapeutic agents, immunotherapeutic agents, immunosuppressive agents, nonsteroidal anti-inflammatory drugs (NSAIDs), colchicine, corticosteroids, and the like. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

Examples of agents for treating diabetes mellitus include insulin formulations (*e.g.*, animal insulin formulations extracted from a pancreas of a cattle or a swine; a human insulin formulation synthesized by a gene engineering technology using microorganisms or methods), insulin sensitivity enhancing agents, pharmaceutically acceptable salts, hydrates, or solvates thereof (*e.g.*, pioglitazone, troglitazone, rosiglitazone, netoglitazone, balaglitazone, rivoglitazone, tesaglitazar, farglitazar, CLX-0921, R-483, NIP-221, NIP-223, DRF-2189, GW-7282TAK-559, T-131, RG-12525, LY-510929, LY-519818, BMS-298585, DRF-2725, GW-1536, GI-262570, KRP-297, TZD18 (Merck), DRF-2655, and the like), alpha-glycosidase inhibitors (*e.g.*, voglibose, acarbose, miglitol, emiglitate and the like), biguanides (*e.g.*, phenformin, metformin, buformin and the like) or sulfonylureas (*e.g.*, tolbutamide, glibenclamide, gliclazide, chlorpropamide, tolazamide, acetohexamide, glyclopyramide, glimepiride and the like) as well as other insulin secretion-promoting agents (*e.g.*, repaglinide, senaglinide, nateglinide, mitiglinide, GLP-1 and the like), amyrin agonist (*e.g.*, pramlintide and the like), phosphotyrosin phosphatase inhibitor (*e.g.*, vanadic acid and the like) and the like.

Examples of agents for treating diabetic complications include, but are not limited to, aldose reductase inhibitors (*e.g.*, tolrestat, epalrestat, zenarestat, zopolrestat, minalrestat, fidareatat, SK-860, CT-112 and the like), neurotrophic factors (*e.g.*, NGF, NT-3, BDNF and the like), PKC inhibitors (*e.g.*, LY-333531 and the like), advanced glycation end-product (AGE) inhibitors (*e.g.*, ALT946, pimagedine, pyradoxamine, phenacylthiazolium bromide (ALT766) and the like), active oxygen quenching agents (*e.g.*, thioctic acid or derivative thereof, a bioflavonoid including flavones, isoflavones, flavonones, procyanidins, anthocyanidins, pycnogenol, lutein, lycopene, vitamins E, coenzymes Q, and the like), cerebrovascular dilating agents (*e.g.*, tiapride, mexiletene and the like).

Anti-hyperlipemic agents include, for example, statin-based compounds which are cholesterol synthesis inhibitors (*e.g.,* pravastatin, simvastatin, lovastatin, atorvastatin, fluvastatin, rosuvastatin and the like), squalene synthetase inhibitors or fibrate compounds having a triglyceride-lowering effect (*e.g.,* fenofibrate, gemfibrozil, bezafibrate, clofibrate, sinfibrate, clinofibrate and the like), niacin, PCSK9 inhibitors, triglyceride lowing agents or cholesterol sequesting agents.

Hypotensive agents include, for example, angiotensin converting enzyme inhibitors (*e.g.,* captopril, enalapril, delapril, benazepril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, trandolapril and the like) or angiotensin II antagonists (*e.g.,* losartan, candesartan cilexetil, olmesartan medoxomil, eprosartan, valsartan, telmisartan, irbesartan, tasosartan, pomisartan, ripisartan forasartan, and the like) or calcium channel blockers (*e.g.,* amlodipine) or aspirin.

Nonalcoholic steatohepatitis (NASH)-treating agents include, for example, ursodiol, pioglitazone, orlistat, betaine, rosiglitazone.

Anti-obesity agents include, for example, central antiobesity agents (*e.g.,* dexfenfluramine, fenfluramine, phentermine, sibutramine, amfepramone, dexamphetamine, mazindol, phenylpropanolamine, clobenzorex and the like), gastrointestinal lipase inhibitors (*e.g.,* orlistat and the like), beta 3-adrenoceptor agonists (*e.g.,* CL-316243, SR-58611-A, UL-TG-307, SB-226552, AJ-9677, BMS-196085 and the like), peptide-based appetite-suppressing agents (*e.g.,* leptin, CNTF and the like), cholecystokinin agonists (*e.g.,* lintitript, FPL-15849 and the like) and the like.

In addition, agents whose cachexia improving effect has been established in an animal model or at a clinical stage, such as cyclooxygenase inhibitors (*e.g.,* indomethacin and the like), progesterone derivatives (*e.g.,* megestrol acetate), glucosteroid (*e.g.,* dexamethasone and the like), metoclopramide-based agents, tetrahydrocannabinol-based agents, lipid metabolism improving agents (*e.g.,* eicosapentanoic acid and the like), growth hormones, IGF-1, antibodies against TNF-α, LIF, IL-6 and oncostatin M may also be employed concomitantly with an RNAi agent according to the present invention. Additional therapeutic agents for use in the treatment of diseases or conditions related to metabolic disorders and/or impaired neurological signaling would be apparent to the skilled artisan and are within the scope of this disclosure.

The RNAi agent and additional therapeutic agents may be administered at the same time or in the same combination, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target gene is decreased, for at least one month. In some embodiments, expression is decreased for at least 2 months, 3 months, or 6 months.

In certain embodiments, administration includes a loading dose administered at a higher frequency, *e.g.,* once per day, twice per week, once per week, for an initial dosing period, *e.g.,* 2-4 doses.

In some embodiments, the RNAi agents useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target gene. Compositions and methods for inhibiting the expression of these genes using RNAi agents can be prepared and performed as described herein.

Administration of the dsRNA according to the methods of the disclosure may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with a target gene-associated disorder. By "reduction" in this context is meant a statistically significant or clinically significant decrease in such level. The reduction can be, for example, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of a RNAi agent targeting a target gene of interest or pharmaceutical composition thereof, "effective against" a target gene-associated disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as an improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating target gene-associated disorders and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and at least 20%, 30%, 40%, 50%, or more can be indicative of effective treatment. Efficacy for a given RNAi agent drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using a RNAi agent or RNAi agent formulation as described herein.

Subjects can be administered a therapeutic amount of dsRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The RNAi agent can be administered over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the RNAi agent can reduce target gene levels, *e.g.,* in a cell, tissue, blood sample or other compartment of the patient by at least 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70,% 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99% or more. In one embodiment, administration of the RNAi agent can reduce target gene levels, *e.g.,* in a cell, tissue, blood sample, or other compartment of the patient by at least 50%.

Before administration of a full dose of the RNAi agent, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (*e.g.,* TNF-alpha or INF-alpha) levels.

Alternatively, the RNAi agent can be administered by oral administration, pulmonary admistration, intravenously, *i.e.,* by intravenous injrection, or subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired, *e.g.,* monthly dose of RNAi agent to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimine may include administration of a therapeutic amount of RNAi agent on a regular basis, such as monthly or extending to once a quarter, twice per year, once per year. In certain embodiments, the RNAi agent is administered about once per month to about once per quarter (*i.e.*, about once every three months).

XI. Target Genes and Target-Gene-Associated Diseases

Target Genes

Without limitations, genes targeted by the siRNAs of the invention include, but are not limited to genes which mediate a skeletal muscle disorder, a cardiac muscle disorder, or an adipose tissue disorder.

Specific exemplary target genes that mediate a cardiac muscle disorder include, but are not limited to, adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4

(KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); or Phosphodiesterase 1 (PDE1).

Specific exemplary target genes that mediate a skeletal muscle disorder include, but are not limited to, myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

Specific exemplary target genes that mediate an adipose tissue disorder include, but are not limited to, Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin; folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); and Activin A Receptor Type 1C (ACVR1C).

Targeting Cardiac Tissue

In some embodiments, the present invention provides a double-stranded iRNA agent that targets ADRB1 for the treatment of obstructive hypertrophic cardiomyopathy (HOCM); familial hypertrophic cardiomyopathy (FHC); heart failure with preserved ejection fraction (HF-pEF); atrial fibrillation (AFIB); ventricular fibrillation (VFIB); angina; myocardial infarction (MI); and/or heart failure or heart failure with reduced ejection fraction (HFREF).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CACNA1C for the treatment of supraventricular tachycardia (SVT); AFIB; Angina; and/or HOCM.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CACNA1G for the treatment of supraventricular tachycardia (SVT); and/or angina.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets AGTR1 for the treatment of HOCM; hypertrophic cardiomyopathy (HCM); and/or HF-pEF.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets SCN2A for the prevention and/or treatment of AFIB.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets HCN1 for the prevention and/or treatment of AFIB; treatment, *e.g.,* rate control, in HOCM.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets HCN4 for the prevention and/or treatment of AFIB; treatment, *e.g.,* rate control, in HOCM.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets HCN3 for the prevention and/or treatment of AFIB; treatment, *e.g.,* rate control, in HOCM.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets KCNA5 for the prevention and/or treatment of AFIB, *e.g.,* AFIB in congestive heart failure (CHF).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets KCNJ3 for the prevention and/or treatment of AFIB.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets KCNJ4 for the prevention and/or treatment of AFIB.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CAMK2D for the prevention and/or treatment of heart failure and/or AFIB.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets PLN for the prevention and/or treatment of HF-rEF, arrhythmia, and/or cardiomyopathy.In some embodiments, the present invention provides a double-stranded iRNA agent that targets PDE1 for the prevention and/or treatment of CHF and/or HF-pEF.

Targeting ADRB1 for the prevention and/or treatment of HOCM, FHC, HFPEF, AFIB, VFIB, angina, MI, and/or HFREF

The beta-adrenergic receptors (ADRBs) are part of a family of membrane proteins known as G-protein coupled receptors where, upon binding of a catecholamine to the receptor, stimulates a conformational change in the ADRB that causes coupling with G-proteins. G-proteins consist of α, β, and γ subunits and ADRB coupling leads to the dissociation of the G-protein into active Gα and Gβ subunits to mediate downstream signaling.

Beta-adrenergic receptors (ADRBs) play an important role in the extrinsic control of cardiac contractility and function, and are important drug targets for cardiovascular conditions such as hypertension and congestive heart failure. Inhaled beta-receptor (*e.g.* "beta-blockers") remain among the mostly commonly prescribed medications in adults to treat cardiovascular disease.

There are three subtypes of ADRBs (ADRB1, ADRB2 and ADRB3). ADRB1s are the predominant subtype expressed in the heart. Multiple ADRB1 have been described and found to be associated with various cardiovascular phenotypes, such as hypertension, heart failure, higher heart rates, or response to beta-blocker therapy. Genetic variants of the ADRB1 have also been shown to modulate the cardiac responses to catecholamine binding. In addition, ADRB1 signaling has also been shown to play an important role in heart failure (HF), where beta-blocking medications are widely used therapeutic agents. Deleterious effects of ADRB1 signaling include apoptosis, myocyte growth, fibroblast hyperplasia, myopathy, fetal gene induction and proarrhythmia (Mann DL, *et al. Circulation.* 1992;85(2):790-804). As an adaptive mechanism in HF, cardiac ADRB1s become less responsive, either downregulating or uncoupling from the G protein pathway (Bristow MR, *et al. N Engl J Med.* 1982;307(4):205-211). With respect to atrial fibrillation, ADRB1 variant carriers have been reported to have an increased risk of atrial fibrillation, and higher heart rates during atrial

fibrillation. ADRB1 polymorphisms are also associated with ventricular fibrillation (VF) in the context of myocardial infarction (MI).

Targeting CACNA1C for the prevention and/or treatment of SVT, AFIB, angina, and/or HOCM

Supraventricular tachycardia (SVT) is a heterogeneous category of cardiac arrhythmias characterized by a fast or tachycardiac rhythm that originates above the atrioventricular (AV) node. The prevalence of SVT is 2.25/1000 persons with a female predominance of 2:1 across all age groups (Lee KW, *et al., Curr Probl Cardiol* 2008;33:467–546). The most common SVTs include atrioventricular nodal re-entrant tachycardia, atrioventricular re-entrant tachycardia and atrial tachycardia. SVT increases patient morbidity, particularly when symptoms are frequent or incessant, and in a small cohort of patients with atrial fibrillation (AF) and ventricular pre-excitation, it can be life-threatening.

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia. The prevalence of AF increases as the population ages (Dang D, *et al. J Natl Med Assoc* 2002;94:1036-48). In AF, the upper chambers of the heart do not function correctly as a result of abnormal electrical signalling (Falk RH. *N Engl J Med* 2001;344:1067-78.). It can be characterized by rapid and irregular atrial depolarisations with a discrete lack of P waves on electrocardiograms. As a result, the blood in the atria remains static and can promote blood clot formation and increase the risk of stroke (Copley DJ, *et al. AACN Adv Crit Care* 2016;27:120-8.). This can cause detrimental symptoms, impair functional status and reduce the quality of life.

Angina represents the most common symptom of ischaemic heart disease, which is a major cause of death and disability worldwide. Nearly 10 million US adults experience stable angina, which occurs when myocardial oxygen supply does not meet demand, resulting in myocardial ischemia. Stable angina is associated with an average annual risk of 3% to 4% for myocardial infarction or death.

Hypertrophic cardiomyopathy is another common heart disorder, usually genetic in origin, that may affect up to 600,000 people in the United States. The disorder, which is characterized by left ventricular hypertrophy, is usually not progressive, but a small subset of patients develop serious complications, such as progressive heart failure, atrial fibrillation, and sudden cardiac death.

Anti-arrhythmic drug therapies, such as calcium channel blockers, are commonly used to treat heart disorders, such as SVT, AFIB, angina and HOCM, by regulating heart function through shaping the action potential and maintaining the rhythm of cardiac contraction.

In particular, calcium channels, such as CACNA1C, play an important role in regulating heart function. CACNA1C encodes for the α-subunit of the CaV1.2 L-type calcium channel (LTCC), which is critical for the plateau phase of the cardiac action potential, cellular excitability, excitation-contraction coupling, and regulation of gene expression. The currently available calcium channel blockers (*e.g.,* dihydropyridines, phenylalkylamines, and benzothiazepines) all act by binding to different sites on CACNA1C and blocking the calcium current.

The CACNA1C calcium channels open and close at specific times to control the flow of calcium ions into cardiomyocytes at each heartbeat. How long the channels are open and closed is regulated to maintain normal heart function. Perturbations of CACNA1C change the structure of calcium channels throughout the body and have been associated with several different cardiac arrhythmia disorders. The altered channels stay open much longer than usual, which allows calcium ions to continue flowing into cells abnormally. The resulting overload of calcium ions within cardiac muscle cells changes the way the heart beats and can cause abnormal heart muscle contraction and arrhythmia.

Increased susceptibility for arrhythmia was observed in patients with gain of function CACNA1C variants under certain conditions (PLoS One. 2014; 9(9): e106982; Splawski I, *et al. Cell*. 2004 Oct 1; 119(1):19-31). Specifically, gain of function mutations of CACNA1C revealed a marked reduction in voltage-dependent inactivation. The consequent increase in calcium influx prolongs the cardiac action potential, and thus the QT interval, and can generate early afterdepolarizations capable of triggering cardiac arrhythmias, such as supraventricular tachycardia and atrial fibrillation. Other CACNA1C variants were also shown to be associated with hypertrophic cardiomyopathy, congenital heart defects, and sudden cardiac death.

Targeting CACNA1G for the prevention and/or treatment of SVT and/or Angina

Supraventricular tachycardia (SVT) is a heterogeneous category of cardiac arrhythmias characterized by a fast or tachycardiac rhythm that originates above the atrioventricular (AV) node. The prevalence of SVT is 2.25/1000 persons with a female predominance of 2:1 across all age groups (Lee KW, *et al., Curr Probl Cardiol* 2008;33:467–546). The most common SVTs include atrioventricular nodal re-entrant tachycardia, atrioventricular re-entrant tachycardia and atrial tachycardia. SVT increases patient morbidity, particularly when symptoms are frequent or incessant, and in a small cohort of patients with atrial fibrillation (AF) and ventricular pre-excitation, it can be life-threatening.

Angina represents the most common symptom of ischaemic heart disease, which is a major cause of death and disability worldwide. Nearly 10 million US adults experience stable angina, which occurs when myocardial oxygen supply does not meet demand, resulting in myocardial ischemia. Stable angina is associated with an average annual risk of 3% to 4% for myocardial infarction or death.

Anti-arrhythmic drug therapies, such as calcium channel blockers, are commonly used to treat heart diseases such as SVT and angina by regulating adult heart function through shaping the action potential and maintaining the rhythm of cardiac contraction.

CACNA1G encodes for the subunit of the CaV3,1 T-type calcium channel, which plays a role in the human sinoatrial node and the conduction system. These channels contribute to the heartbeat by influencing pacemaking and the atrioventricular node. Inactivation of *CACNA1G* significantly slowed the intrinsic in vivo heart rate, prolonged the sinoatrial node recovery time, and slowed pacemaker

activity of individual sinoatrial node cells through a reduction of the slope of the diastolic depolarization (Mangoni, *et al., Circulation Research* 2006, 1422-1430). Thus, selective blockers of CaV3.1 channels hold promise for the therapeutic management of the cardiac diseases that require moderate heart rate reduction, such as SVT. The T-type calcium channels also constitute a promising pharmacological target for the treatment of human diseases, such as epilepsy and chronic pain (Birch PJ, *et al. Drug Discov Today*. 2004;9:410 – 418).

Targeting AGTR1 for the prevention and/or treatment of HOCM, HCM, and/or HFpEF

Hypertrophic cardiomyopathy (HCM) is the most common inheritable cardiac disorder with a phenotypic prevalence of 1:500. It is defined by the presence of left ventricular hypertrophy (LVH) in the absence of loading conditions (hypertension, valve disease) sufficient to cause the observed abnormality.

The obstructive HCM (hypertrophic obstructive cardiomyopathy or HOCM) is subtype of HCM. In HOCM, the wall (septum) between the bottom chambers of the heart thickens. The walls of the pumping chamber can also become stiff. The thickened septum may cause a narrowing that can block or reduce the blood flow from the left ventricle to the aorta, which is a condition called "outflow tract obstruction."

Increased blood pressure causes a concentric pattern of LVH, which may progress to ventricular dilation and heart failure with preserved ejection fraction (HFpEF). Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome in which patients have symptoms and signs of heart failure as the result of high ventricular filling pressure despite normal or near normal left ventricular ejection fraction (LVEF ≥50 percent). At a cellular level, cardiac myocytes in patients with HFpEF are thicker and shorter than normal myocytes, and collagen content is increased. At the organ level, affected individuals may have concentric remodeling with or without hypertrophy. Increases in myocyte stiffness are mediated in part by relative hypophosphorylation of the sarcomeric molecule titin, due to cyclic guanosine monophosphate (cGMP) deficiency thought to arise primarily as a consequence of increased nitroso-oxidative stress induced by comorbid conditions such as obesity, metabolic syndrome and aging. Cellular and tissue characteristics may become more pronounced as the disease progresses.

Genetic variants in the renin–angiotensin–aldosterone system (RAAS) are considered candidates for these modifying effects. The RAAS system contributes to LVH through effects mediated by circulating angiotensin as well as local activation of RAAS in the myocardium. Angiotensin (Ang) I, produced from angiotensinogen (AGT), is converted to Ang II predominantly by angiotensin-converting enzyme (ACE) and possibly by chymase 1 (CMA1). Ang II binds primarily to the Ang II type 1 receptor (AGTR1) to promote cell growth and hypertrophy. It also stimulates aldosterone by aldosterone synthase (CYP11B2) synthesis, thereby increasing the release of aldosterone, which promotes fluid retention and cardiac fibrosis.

Previous studies suggested a role for specific genetic variants in genes encoding components of the RAAS pathway in modulation of the severity of LVH in patients with HCM (Orenes-Piñero E, *et al. J Renin Angiotensin Aldosterone Syst* 2011; 12: 521–530; Ortlepp JR, *et al., Heart* 2002; 87: 270–275). In particular, a specific A>C polymorphism of the *AGTR1* gene was considered as the pro-LVH allele. Carriers that harbor the pro-LVH allele had greater left ventricular muscle mass and interventricular septum thickness compared to those without the pro-LVH allele (Kolder *et al. Eur J Hum Genet.* 2012 Oct; 20(10): 1071–1077). Additional studies further demonstrated that left ventricular mass is associated with the AGTR1 polymorphism, and cardiac hypertrophy was improved by down-regulating AGTR1 (Y. Yang, *et al. Exp. Ther. Med.,* 12 (3) (2016), pp. 1556-1562).

Targeting SCN2A for the prevention and/or treatment of AFIB.

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia. The prevalence of AF increases as the population ages (Dang D, et al. J Natl Med Assoc 2002;94:1036-48). In AF, the upper chambers of the heart do not function correctly as a result of abnormal electrical signalling (Falk RH. N Engl J Med 2001;344:1067-78.). It can be characterized by rapid and irregular atrial depolarisations with a discrete lack of P waves on electrocardiograms. As a result, the blood in the atria remains static and can promote blood clot formation and increase the risk of stroke (Copley DJ, et al. AACN Adv Crit Care 2016;27:120-8.). This can cause detrimental symptoms, impair functional status and reduce the quality of life.

Atrial fibrillation can cause syncope or a temporary loss of consciousness caused by a fall in blood pressure. There is also a possibility of atrial fibrillation developing secondary to an epileptic seizure in cases of atrial fibrillation and transient loss of consciousness. Epileptic seizures are often associated with changes in cardiac autonomic function.

Sodium voltage-gated channel alpha subunit 2 (SCN2A) is one of the genes most commonly associated with early-onset epilepsy, and has recently been linked to autism spectrum disorder and developmental delay. SCN2A encodes the Nav1.2 subunit of voltage-gated sodium channel in neurons, which is important for action potential initiation and conduction. SCN2A gain-of-function mutations have been identified, and the phenotypes range from benign neonatal or infantile seizures to severe epileptic encephalopathy. SCN2A gene deletion acts as protective genetic modifier of sudden unexpected death in epilepsy (SUDEP) and suggest measures of brain–heart association as potential indices of SUDEP susceptibility (V Mishra et al., Hum Mol Genet. 2017 Jun 1;26(11):2091-2103). In addition to epilepsy and developmental delays, other manifestations of SCN2A deletion can include movement disorders such as dystonia, abnormal gait, ADHD, autism, dysautonomia (*i.e.* problems with heart rate, blood pressure, and temperature regulation), and GI problems such as feeding difficulties or reflux.

Targeting HCN1 for the prevention and/or treatment of AFIB; treatment, e.g., rate control, in HOCM.

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Hypertrophic cardiomyopathy is another common heart disorder, usually genetic in origin, that may affect up to 600,000 people in the United States. The disorder, which is characterized by left ventricular hypertrophy, is usually not progressive, but a small subset of patients develop serious complications, such as progressive heart failure, atrial fibrillation, and sudden cardiac death.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are voltage-gated channels encoded by the HCN1-4 gene family. These channels are primarily expressed in the heart and in the central and peripheral nervous systems. HCN channels conduct K+ and Na+ ions at a ratio of 3:1 to 5:1. They are activated by hyperpolarization of membrane voltage to -50 mV or below, and conduct the hyperpolarization-activated current, termed I_f in heart. In the sino-atrial node (SAN), I_f plays an essential role in setting the heart rate and mediating its autonomic control.

HCN1 is highly expressed in the SAN and, in addition, non-pacemaking atrial and ventricular cardiomyocytes also express HCN channels, with an increase in I_f activity in ventricular myocytes reported in hypertrophy, ischemic cardiomyopathy and heart failure due to re-expression of HCN genes. Studies have shown that I_f current density and occurrence is significantly greater in hypertrophic cardiomyocytes and end-stage failing hearts and this is directly related to the arrhythmias

Genetic variants in HCN channels are linked to sinus node dysfunction, atrial fibrillation, ventricular tachycardia, atrio-ventricular block, Brugada syndrome, sudden infant death syndrome, and sudden unexpected death in epilepsy. HCN1 deficient mice display congenital sinus node dysfunction with severely reduced cardiac output.

Several HCN channel blockers including ZD7288, zatebradine, cilobradine and ivabradine are available. The first clinically approved substance from this new class of drugs is ivabradine.

Targeting HCN4 for the prevention and/or treatment of AFIB; treatment, e.g., rate control, in HOCM.

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia. The prevalence of AF increases as the population ages (Dang D, *et al. J Natl Med Assoc* 2002;94:1036-48). In AF, the upper chambers of the heart do not function correctly as a result of abnormal electrical signalling (Falk RH. *N Engl J Med* 2001;344:1067-78.). It can be characterized by rapid and irregular atrial depolarisations with a discrete lack of P waves on electrocardiograms. As a result, the blood in the atria remains static

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HCN4 constitutes the predominant isoform in the sino-atrial node (SAN) at both transcript and protein level.

Gain of function variants in HCN4 have been shown to cause rhythm abnormalities, including symptomatic or asymptomatic bradycardia ventricular premature beats, tachycardia–bradycardia syndrome and atrial fibrillation (AF), complete atrioventricular (AV) block, long QT syndrome (LQTS) and torsades de pointes.

Drugs that specifically block HCN channels, *e.g.,* ivabradine, slow the diastolic depolarisation of pacemaker cells, hence cardiac rate, with limited adverse cardiovascular side effects. Selective and quantitatively controlled slowing of heart rate provides an important therapeutic advantage in a variety of cardiac conditions.

Targeting HCN3 for the prevention and/or treatment of AFIB; treatment, e.g., rate control, in HOCM.

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Although the cardiac expression of HCN3 channels is low, a ventricular phenotype caused by global deletion of HCN3 has been described. Epicardial myocytes of HCN3 knockouts displayed a reduction of I_f density by about 30% and a shortening of action potential duration caused by changes during the late repolarization phase. ECG recordings displayed a slight prolongation of the QT interval combined with increased T-wave amplitudes. These alterations were present only at low heart rates.

Thus, HCN3 contributes to the resting membrane potential and acts as a functional antagonist of hyperpolarizing K currents in late repolarization. Lack of this activity leads to a shortening of action potential duration.

Targeting KCNA5 for the prevention and/or treatment of AFIB, e.g., AFIB in congestive heart failure (CHF).

Atrial fibrillation (AF) is the most common cardiac rhythm disorder in clinical practice. During the lifetime of men and women aged ⩾40 years, there is about 25% risk for the development of AF. This arrhythmia may result in irregular ventricular response, tachycardia-mediated cardiomyopathy, heart failure and thromboembolism. AF accounts for nearly one-third of strokes in individuals above 65 years of age, and is also an independent predictor of mortality. AF is often associated with structural heart diseases or systemic disorders, such as hypertension, coronary artery disease, heart failure, rheumatic heart disease, hyperthyroidism and cardiomyopathies. However, in nearly 10–20% of cases, the underlying etiology for AF cannot be identified by routine examination, and such AF is termed 'idiopathic'.

Heart failure (HF), including CHF and HF-pEF, affects an estimated 30–50 million patients worldwide. Despite recent therapeutic advances, its prevalence is increasing, partly due to a fall in mortality, but also from higher rates of major co-morbidities such as obesity, diabetes, and age. A major factor underlying cardiac dysfunction in HF resides in second messenger signaling defects coupled to 3',5'-cyclic adenosine and guanosine monophosphate (cAMP, cGMP). Cyclic AMP stimulates protein kinase A (PKA) and exchange protein activated by cAMP (EPAC), acutely enhancing excitation-contraction coupling and sarcomere function. Cyclic GMP acts as a brake on this signaling by activating protein kinase G (PKG). Both cyclic-nucleotides have relevant vascular and fibroblast activity, reducing vessel tone, altering permeability and proliferation, and suppressing fibrosis. They are synthesized by adenylyl or guanylyl cyclases and degraded (hydrolyzed) by phosphodiesterases (PDEs), to provide tissue and cell specific intracellular nano-regulation.

K+ channels are members of a large family of transmembrane proteins that allow K+ to cross biological membranes selectively. Like Ca2+ and Na+ channels, voltage-gated K+ channels undergo conformational changes to open and close a gate in response to membrane depolarization. The K+ channel family is formed by a complex and diverse group of proteins that is known to exist in all three domains of organisms, eubacteria, archaebacteria, and eukaryotes. K+ channels have a wide range of functions that includes setting the resting membrane potential, modulating electrical excitability, and regulating cell volume.

Cardiac potassium channels maintain the rhythmicity of the heartbeat by repolarizing cardiomyocytes such that the electrical and contractile machineries stay in sync. They alternate between opened and closed conformations in response to the voltage difference across the membrane and form functional homotetrameric channels and heterotetrameric channels that contain variable proportions of KCNA1, KCNA2, KCNA4, KCNA5, and possibly other family members as well.

Dominant-negative mutations in KCNA5 have been demonstrated to fail to generate the ultrarapid delayed rectifier current vital for atrial repolarization and exerted an effect on wild-type current.

KCNJ3 for the prevention and/or treatment of AFIB.

As the population ages globally, atrial fibrillation (AF or AFIB) is predicted to affect 6–12 million people in the USA by 2050 and 17.9 million in Europe by 2060. Subjects with atrial fibrillation are 5 to 7 times more likely to have a stroke than the general population. Clots can also travel to other parts of the body (kidneys, heart, intestines), and cause other damage. Atrial fibrillation can also decrease the heart's pumping ability. The irregularity can make the heart work less efficiently. In addition, atrial fibrillation that occurs over a long period of time can significantly weaken the heart and lead to heart failure.

Sinus node cells, located in the right atrium, spontaneously produce an electric impulse (*i.e.*, action potential) that propagates along the cardiac conduction system and causes contraction of the heart muscle.1Thus, heart rate is precisely regulated within the proper range by both intrinsic and extrinsic mechanisms. The acetylcholine-activated potassium channel (I_{KACh} channel) expressed in the sinus node, atrium, and atrioventricular node contributes to heart rate slowing triggered by the parasympathetic nervous system. The IKACh channel is a heterotetramer of 2 inwardly rectifying potassium channel proteins, Kir3.1 and Kir3.4, encoded by the genes KCNJ3 and KCNJ5, respectively. As indicated above, KCNJ5 mutation has been associated with atrial fibrillation (AF). However, the molecular basis of IKACh channel pathology remains poorly understood and, to date, rare mutations showing a large effect have not been reported for cardiac diseases.

Autosomal dominant mutations in KCNJ3 have been associated with symptomatic sinus bradycardia, and chronic AF with slow ventricular response. Because I_{KACh} channels are expressed more abundantly in the atrium than in the ventricle, the blockage of IKACh is a target for atrial-selective AF therapy with a lower risk of ventricular arrhythmia.

Targeting KCNJ4 for the prevention and/or treatment of AFIB

As the population ages globally, atrial fibrillation (AF or AFIB) is predicted to affect 6–12 million people in the USA by 2050 and 17.9 million in Europe by 2060. Subjects with atrial fibrillation are 5 to 7 times more likely to have a stroke than the general population. Clots can also travel to other parts of the body (kidneys, heart, intestines), and cause other damage. Atrial fibrillation can also decrease the heart's pumping ability. The irregularity can make the heart work less efficiently. In addition, atrial fibrillation that occurs over a long period of time can significantly weaken the heart and lead to heart failure.

Potassium channels, such as KCNJ4, play an important role in regulating adult heart function through shaping the action potential and maintaining the rhythm of cardiac contraction.

KCNJ4 has been identified as a target for antiarrhythmic drugs, as it is expressed at ≈10-fold greater levels in the atria relative to the ventricles of animal model and expression of KCNJ4 Has been shown to be upregulated in left atrial appendage tissues from subjects having AFIB. In addition, gain-of-function mutations of KCNJ4 have been associated with familial forms of AF, and KCNJ4 upregulation contributes to the stabilization/perpetuation of AFIB.

Targeting PLN for the prevention and/or treatment of HF-rEF, arrhythmia, and/or cardiomyopathy

Heart failure, such as heart failure with reduced ejection fraction (HF-rEF), is a major cause of death and disability. The hallmarks of heart failure are impaired cardiac contraction and relaxation accompanied by abnormalities in calcium handling and β-adrenergic signaling (Lou, Q, *et al. Adv Exp Med Biol.* 2012;740:1145–1174). In cardiomyocytes, cytosolic calcium regulates cardiac contraction and relaxation by an excitation–contraction coupling mechanism. The Ca2+ influx via L-type calcium channels elicits Ca2+-induced Ca2+ release from sarco(endo)plasmic reticulum (SR) through ryanodine receptors, and increased cytosolic Ca2+ leads to cardiac contraction. The sequestration of Ca2+ from the cytosol into the SR, which determines active relaxation, is caused by a calcium pump on the SR called SR Ca2+ ATPase (SERCA2a), the activity of which is regulated by a small phosphoprotein, phospholamban (PLN) (Kranias, EG, *et al., Circ Res.* 2012;110(12):1646–1660).

In physiological conditions, β-adrenergic receptor (βAR) stimulation enhances myocyte contraction by activating cyclic adenosine monophosphate–dependent kinase (protein kinase A [PKA]), which phosphorylates multiple Ca2+ cycling proteins, including PLN. Phospholamban inhibits SERCA2a activity through protein–protein interaction. Phosphorylation of PLN by PKA alters its interaction with SERCA2a to activate Ca2+ reuptake to the SR, resulting in enhanced SR Ca2+ loading and Ca2+ cycling. In the failing myocyte, dysfunctional βAR signaling leads to less PKA activation and activation of alternate pathways, such as calcium/calmodulin-dependent kinase II signaling to cause pathological hypertrophy.Consequently, the usefulness of positive inotropic agents in HF is strongly limited, and direct activation of Ca2+ cycling, which can circumvent dysfunctional βAR activity, is required. Thus, inhibition of PLN is one of the most promising strategies in this context. Several reports have demonstrated that PLN inhibition alleviates cardiac failure in various

animal models of cardiac pathologies, including myocardial infarction in rats, genetic cardiomyopathy in hamsters, and dilated cardiomyopathy in mice (Iwanaga, Y, *et al., J Clin Invest.* 2004;113(5):727–736; Hoshijima, M, *et al., Nat Med.* 2002;8(8):864–871; Minamisawa, S. *et al., Cell.* 1999;99(3):313–322). In addition, modulation of PLN improves contractility in human cardiomyocytes from patients with advanced HF (del Monte, F, *et al. Circulation.* 2002;105(8):904–907), suggesting that targeting PLN is a bona fide therapy for failing hearts.

In particular, the ablation of PLN in mice prevents SERCA2a inhibition and enhances cardiac contractility by increasing the SR Ca2+ store. The ablation of PLN also reverses heart failure in some cardiomyopathic animal models, indicating the possibility of therapeutic approaches. The overexpression of PLN in mouse heart depresses cardiac function and proves that only ~40% of SERCA pumps are normally regulated by PLN in mouse heart. The superinhibition of SERCA by specific PLN mutants impairs cardiac function and leads to cardiac remodelling and early death if the effects of the mutation cannot be reversed by β-agonists. In human and animal models of heart failure, the PLN–SERCA inhibited complex increases. Interventions that diminish the PLN–SERCA complex have been beneficial in some mouse models of heart failure (MacLennan and Kranias. 2003. *Nat Rev Mol Cell Biol* 4:566-77).

Dilated cardiomyopathy (DCM) is the second most common cause of heart failure with reduced ejection fraction (HFrEF) after coronary artery disease. It has been estimated that up to 40% of DCM cases have a genetic cause. The p.(Arg14del) pathogenic variant of the PLN gene (PLN-R14del) is a Dutch founder mutation with a high prevalence in DCM and arrhythmogenic cardiomyopathy (ACM) patients. Cardiomyopathy caused by the p.(Arg14del) pathogenic variant of the PLN gene is characterized by intracardiomyocyte PLN aggregation and can lead to severe DCM. Depletion of PLN attenuated heart failure in several cardiomyopathy models. Specifically, PLN knockdown was shown to reduce protein aggregation, normalize autophagy markers, improve cardiomyopathy and survival (Eijgenraam *et al.* 2022. *Int J Mol Sci* 23:2427. 4). PLN knockdown also reversed the heart failure phenotype in a genetic dilated cardiomyopathy mouse model, and prevented progression of left ventricular dilatation and improveed left ventricular contractility in rats with myocardial infarction (Grote Beverborg *et al.* 2021. *Nat Comm* 12:5180). PLN abalation was also shown to reduce susceptibility to ventricular arrhythmias in mouse model of catecholaminergic polymorphic ventricular tachycardia (Mazzocchi *et al. 2016. J Physiol* 594: 3005-3030).

Thus, inhibition of PLN is an effective strategy in treating and/or preventing genetic cardiomyopathy, arrhythmia, as well as heart failure, in particular HF with reduced ejection fraction (HF-rEF).

Targeting CAMK2D for the prevention and/or treatment of heart failure and/or AFIB

CAMK2D has been shown to associate with the development of cardiac disease, such as heart failure, and arrhythmias (Maier and Bers, 2002, *J. Mol. Cell. Cardiol.* 34, 919–939; Swaminathan et al., 2012, *Circ. Res.* 110, 1661–1677). Animal models have shown proof-of-concept studies that

transgenic overexpression of CAMK2D is sufficient to induce structural and electrical remodeling in the heart, leading to compromised contractility and increased risk for sudden cardiac death (Zhang et al., 2002, *J. Biol. Chem.* 277, 1261–1267; Wagner et al., 2011, *Circ. Res.* 108, 555–565). Likewise, genetic and chemical inhibition of CAMK2D has been shown to confer protection from the development of dilated cardiomyopathy and sustained contractile performance, following both pressure overload and ischemic stress (Backs et al., 2009, *J. Clin. Invest.* 116, 1853–1864.; Ling et al., 2009, *J. Clin. Invest.* 119, 1230–1240.). Human heart failure has also been associated with an increased expression/activity of CAMK2D (Hoch et al., 1999, *Circ. Res.* 84, 713–721). The central role for CAMK2D in development of disease stems from its regulation of proteins involved in critical cell functions such Ca2+ cycling. CAMK2D has been implicated in pathologic phosphorylation of a number of Ca2+ handling proteins including phospholamban, leading to activation of the sarcoplasmic reticulum (SR) ATP-driven Ca2+ pump SERCA2a (Mattiazzi and Kranias, 2014, *Front. Pharmacol.* 5, 5.); the ryanodine receptor SR Ca2+ release channel (RyR2) (Witcher et al., 1991, *J. Biol. Chem. 266,* 11144–11152), promoting increased channel open probability and SR Ca2+ leak; and the L-type Ca2+ channel Cav1.2 and associated β-subunits, potentiating current amplitude and slowing inactivation (Hudmon et al., 2005, *J. Cell Biol.* 171, 537–547). Collectively, these events not only promote activation of hypertrophic remodeling cascades but also heighten the risk for inappropriate membrane potential depolarizations (afterdepolarizations) that serve as arrhythmia triggers (Wu et al., 2002, *Circulation* 106, 1288–1293).

In addition to association with heart failure, CAMK2D has also been found to be a GWAS locus for atrial fibrillation (Roselli C *et al.* 2018. *Nat Genet*; 50:1225–1233; Ramirez J *et al.* 2020. *Am J Hum Genet* 106:764-78). Pathological activation of CAMK2D promotes arrhythmia and heart failure (Veitch CR *et al.* 2021 *Front Pharmacol* 12: 695401; Nassal D *et al.* 2020. *Front Pharmacol* 11:35). In humans, CAMK2D levels and activity are increased in atrial fibrillation and heart failure. In animal models, sustained CAMK2D activation induces adverse structural and electrical remodeling of the heart via phosphorylation of target proteins. Pharmacological and genetic inhibition were shown to prevent these changes. Transagenic expression in the atria of a CAMK2D inhibitory peptide was shown to prevent adverse atrial structure and electrical remodeling (Liu Z *et al.* 2019. *Heart Rhythm* 16:1080-1088). In addition, CAMK2D knockout protects against pathological cardiac hypertrophy in a mouse model of heart failure (Backs J *et al. PNAS* 2009;106:7:2342-2347).

Thus, suppressing CAMK2D expression is an effective strategy in treating and/or preventing heart failure and/or atrial fibrillation.

Targeting PDE1 for the prevention and/or treatment of CHF and/or HF-pEF

Heart failure (HF), including CHF and HF-pEF, affects an estimated 30–50 million patients worldwide. Despite recent therapeutic advances, its prevalence is increasing, partly due to a fall in mortality, but also from higher rates of major co-morbidities such as obesity, diabetes, and age. A major factor underlying cardiac dysfunction in HF resides in second messenger signaling defects

coupled to 3',5'-cyclic adenosine and guanosine monophosphate (cAMP, cGMP). Cyclic AMP stimulates protein kinase A (PKA) and exchange protein activated by cAMP (EPAC), acutely enhancing excitation-contraction coupling and sarcomere function. Cyclic GMP acts as a brake on this signaling by activating protein kinase G (PKG). Both cyclic-nucleotides have relevant vascular and fibroblast activity, reducing vessel tone, altering permeability and proliferation, and suppressing fibrosis. They are synthesized by adenylyl or guanylyl cyclases and degraded (hydrolyzed) by phosphodiesterases (PDEs), to provide tissue and cell specific intracellular nano-regulation.

PDE1 is constitutively and robustly expressed in the heart. It is activated by a Ca2+/calmodulin-binding domain and provides a substantial percent of in vitro cAMP and cGMP hydrolytic activity in mammals, including humans.

It has been shown that inhibition of PDE1 prevents phenylephrine-induced myocyte hypertrophy in neonatal and adult rat ventricular myocytes reduces angiotensin II or TGF--induced activation of rat cardiac fibroblasts, and attenuates isoproterenol-induced interstitial fibrosis in mice. Cellular senescence in vascular smooth muscle myocytes leads to elevated PDE1 expression, and PDE1 inhibition restores vasodilatory responses to sodium nitroprusside in aging mice. PDE1 expression in vascular smooth muscle cells in vitro increases with the transition from the contractile to the synthetic phenotype, and PDE1 inhibition attenuates proliferation and migration of vascular smooth muscle cells in culture. PDE1 expression is increased in mouse vascular injury models in vivo and in neointimal smooth muscle cells of human coronary arteries, and injury-induced neointimal formation is reduced by PDE1 inhibition in coronary arteries of mice. Knockout of the PDE1C gene has antihypertrophic, antifibrotic, and antiapoptotic actions in mouse hearts. These observations suggest that PDE1 is a therapeutic target for cardiovascular disease. Indeed, recently a selective small molecule PDE1 inhibitor, ITI-214, was demonstrated to improve cardiac output by increasing heart contractility and decreasing vascular resistance in a Phase I/II study of heart failure patients.

Targeting Skeletal Muscle Tissue

In some embodiment, , the present invention provides a double-stranded iRNA agent that targets myostatin for the treatment of Myostatin-related muscle dystrophy.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CHRNA1 for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CHRNB1 for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CHRBD for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CHRNE for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CHRNG for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets COL13A1 for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets LRP4 for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets MUSK for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets RAPSN for the treatment of congenital myasthenic syndrome.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets SCN4A for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets DOK7 for the treatment of congenital myasthenic syndrome (CMS).

In some embodiments, the present invention provides a double-stranded iRNA agent that targets DUX4 for the treatment of Facioscapulohumeral muscular dystrophy (FSHD).

Targeting myostatin for the prevention and/or treatment of Myostatin-related muscle dystrophy

Myostatin, also known as growth differentiation factor 8 (GDF8), is a negative regulator of muscle mass and a member of the TGF-β superfamily of proteins. Myostatin is initially synthesized by myocytes as a pre-promyostatin molecule composed of an N-terminal signal sequence (for secretion), an N-prodomain region (essential for proper folding of myostatin and subsequently proteolytically processed), and the biologically active C-terminal domain. The precursor pre-promyostatin must undergo proteolytic cleavage to form the biologically active myostatin molecule, which exists as a disulfide-linked dimer of two C-terminal domains. The cleaved propeptide domain also plays a regulatory role through non-covalent binding to the active myostatin C-terminal domain to form an inactive latent myostatin complex. Myostatin is also capable of effecting a non-canonical signaling cascade involving the cellular energy-sensing enzyme AMP-activated kinase (AMPK) and a regulatory protein kinase transforming growth factor-β-activated kinase 1.

Genetic deletion of myostatin has been associated with increasing muscle mass in mice, cattle, dogs, horses, and other species, indicating its evolutionary conservation (McPherron AC, *et al., Nature* 1997; 387:8390). Discovery of a hypermuscular child who was homozygous for an splice site mutation, which resulted in a premature stop codon, suggested that inhibition of myostatin might confer therapeutic benefits for muscle wasting disease in humans (Schuelke M, *et al. New Engl J Med* 2004; 350:26822688).

Various myostatin inhibitors have been developed and evaluated as potential treatments for differnet types of muscular dystrophy. These inhibitors have been shown to ameliorate the phenotype of muscular dystrophy, *e.g.,* by improving muscle mass and strength.

Targeting CHRNA1, CHRNB1, CHRND, CHRNE and CHRNG for the prevention and/or treatment of CMS

Congenital myasthenic syndromes (CMS) are a heterogeneous group of rare inherited neuromuscular disorders characterized by fatigable weakness of skeletal muscle owing to compromised function of the neuromuscular junction (NMJ). The phenotype is caused by failure of transmission across this synapse connecting the nerve with the muscle, whereby an incoming nerve stimulus does not consistently lead to muscle excitation and contraction. Neuromuscular transmission is mediated by the generation of an action potential causing the release of acetylcholine from the nerve terminal into the synaptic cleft, its binding to the acetylcholine receptor (AChR) with the opening of its ion channel and the enzymatic breakdown of acetylcholine by acetylcholinesterase (AChE). The AChR controls electrical signalling between nerve and muscle cells by opening and closing a gate, membrane-spanning pore to trigger muscle contraction. It has five subunits of four different types: two alpha and one each of beta, gamma (or epsilon), and delta subunits ((*i.e.*, CHRNE, CHRNA1, CHRNB1, CHRND, and CHRNG). Mutations affecting subunits of the AChR pore cause CMS in humans.

Pathophysiological mechanisms acting on any part of this chain and resulting in a reduction in the amount of acetylcholine released, the impairment of the AChR, reduction in the number of receptors or defective breakdown of acetylcholine may lead to CMS. The majority of CMS types are caused by defects in the AChR itself, but they can also result from causative variants affecting presynaptic proteins or proteins associated with the synaptic basal lamina or variants causing defects in endplate development and maintenance or defects in protein glycosylation. Defective neuromuscular transmission presents clinically as fatigable weakness due to increasing impairment of transmission across the NMJ with repeated activation.

Generalized and fatigable skeletal muscle weakness is the most common clinical sign of CMS, but locus and allelic heterogeneity determine variable severity and additional symptoms. CMS can result from recessive missense, non-sense, or splice site and promoter region mutations in any of the AChR subunits, but most occur in the gamma (or epsilon) subunit.

Diagnosis of CMS is established with clinical and electrodiagnostic features and identification of a causative mutation. In some instances, a clinical diagnosis can be made without finding a causative gene (*e.g.*, individuals who exhibit fatigable weakness, especially of ocular and other cranial muscles, at birth or early childhood). Clinical diagnosis may rely on history, clinical exams, blood tests, incremental or decremental responses or abnormal single-fiber EMG (SF-EMG) study results, lung function tests, polysomnography, the Tensilon test, and muscle biopsy. In rarer cases when symptoms manifest in adolescence or adulthood, symptom presentation may differ from that seen in infants and young children and can include proximal and axial muscle weakness associated with a decremental response requiring prolonged stimulation.

Mutations in about 32 genes that encode proteins involved in this signaling pathway are known to cause CMS. Eight proteins are associated with presynaptic CMS, four with synaptic CMS,

fifteen with post-synaptic CMS, and five with glycosylation defects. Proteins affected in CMS have different functions, such as ion channels (AchR,), structural proteins (COL13A1, RAPSN), signalling molecules (LRP4, MUSK, DOK7), catalytic enzymes, sensor proteins, or transport proteins. Various gene mutations in presynaptic, synaptic, and postsynaptic proteins have been demonstrated in patients, with more than 50% of the mutations involving aberrations in postsynaptic AChR subunits (*i.e.,* CHRNE, CHRNA1, CHRNB1, CHRND, and CHRNG). Mutations in RAPSN, COLQ, and DOK7 comprise another 35% to 50% of cases.

The CHRNA1 gene encodes the alpha-subunit of the nicotinergic, post-synaptic AchR. CHRNA1 mRNA undergoes alternative splicing and two splice variants (P3A- and P3A+) are produced. Mutations in CHRNA1 result in imbalance between the two splice variants with an increase in P3A+. CHRNA1 mutations reduce the number of AchR at the post-synaptic membrane. The pattern of inheritance is autosomal dominant if CHRNA1 mutations cause a slow channel CMS (SCCMS), or autosomal recessive in case of primary AchR-deficiency. The first CHRNA1-related CMS were reported in 2008. Patients presented already prenatally with growth retardation, reduced movements, edema, contractures, and postnatally with dysmorphism, muscle wasting, scoliosis, contractures, and pterygia. Antisense oligonucleotides (AONs) have been shown to restore the balance between the two splice variants and are thus expected to be beneficial in patients carrying such mutations.

The CHRNB1 gene encodes for the beta-subunit of the nicotinergic, post-synaptic acetylcholine receptor (AChR). Non-synonymous mutations in the human CHRNB1 gene encoding the cholinergic receptor nicotinic beta 1 subunit are known to cause dominant and recessive forms of CMS. The first mutations in CHRNB1 causing CMS were reported in a Brazilian study in 2008. The first patient published was a 28 year old male manifesting since birth with ptosis, ophthalmoparesis, dysphagia, proximal limb muscle weakness, scapular winging, weakness of axial muscles, wasting, and scoliosis. He showed a decremental response to RNS, had double discharges, and a myopathic EMG. The course was progressive but he benefitted from fluoxetine (Mihaylova V, *et al. J Neurol Neurosurg Psychiatry.* 2010;81:973–977). The second patient carrying a CHRNB1 mutation was a 3wo male manifesting with ptosis, facial weakness, severe hypotonia, and respiratory insufficiency requiring assisted ventilation (Shen XM, *et al., Hum Mutat.* 2016;37:1051–1059). The response to LF-RNS was decremental. In a Spanish study of a CMS cohort, a third patient with a CHRNB1 mutation was identified but no clinical details were provided (atera-de Benito D, *et al., Neuromuscul Disord* 2017. pii: S0960–8966(17)30475–3).

The CHRND gene encodes the delta-subunit of the nicotinergic, post-synaptic AchR. The first mutation in CHRND causing CMS was reported in a German patient with early-onset CMS manifesting with feeding difficulties, moderate, generalised weakness, and recurrent episodes of respiratory insufficiency provoked by infections (Müller JS, *et al., Brain.* 2006;129:2784–2793). The second patient was a 20 year old female with moderate to severe myasthenic manifestations since birth (Shen XM, *et al., J Clin Invest.* 2008 May; 118(5):1867-76). She had a marked decremental response to LF-RNS. One of her siblings with a similar presentation had died at age 11 m. Two

further patients were reported in a study of CMS patients from Israel but no clinical details were provided (Aharoni S, *et al., Neuromuscul Disord.* 2017 Feb; 27(2):136-140).

The CHRNE gene encodes for the epsilon-subunit of the AchR. The first mutation in the CHRNE gene causing a CMS has been reported already in 2000 (Sieb JP, *et al., Hum Genet.* 2000;107:160–164). Since then various different types of mutations have been reported and it is estimated that up to half of the patients with a CMS carry a CHRNE mutation, thus representing the gene most frequently mutated in CMS. In a study of 64 CMS patients from Spain, CHRNE mutations were detected in 27% of the patients (Natera-de Benito D, *et al., Neuromuscul Disord* 2017. pii: S0960–8966(17)30475–3). In a study of 45 patients from 35 Israeli CMS families, CHRNE mutations were found in 7 kinships (Aharoni S, *et al., Neuromuscul Disord.* 2017 Feb; 27(2):136-140). In a study of 23 families with CMS from Maghreb countries, the founder mutation c.1293insG was found in 60% of these patients (Richard P, *et al., Neurology.* 2008 Dec 9; 71(24):1967-72). Type and severity of clinical manifestations of CHRNE mutations may vary considerably between affected families. Some patients may present with only ptosis whereas others may present with severe generalised myasthenia. Most patients present at birth with mildly progressive bulbar, respiratory, or generalized limb weakness with ptosis or ophthalmoplegia. Single patients may die prematurely in infancy from respiratory failure. Some patients may have myasthenic symptoms since birth and achieve ambulation late or not at all. Single patients present with a fluctuating course. Single patients develop severe scoliosis. RNS may be decremental or may be normal. Single-fiber EMG (SF-EMG) may reveal an increased jitter. Some patients may show repetitive CMAPs. Most patients respond favourably to AchE inhibitors.

The CHRNG gene encodes for the fetal gamma-subunit of the AchR. Mutations in the CHRNG gene cause CMS with multiple ptyerygia (lethal multiple pterygia syndrome (LMPS) or the Escobar variant of multiple pterygia syndrome (EVMPS)) (Hoffmann K, *et al., Am J Hum Genet.* 2006;79:303–312). In a study of seven families with Escobar syndrome (contractions, multiple pterygia, respiratory distress), mutations in the CHRNG gene were detected in 12 family members. The female to male ratio was 7:5. Some patients presented with decreased fetal movements, facial weakness, respiratory distress, arthrogryposis, short stature, kyphosis/scoliosis, dysmorphism, high-arched palate, cleft palate, arachnodactyly, or cryptorchism. None presented with myasthenic manifestations postnatally. CHRNG mutations may be also responsible for the allelic disease fetal akinesia deformation sequence (FADS). In a study of 46 CMS patients from Spain, five carried a mutation in the CHRNG gene (Natera-de Benito D, *et al., Neuromuscul Disord* 2017. pii: S0960–8966(17)30475–3). They all presented with arthrogryposis and delayed motor milestones, and some of them with poor sucking. Interestingly, none of them received drugs usually given for CMS. In a study of three Iranian CHRNG-related CMS patients, no drug treatment was applied. One of the patients presented with short neck, mild axillar pterygia, elbows and knees, joint contractures, clenched hands with thumbs held across palm and club feet (varus). The patient had rockerbottom feet, with almost no movement in ankles. Facial dysmorphism included hemangioma over forehead

and nose, strabismus, flat nasal bridge, and downturned corners of mouth (Kariminejad A, *et al., BMC Genet.* 2016 May 31; 17(1):71).

Overall, CMS can result from missense, non-sense, or splice site and promoter region mutations in any of the AChR subunits, but most occur in the gamma (or epsilon) subunit. The high frequency of mutations in the epsilon subunit compared with other subunits has been attributed to phenotypic rescue by substitution of the fetal gamma subunit for the defective epsilon subunit (Ohno K, *et al. Hum Mol Genet.* 1997;6:753–66). Individuals harboring null mutations in both alleles of CHRNA1, CHRNB1, or CHRND cannot survive because no substituting sub-units exist and hence these individuals probably die in utero (Engel AG, *et al., Lancet Neurol.* 2015;14:420–34). Patients with heterozygous or homozygous low-expressor mutations in the non-epsilon subunit are severely affected have high mortality in infancy or early childhood. Thus, given the importance of AchR in CMS, manipulation of AchR subunits are expected to ameliorate the CMS phenotype in patients.

Targeting COL13A1 for the prevention and/or treatment of CMS

Mutations in gene encoding synaptic proteins can cause CMS. Collegan XIII is a non-fibrillar transmembrane collagen which has been long recognized for its critical role in synaptic maturation of the neuromuscular junction. The COL13A1 gene encodes the α-chain of collagen XIII with a single transmembrane domain. COL13A1 is localised to the NMJ, where it is responsible for clustering of the AchR during myotube differentiation. Unlike most of the collagens, COL13A1 is anchored to the plasma membrane by a hydrophobic transmembrane segment. The presence of a proprotease recognition site in the ectodomain allows the C-terminus to be proteolytically cleaved into a soluble form that is part of the basal lamina.

Mutations in this gene manifest clinically as CMS, which has been reported in three patients from two families (Logan CV, *et al. Am J Hum Genet.* 2015;97:878–85). Two of these patients manifested with congenital respiratory insufficiency, bulbar weakness, or facial weakness. All three patients presented with feeding difficulties, ptosis, limb weakness, and dysmorphism. Two patients each presented with spinal stiffness or distal joint laxity, and one patient with ophthalmoparesis and cognitive impairment. Two showed a decremental response to RNS and two an increased jitter. Two required non-invasive positive pressure ventilation.

COL13A1 loss-of-function mutations were also identified in six additional CMS patients from three unrelated families (Dusl M. *et al., Journal of Neurology*, 2019; 255: 1107-1112). The phenotype of these cases was similar to the previously reported patients including respiratory distress and severe dysphagia at birth that often resolved or improved in the first days or weeks of life. All individuals had prominent eyelid ptosis with only minor ophthalmoparesis as well as generalized muscle weakness, predominantly affecting facial, bulbar, respiratory and axial muscles. Response to acetylcholinesterase inhibitor treatment was generally negative while salbutamol proved beneficial. These data further support the causality of COL13A1 variants for CMS and suggest that this type of CMS might be clinically homogenous and requires alternative pharmacological therapy.

Targeting LRP4 for the prevention and/or treatment of CMS

Some CMS are due to mutations in genes encoding post-synatic proteins. Post-synaptic CMSs represent the vast majority of the CMS subtypes. Post-synaptic CMS are subdivided into primary AchR deficiency, kinetic abnormalities of the AChR, and defects within the AChR-clustering pathway. Mutations in LRP4 cause defects within the AChR-clustering pathway.

The LRP4 gene encodes for lipoprotein receptor-related protein 4, which functions as a receptor for agrin. Agrin, which is released from motor nerve terminals, binds to LRP4 in muscle, stimulating the formation of a complex between LRP4 and muscle-specific kinase (MUSK), a receptor tyrosine kinase that acts as a master regulator of synaptic differentiation. LRP4, once clustered in the postsynaptic membrane as a consequence of MUSK activation, also signals directly back to motor axons to stimulate presynaptic differentiation. Activated MUSK together with DOK7 stimulates rapsyn to concentrate and anchor AchR at the post-synaptic membrane and interacts with other proteins implicated in the assembly and maintenance of the NMJ. LRP4 is thus essential for pre- and post-synaptic specialisation of the NMJ.

The first mutation in the LRP4 gene causing CMS was reported in 2014. A newborn female presented with respiratory arrest and feeding difficulties, and required feeding and ventilator support until 6 m of age. Motor milestones were delayed and she developed easy fatigability with temporary wheelchair-dependency. At ages 9 and 14y she presented with ptosis, ophthalmoparesis, and limb weakness. RNS evoked a decremental response, which improved upon application of edrophonium. AchE inhibitors worsened the clinical manifestations. A second kinship harbouring LRP4 mutations was reported in 2015. The two sisters, aged 34 and 20y, presented with delayed motor milestones, slight chewing and swallowing difficulties, and later developed limb weakness. Albuterol was highly effective.

Targeting MUSK for the prevention and/or treatment of CMS

Mutations in MUSCK cause defects within the AChR-clustering pathway. MUSK encodes for a protein that is involved in endplate maturation, maintenance of the endplate functions, proper functioning of rapsyn, and functioning of the AchR. MUSK forms a co-receptor for agrin with LRP4. Activation of MUSK by agrin and DOK7 results in the recruitment of several downstream kinases and phosphorylation of the AChR β-subunit, leading to the reorganization of the actin cytoskeleton and AChR clustering. The fundamental role of the MUSK-signaling pathway is supported by the fact that mice deficient in agrin, MUSK, rapsyn or Dok-7 lack postsynaptic differentiation and die at birth from respiratory failure.

CMS due to MUSK mutations manifests as respiratory insufficiency, neonatal ptosis, proximal limb muscle weakness, and weak bulbar, facial, or ocular muscles. A 30yo Chinese male with the LGMD-type of MUSK-related CMS developed mild atrophy of the leg muscles. LF-RNS was decremental. Pyridostigmin deteriorated the clinical manifestations. Another male infant manifested with congenital respiratory failure requiring mechanical ventilation, axial weakness with

head drop, facial weakness, proximal limb weakness, and ophthalmoparesis. Salbutamol was effective but 3,4-DAP had only a mild effect, and AchE inhibitors worsened the phenotype. In a female with congenital hypotonia and respiratory distress requiring mechanical ventilation for 8 m, respiratory distress and nocturnal apnea with vocal cord paralysis recurred at age 8y. 3,4-DAP was effective. In two Turkish brothers MUSK mutations manifested as LGMD-type CMS. MUSK-related CMS may also manifest as congenital ptosis and later in life with fatigability. In another patient with MUSK-related CMS and congenital respiratory insufficiency, albuterol was moderately effective but AchE inhibitor, 3,4-DAP, and ephedrine were ineffective.

Targeting RAPSN for the prevention and/or treatment of CMS

Mutations in RAPSN cause defects within the AChR-clustering pathway. RAPSN encodes for rapsyn, a post-synaptic membrane protein that anchors the nicotinic AchR to the motor endplate and also binds to β-dystroglycan. Rapsyn is essential for clustering of the AchR at the post-synaptic membrane and required for the phosphorylation of CHRNB1. Mutant mice lacking rapsyn show absence of aggregation of AChRs and lack of accumulation of cytoskeletal proteins such as β-dystroglycan, and utrophin.

RAPSN mutations are a common cause of post-synaptic CMS. Humans with mutations in the RAPSN gene are affected with a postsynaptic form of CMS characterized by impairment of the morphologic development of the postsynaptic region. The severity of symptoms in this form of CMS is variable. The most common of the RAPSN mutation is N88G, and patients are either homozygous or heterozygous for the N88K mutation (Ohno K, *et al.,* (2002) *Am J Hum Genet* 70(4):875–885).

Clinically, patients present with fluctuating ptosis, occasionally bulbar symptoms, neck muscle and mild proximal limb muscle weakness. Infections can precipitate exacerbation of clinical manifestations. In single patients prominent hyperlordosis can occur. Usually, the response to AchE inhibitor is favourable but can be improved by adding 3,4 DAP. Fluoxetine may worsen the decremental response in single patients. In some patients general anesthesia may exacerbate muscle weakness. The overall course is stable with intermittent worsenings.

Targeting DOK7 for the prevention and/or treatment of CMS

Mutations in DOK7 cause defects within the AChR-clustering pathway, and are responsible for about 10-20% of all cases of CMS. The DOK7 (downstream-of-kinase) gene encodes for the protein DOK7, which is involved in signaling downstream of receptor and non-receptor phosphotyrosine kinases. DOK7 is a cytoplasmic activator of muscle-specific receptor-tyrosine kinase (MuSK). Both DOK7 and MuSK are required for neuromuscular synaptogenesis. Mutations in DOK7 underlie a congenital myasthenic syndrome (CMS) associated with small and simplified neuromuscular synapses likely due to impaired DOK7/MuSK signaling. The overwhelming majority of patients with DOK7 CMS have at least one allele with a frameshift mutation that causes a truncation in the COOH-terminal region of DOK7 and affects MuSK activation.

Concerning the frequency of DOK7-related CMS, it was the second most frequent subtype in a Brasilian cohort. Clinical onset is characterised by gait disturbance due to muscle weakness after normal motor milestones. Proximal limb muscles are more strongly affected than distal limb muscles (LGMD-like pattern). Congenital DOK7-related CMS may manifest as stridor due to vocal cord paralysis, occasionally requiring intubation and artificial ventilation. Occasionally, patients present with ptosis but only rarely with ophthalmoparesis. Fatigability is often absent but prolonged periods of weakness may occur. Feeding difficulties may require nasogastral tube feeding or even PEG implantation. Muscle biopsy may show lipidosis and defective branching of terminal axons, which results in a unique terminal axon contacting en passant post-synaptic cups. AchE inhibitors are usually ineffective and may even worsen clinical manifestations. Ephedrine (initially 25 mg/d and increased to 75-100 mg/d) seems to be an effective alternative. Salbutamol may be effective in DOK7-related CMS as well. Single patients profit from albuterol, which can prevent progression of muscle weakness in LGMD-type DOK7-related CMS.

Targeting SCN4A for the prevention and/or treatment of CMS

Mutations in SCN4A cause defects within the AChR-clustering pathway. SCN4A encodes for a post-synaptic Nav1.4 voltage-gated sodium channel responsible for the initiation and propagation of the action potential in the muscle fibres that results in muscle contraction.

Several allelic disorders of skeletal muscle are caused by mutations of SCN4A. Missense mutations with gain-of-function changes (too much inward Na+ current) are found in hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita, and several variants of sodium channel myotonia. Leaky channels resulting from mutations of arginine residues in the voltage sensor domain cause hypokalemic periodic paralysis (HypoPP) type 2. These traits are all dominantly inherited.

Loss-of-function (LOF) mutations of SCN4A are associated with recessively inherited phenotypes. A congenital myasthenic syndrome (CMS) has been associated with missense mutations of SCN4A that cause a LOF by markedly enhancing channel inactivation. More recently, congenital myopathy with neonatal hypotonia has been reported in patients with null mutations in SCN4A. A homozygous null is embryonic lethal, while compound heterozygous mutations (null allele plus an LOF allele) result in congenital myopathy with survival to adulthood. Remarkably, family members with a single SCN4A null allele are healthy.

Phenotypically, mutations in this gene manifest in infancy with global hypotonia, impaired sucking, dysphagia, delayed postural and motor development and later in life with episodic, fluctuating muscle weakness like in periodic paralysis, bilateral facial palsy, ptosis, and ophthalmoparesis. Episodes of periodic weakness could not be triggered by exercise, rest, potassium loading, or food, like in periodic paralysis. In older patients, SCN4A-related CMS may manifest exclusively as easy fatigability. In a 20yo normokalemic female, SCN4A-related CMS manifested as sudden attacks of respiratory and bulbar paralysis since birth, lasting 3–30 min and recurring one to three times per month, delayed motor development, easy fatigability, ptosis, ophthalmoparesis, and

later as persisting facial, truncal, or limb weakness. Some patients present with dysmorphism, such as high-arched palate, adduction deformity of the knees or ankles, and increased lumbar lordosis. Some patients are mentally retarded with cerebral atrophy on MRI. RNS may be normal but higher stimulus frequency may trigger a decremental response. AchE inhibitors are only marginally effective. Acetazolamide together with potassium was ineffective.

Targeting DUX4 for the prevention and/or treatment of FSHD

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder primarily characterized by asymmetric, progressive muscle weakness beginning at the face, shoulders, and upper limbs, which spreads to the lower regions of the body with age. It is the third most common muscular dystrophy, with about 1:8,000–1:22,000 people affected worldwide. Age of onset is variable, ranging from birth to adulthood. Patients with the rare infantile form of FSHD, presenting symptoms before 5 y of age, follow a more severe and rapid course of the disease. At present, FSHD is incurable.

The majority of FSHD patients (~95%, FSHD1) have a contraction of the D4Z4 repeat array in chromosome 4q35. Each D4Z4 repeat contains the first two exons of the double homeobox protein 4 (DUX4) gene, with its third (and final) exon located immediately downstream of the array. The D4Z4 array is normally hypermethylated in the course of development. Studies show that the contraction relaxes the chromatin and demethylates DNA in this region, resulting in aberrant DUX4 expression in skeletal muscle.

The aberrant expression of DUX4 in skeletal muscle is thought to cause FSHD. DUX4 encodes a transcription factor that activates pathways involved in muscle degeneration and apoptosis, events observed in patient muscles. DUX4 also inhibits myogenic differentiation and increases the sensitivity of muscle cells to oxidative stress. DUX4 is normally expressed during early embryonic development, and is then effectively silenced in all tissues except the testis and thymus. Its reactivation in skeletal muscle disrupts numerous signalling pathways that mostly converge on cell death. Thus, DUX4 serves as an attractive therapeutic target and inhibition of DUX4 expression represents be a potential therapy approach for FSHD.

Targeting Adipose Tissue

In some embodiments, the present invention provides a double-stranded iRNA agent that targets DEGS1 for the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets leptin for the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets FLCN for the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets ZFP42 3for the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets CDK6 the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets mTOR the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets RPTOR the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets FOXP1 the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets PDE3B the treatment of a metabolic disorder.

In some embodiments, the present invention provides a double-stranded iRNA agent that targets ACVR1C the treatment of a metabolic disorder.

Targeting DEGS1 for the prevention and/or treatment of a metabolic disorder

Obesity is a medical condition in which excess body fat has accumulated to the extent that it impairs health. The global epidemic of obesity is leading to unprecedented rates of diabetes and liver disease, and their late-stage complications, including cardiovascular and kidney disease.

The sphingolipid ceramide, which is a precursor to sphingomyelins and gangliosides that has both structural and signaling functions, is an important driver of the metabolic perturbations that underlie these diseases.

Dihydroceramide desaturase 1 (DEGS1), an enzyme that catalyzes the final step in the *de novo* synthesis of ceramide, is a particularly attractive therapeutic target. Indeed, studies in rodents reveal that inhibitors of ceramide synthesis prevent or reverse the pathogenic features of a metabolic disorder, including type 2 diabetes, nonalcoholic fatty liver disease, atherosclerosis, and cardiomyopathy. For example, Indeed, studies in rodents reveal that fenretinide, which directly targets and irreversibly inhibits DEGS1, prevented high fat diet-induced obesity and insulin resistance and hepatic steatosis without changes in energy expenditure or caloric intake (Preitner F, *et al. Am J Physiol Endocrinol Metab*. 2009; 279:E1420-E1429). Additionally, this DEGS1 inhibitor has been shown to sensitize obese post-menopausal women to insulin (Johansson H, *et al. Cancer Res.* 2008:68:9512-9518).

Targeting leptin for the prevention and/or treatment of a metabolic disorder

Leptin, the product of the Lep gene, is a 167-residue peptide hormone. It is primarily secreted by adipose tissue. Functional inactivation of the Lep gene leads to undetectable levels of leptin in circulation.

Most common forms of obesity are associated with excessive circulating levels of leptin (coined "hyperleptinemia"), which results in a still ill-defined state of "leptin resistance". The most

accepted definition of leptin resistance is the inability of pharmacological doses of leptin to suppress food intake and body weight.

Hyperleptinemia is correlated with pro-inflammatory responses and with the chronic sub-inflammatory state observed in obesity. On one hand, leptin enhances the production of inflammatory cytokines, and on the other hand, cytokines such as IL-6 and TNF-α promote leptin production by the adipose tissue.

Increased leptin resistance associated with high levels of free fatty acid and inflammatory cytokines may contribute to the reduction in lipid oxidation in insulin-sensitive organs, leading to accumulation of lipids (lipotoxicity) and insulin resistance. In addition, leptin induces cholesterol uptake by macrophages, angiogenesis, platelet aggregation, stimulates the oxidative stress in endothelial cells and inhibits vasorelaxation, increasing the risk of atherosclerosis. Furthermore, in humans, leptin is an independent risk factor for coronary artery disease (Paz-Filho G, *et al. Arq Bras Endocrinol Metab*. 2012; 56/9:597-607).

Targeting FLCN for the prevention and/or treatment of a metabolic disorder

Non-alcoholic steatohepatitis (NASH) represents a major economic burden and is characterized by triglyceride accumulation, inflammation, and fibrosis. No pharmacological agents are currently approved to treat this condition.

Autophagy has been demonstrated to play a significant role in this condition, which serves to degrade intracellular lipid stores, reduce hepatocellular damage, and dampen inflammation. Autophagy is primarily regulated by the transcription factors TFEB and TFE3, which are negatively regulated by mTORC1. Given that FLCN is an mTORC1 activator via its GAP activity towards RagC/D, a liver specific Flcn knockout mouse model was generated to study its role in NASH progression. It was demonstrated that loss of FLCN results in reduced triglyceride accumulation, fibrosis, and inflammation in mice exposed to a NASH-inducing diet. (Paquette M, *et al. BioRχiv*. 2020).

It has also been demonstrated that FLCN regulates adipose tissue browning via mTOR and the transcription factor TFE3. Adipose-specific deletion of FLCN relieves mTOR-dependent cytoplasmic retention of TFE3, leading to direct induction of the PGC-1 transcriptional coactivators, drivers of mitochondrial biogenesis and the browning program.

Targeting ZFP423 for the prevention and/or treatment of a metabolic disorder

Obesity is a medical condition in which excess body fat has accumulated to the extent that it impairs health. The global epidemic of obesity is leading to unprecedented rates of diabetes and liver disease, and their late-stage complications, including cardiovascular and kidney disease.

Due to the global epidemic of obesity, there is an urgency to understand mechanisms regulating adipose development. Adipogenesis is initiated by the expression of ZFP423, which

induces the expression of peroxisome proliferator-activated receptor γ (PPARGγ) and CCAAT-enhancer-binding proteins (C/EBPs), and genes specific for adipocytes.

Recently, ZFP423 was found to maintain white adipocyte identity by suppressing beige cell thermogenic gene progra). It has been shown in mice that adipocyte-specific inactivation of ZFP423 induced in adult mice leads to accumulation of beige-like adipocytes. The literature supports the notion that beige adipocytes can exert beneficial effects on glucose homeostasis and increase energy expenditure. Indeed, mice lacking adipocyte ZFP423 were resistant to diet-induced obesity. Furthermore, ZFP423 deficiency, combined with b3-adrenergic receptor activation, led to a reversal of weight gain and improved glucose tolerance when induced in obese animals (Shao M, *et al. Cell Metabolism*, 2016; 23:1167–1184).

Targeting CDK6 for the prevention and/or treatment of a metabolic disorder

Obesity has long been known to be the most important risk factor for the development of type II diabetes and other metabolic diseases. In rodents and humans, fat is deposited as energy storage in white adipose tissue (WAT), whereas fat is consumed to produce heat in the mitochondria-rich brown adipose tissues (BAT). As a thermogenic tissue, inducible-brown adipocytes (also called beige or brite cells) are found sporadically in WAT of adult animals with similar features as classical brown adipocytes. Importantly, the activation of beige cells is associated with a protection against obesity and metabolic diseases in rodent models and correlated with leanness in human

It has been shown that CDK6 regulates beige adipocyte formation and that mice lacking the CDK6 protein or its kinase domain (K43M) exhibit significant increases beige cell formation, enhanced energy expenditure, better glucose tolerance, and improved insulin sensitivity, and are more resistant to high-fat diet-induced obesity. Re-expression of CDK6 in Cdk6–/– mature or precursor cells, or ablation of RUNX1 in K43M mature or precursor cells, reverses these phenotypes (Hou X, *et al. Nature Comm*, 2018, 9: 1023). Additionally, overexpression of microRNA-107 (miR-107), which directly targets and downregulates CDK6, has been shown to reduce expression of CDK6 and its effectors and impairs adipocyte differentiation (Ahonen MA, et al. Molecular & Cellular Endocrin, 2019; 479:110-116). Thus, downregulation of CDK6 can potentially prevent and slow down progression of obesity..

Targeting RPTOR for the prevention and/or treatment of a metabolic disorder

Overnutrition causes hyperactivation of mTORC1-dependent negative feedback loops leading to the downregulation of insulin signaling and development of insulin resistance. Insulin signaling plays a crucial role in the control of systemic glucose homeostasis.

It has been shown that knockout of Rptor in different specific tissues of are protective against weight-gain and obesity. For example, a study in mice with Rptor conditionally deleted in osteoblast (Rptorob–/–) shows that, as compared to controls, chow-fed Rptorob–/– mice had substantially less fat mass and exhibited adipocyte hyperplasia. Remarkably, upon feeding with high-fat diet, mice with

pre- and post-natal deletion of Rptor were protected from diet-induced obesity and exhibited improved glucose metabolism with lower fasting glucose and insulin levels, increased glucose tolerance and insulin sensitivity. This leanness and resistance to weight gain was not attributable to changes in food intake, physical activity or lipid absorption but instead was due to increased energy expenditure and greater whole-body substrate flexibility. RNA-seq revealed an increase in glycolysis and skeletal insulin signaling pathways, which correlated with the potentiation of insulin signaling and increased insulin-dependent glucose uptake in Rptor-knockout (Tangseefa P, *et al. Bone Research*, 2021; 9:10).

In another study, mice with an adipose-specific knockout of Rptor was generated, and these mice had substantially less adipose tissue, were protected against diet-induced obesity and hypercholesterolemia, and exhibited improved insulin sensitivity. Leanness was in spite of reduced physical activity and unaffected caloric intake, lipolysis, and absorption of lipids from the food (Polak P, *et al. Cell Metabolism*, 2008; 8(5):399-410). With similar profile, mice with Rptor knockout specifically in intestinal epithelial cells consistently gained less body weight on a high-fat diet compared to wildtype mice secondary to significantly reduced food intake. Importantly, the intestinal epithelial cell-specific Rptor knockout mice did not appear to be malnourished, demonstrated by their preservation of lean body mass, and also maintained a normal metabolic profile without significant changes in triglyceride or fasting glucose levels (Onufer E, *et al. Biochem Biophys Res Comm*, 2018; 505(4): 1174-1179)..

Targeting mTOR for the prevention and/or treatment of a metabolic disorder

The mTOR kinase is encoded by a single gene in mammals, but it exerts its main cellular functions by forming mTORC1 and mTORC2 through assembly with specific adaptor proteins. mTORC1 controls protein synthesis, cell growth and proliferation, and mTORC2 is a regulator of the actin cytoskeleton, and promotes cell survival and cell cycle progression. Cellular adenosine triphosphate (ATP) levels increase mTOR activity, and the mTOR kinase itself serves as a cellular ATP sensor. mTOR, thus, works as a critical checkpoint by which cells sense and decode changes in energy status

Overnutrition causes hyperactivation of mTORC1-dependent negative feedback loops leading to the downregulation of insulin signaling and development of insulin resistance. Insulin signaling plays a crucial role in the control of systemic glucose homeostasis.

In vitro experiments have demonstrated that mTORC1 is essential for the differentiation and maintenance of white adipocytes. Indeed, mTORC1 activation is necessary for insulin- or nutrient (amino acid)-induced adipogenesis and expression of SREBP1 and PPARγ, which are master transcriptional regulators of adipocyte differentiation and lipid homeostasis. Conversely, mTOR's inhibitor rapamycin impairs adipocyte differentiation by inhibiting PPARγ transactivation activity. In diet-induced obesity, overactivity of the mTORC1 signaling favors the expansion of the white adipose tissue mass, leading to adipocytes insulin resistance (Catania C, *et al. Int J Obes*, 2011; 35:751-761).

Targeting FOXP1 for the prevention and/or treatment of a metabolic disorder

Obesity and its related complications such as type 2 diabetes mellitus, coronary heart disease and obstructive sleep apnea, have been considered significant health problems. Although dietary management, exercise, and pharmacological intervention have been proven to control weight, these approaches are largely inefficient for maintaining healthy long-term weight loss. Therefore, effective therapies for treating obesity and related metabolic disorders are needed.

Increasing brown adipose tissue (BAT) mass or/and activity in mice and humans has been demonstrated to help lose weight and improve whole-body metabolism.

In addition, adipose-specific deletion of FOXP1 led to an increase of brown adipose activity and browning program of white adipose tissues. The FOXP1-deficient mice showed an augmented energy expenditure and are protected from diet-induced obesity and insulin resistance. Consistently, overexpression of Foxp1 in adipocytes impaired adaptive thermogenesis and promotes diet-induced obesity.

In addition, deletion of the FOXP1 gene in osteoblasts led to augmentation of AdipoQ levels accompanied by fueled energy expenditure in adipose tissues. Adiponectin (AdipoQ) is a hormone abundantly secreted by adipose tissues, has multiple beneficial functions, including insulin sensitization as well as lipid and glucose metabolism. In contrast, overexpression of FOXP1 in bones impaired AdipoQ secretion and restrained energy consumption. Chromatin immunoprecipitation sequencing analysis revealed that AdipoQ expression, which increases as a function of bone age, is directly controlled by FOXP1 (Zhang W, *et al. J Bone Miner Res*, 2021).

Targeting PDE3B for the prevention and/or treatment of a metabolic disorder

The incidence of obesity in the developed world is increasing at an alarming rate. Concurrent with the increase in the incidence of obesity is an increase in the incidence of type 2 diabetes. Cyclic AMP (cAMP) and cGMP are key second messengers in all cells; for example, when it comes to processes of relevance for the regulation of energy metabolism, cAMP is a key mediator in the regulation of lipolysis, glycogenolysis, gluconeogenesis and pancreatic β cell insulin secretion.

PDE3B, one of several enzymes which hydrolyze cAMP and cGMP, is expressed in cells of importance for the regulation of energy homeostasis, including adipocytes, hepatocytes, hypothalamic cells and β cells. In adipocytes, PDE3B (phosphodiesterase 3B) is an important regulatory effector in signalling pathways controlled by insulin and cAMP-increasing hormones. Previous results from PDE3B-transgenic mice indicate that PDE3B plays an important role in modulation of energy metabolism. In epididymal white adipose tissue of PDE3B KO mice on a SvJ129 background, cAMP/protein kinase A (PKA) and AMP-activated protein kinase (AMPK) signaling pathways are activated, resulting in "browning" phenotype, with a smaller increase in body weight under high-fat diet, smaller fat deposits, increased β-oxidation of fatty acids (FAO) and oxygen consumption (Chung YW, *et al. Scientific Report*, 2017:7:40445).

In human, genome-wide analysis of array-based rare, non-synonymous variants in 184,246 individuals of UK Biobank and exome-sequence-based rare loss of function gene burden testing indicated that loss-of function of PDE3B is associated with a beneficial impact on waist-to-hip ration adjusted for BMI (WHRadjBMI).

Targeting ACVR1C for the prevention and/or treatment of a metabolic disorder

Body fat distribution strongly influences the development of type 2 diabetes. In a Mendelian randomization study of 296,291 individuals, it was previously found that a genetic predisposition to increased abdominal fat distribution was associated with elevated triglyceride levels, elevated blood pressure, and an increased risk of coronary artery disease, independent of overall adiposity. Furthermore, a genetic predisposition to increased abdominal fat distribution was strongly associated with the development of type 2 diabetes. For each 1 SD genetic increase in waist-to-hip ratio adjusted for BMI (WHRadjBMI) (a measure of body fat distribution), risk of type 2 diabetes increased by 77% (12). These findings were replicated in a separate Mendelian randomization study.

Recent studies of ALK7 indicate that ALK7's primary function in metabolic regulation is to limit catabolic activities and preserve energy. ALK7-knockout mice showed reduced diet-induced weight gain and fat accumulation when subjected to a high fat diet (Ibanez CA, FEBS J, 2021). In human, it has been demonstrated that variants predicted to lead to loss of function of the gene ACVR1C, which encodes the activin receptor-like kinase 7 (ALK7), influence body fat distribution and protect against type 2 diabetes (Emdin CA, *et al. Diabetes*, 2019:68(1):226-234). Genome-wide analysis of array-based rare, non-synonymous variants in 184,246 individuals of UK Biobank and exome-sequence-based rare loss of function gene burden testing indicated that loss-of function of ALK7 is associated with a beneficial impact on waist-to-hip ration adjusted for BMI (WHRadjBMI).

XII. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a dsRNA agent of the invention. In certain embodiments, the individual components of the pharmaceutical formulation may be provided in one container. Alternatively, it may be desirable to provide the components of the pharmaceutical formulation separately in two or more containers, *e.g.*, one container for a dsRNA agent and at least another for a pharmaceutically acceptable carrier, *e.g.*, PBS. The kit may be packaged in a number of different configurations such as one or more containers in a single box. The different components can be combined, *e.g.*, according to instructions provided with the kit. The components can be combined according to a method described herein, *e.g.*, to prepare and administer a pharmaceutical composition. The kit can also include a delivery device.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the RNAi agents and methods featured in the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

An inforrmal Sequence Listing is filed herewith and forms part of the specification as filed.

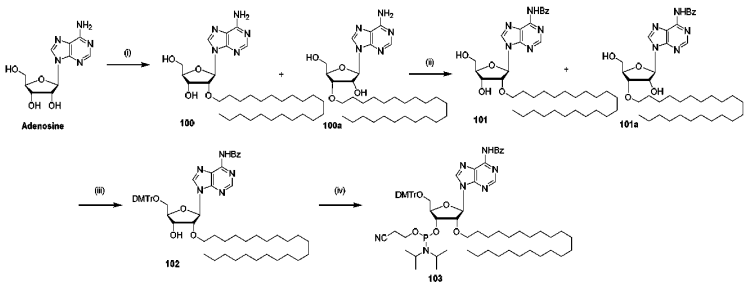
This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the informal Sequence Listing and Figures, are hereby incorporated herein by reference.

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EXAMPLES

Example 1: Synthesis of C22-Nucleoside Phosphoramidites for the Synthesis of dsRNA Agent Conjugates

Scheme 1



Scheme 1. Synthesis of 2'-C22 A phosphoramidite. (i) 1-Bromodocosane, KOH, DMF, 60 °C, overnight. (ii) (1) TMSCl, Pyridine, 0 °C to rt, 3 h, (2) Bz_2O, 0 °C to rt, overnight, (3) ammonium hydroxide, 0 °C to rt, 5 h. (iii) DMTrCl, Pyridine, rt, 6 h. (iv) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, EtOAc, 0 °C to rt, 1 h.

Compound 100: Adenosine (25 g, 93.6 mmol) and DMF (250 mL) were added into a 500 mL round-bottom flask, and then the suspension was warmed to 60 °C. 1-Bromodocosane (54.7 g, 140 mmol) and KOH (10.5 g, 187 mmol) were added into the suspension and the reaction mixture was stirred at 60 °C overnight (16 h). The reaction was cooled to room temperature (a lot of insoluble matters were observed) and quenched by addition of NH_4Cl (10 g). The mixture, including the insoluble matter, was poured into a 2 L separating funnel and diluted with CH_2Cl_2 and H_2O. (3 phases; organic phase, aqueous phase and emulsion phase, were observed.) The aqueous and emulsion phases were extracted with CH_2Cl_2 3 times. TLC indicated that a major product spot was detected in the organic phase (5% MeOH in ethyl acetate, Rf = 0.5). The collected organic phase was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2 for 60 min) to obtain a mixture of 2'-C22 product (compound **100**) and 3'-C22 product (**100a**) as a white solid (28.2 g, 52%; 2'-C22/3'-C22 = ca. 9:1). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.38–8.33 (m, 1H), 8.13 (s, 1H), 7.35 (brs, 2H), 5.98 (d, *J* = 6.3 Hz, 0.9H), 5.78 (d, *J* = 6.2 Hz, 0.1H), 5.47–5.40 (m, 1H), 5.19–5.16 (m, 1H), 4.73 (q, *J* = 5.8 Hz, 0.1H), 4.47 (dd, *J* = 4.8, 6.4 Hz, 0.9H), 4.30–4.28 (m, 0.9H), 4.04–4.03 (m, 0.1H), 3.99–3.97 (m, 0.9H), 3.94–3.92 (m, 0.1H), 3.69–3.66 (m, 1H), 3.58–3.53 (m, 2H), 3.37–3.30

(m, 1H), 1.58–1.53 (m, 0.2H), 1.39–1.36 (m, 1.8H), 1.29–1.08 (m, 38H), 0.85 (t, J = 6.6 Hz, 3H). **LCMS** (ESI) calculated for $C_{32}H_{58}N_5O_4$ $[M+H]^+$ m/z = 576.45, found 576.4.

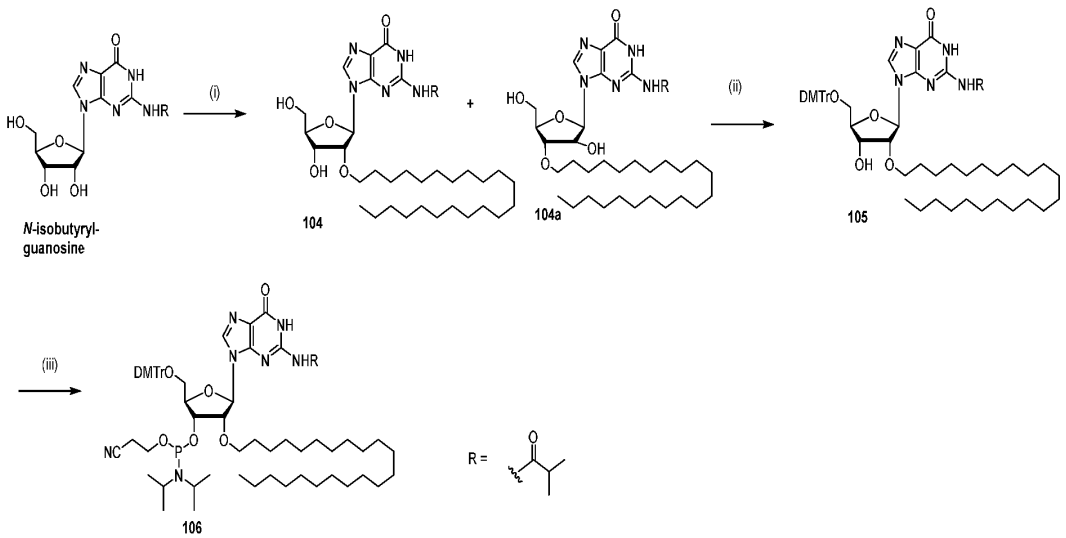
Compound 101: To a suspension of compound **100** and 3'-C22 **100a** (mixture, 28 g, 48.6 mmol) in pyridine (40 mL) was added dropwise TMSCl (2.98 mL, 23.4 mmol) at 0 °C and the mixture was warmed to room temperature and stirred for 3 h. TLC indicated that compound **100 and 100a** was consumed, and a new major spot was detected (50% ethyl acetate in hexane, Rf = 0.7). The reaction mixture was cooled to 0 °C and benzoic anhydride (2.12 g, 9.38 mmol) was added. The resulting solution was wormed to room temperature and stirred overnight (14 h). TLC indicated that the protected intermediate with TMS was consumed, and a new major spot was detected (50% ethyl acetate in hexane, Rf = 0.8). The reaction was cooled to 0 °C and quenched with H_2O. The resulting solution was warmed to room temperature and stirred for 5 h. The mixture was cooled to 0 °C and 28% ammonium hydroxide solution (40 mL) was added. The resulting mixture was warmed to room temperature and stirred for 5 h. TLC indicated that the fully protected intermediate with TMS and Bz groups was consumed, and a new major spot was detected (100% ethyl acetate, Rf = 0.5). The reaction was diluted with ethyl acetate and the organic layer was washed with water, brine and dried (Na_2SO_4) and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–5% MeOH in CH_2Cl_2 for 30 min) to obtain a mixture of 2'-C22 product (compound **101**) and 3'-C22 product (**101a**) as a white solid (24.9 g, 75%; 2'-C22/3'-C22 = ca. 9:1). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 11.22 (s, 1H), 8.75 (s, 1.8H), 8.73 (s, 0.2H), 8.06–8.04 (m, 2H), 7.66–7.63 (m, 1H), 7.57–7.54 (m, 2H), 6.14 (d, J = 5.9 Hz, 0.9H), 6.04 (d, J = 5.8 Hz, 0.1H), 5.53 (d, J = 6.2 Hz, 0.1H), 5.23 (d, J = 5.3 Hz, 0.9H), 5.17 (brs, 1H), 4.79 (q, J = 5.5 Hz, 0.1H), 4.52 (dd, J = 5.0, 6.1 Hz, 0.9H), 4.34 (q, J = 4.2 Hz, 0.9H), 4.06 (q, J = 3.9 Hz, 0.1H), 4.04–3.98 (m, 1H), 3.71–3.69 (m, 1H), 3.62–3.59 (m, 2H), 3.52–3.48 (m, 0.1H), 3.42–3.39 (m, 0.9H), 1.58–1.53 (m, 0.2H), 1.44–1.40 (m, 1.8H), 1.26–1.11 (m, 38H), 0.84 (t, J = 6.8 Hz, 3H). **LCMS** (ESI) calculated for $C_{39}H_{62}N_5O_5$ $[M+H]^+$ m/z = 680.48, found 680.6.

Compound 102: To a solution of compound **101** and 3'-C22 product **101a** (mixture, 25 g, 36.8 mmol) in pyridine (300 mL) was added 4,4'-dimethoxytriphenyl chloride (12.5 g, 36.8 mmol) and the mixture was stirred at room temperature for 6 h. TLC indicated that compound **101** was consumed, and a new major spot was detected (90% ethyl acetate in hexane, Rf = 0.8). The reaction was quenched with saturated $NaHCO_3$ (aq.) and diluted with ethyl acetate. The organic layer was washed with water, brine and dried (Na_2SO_4) and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexane for 10 min and then kept 50% ethyl acetate in hexane for 10 min) to obtain compound **102** as a light-yellow form (28.5 g, 79%). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 11.23 (brs, 1H), 8.66 (s, 1H), 8.60 (s, 1H), 8.06–8.04 (m, 2H), 7.66–7.63 (m, 1H), 7.56–7.54 (m, 2H), 7.38–7.36 (m, 2H), 7.27–7.18 (m, 7H), 6.85–6.82 (m, 4H), 6.15 (d, J = 5.3 Hz, 1H), 5.26 (d, J = 5.8 Hz, 1H), 4.66 (t, J = 5.1 Hz, 1H), 4.39 (q, J = 5.1 Hz,

1H), 4.12 (q, *J* = 4.5 Hz, 1H), 3.72 (s, 6H), 3.61 (dt, *J* = 6.5, 9.7 Hz, 1H), 3.45 (dt, *J* = 6.5, 9.7 Hz, 1H), 3.30–3.24 (m, 2H), 1.47–1.42 (m, 2H), 1.27–1.12 (m, 38H), 0.84 (t, *J* = 6.8 Hz, 3H). **LCMS** (ESI) calculated for $C_{60}H_{80}N_5O_7$ $[M+H]^+$ m/z = 982.61, found 982.6.

Compound 103: To a solution of compound **102** (5 g, 5.09 mmol) in ethyl acetate (30 mL) was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (1.48 mL, 6.62 mmol) at 0 °C and the mixture was stirred at room temperature for 1 h. TLC indicated that compound **102** was consumed, and a new major spot was detected (40% ethyl acetate in hexane, Rf = 0.4). The reaction mixture was quenched with saturated $NaHCO_3$ (aq.) and then the organic layer was washed with water, brine, dried (Na_2SO_4) and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–40% ethyl acetate in hexane for 15 min and then 40–50% ethyl acetate in hexane for 20 min) to obtain compound **103** as a white form (4.58 g, 76%). **^{1}H NMR** (600 MHz, CD_3CN) δ 9.36 (brs, 1H), 8.62–8.60 (m, 1H), 8.31–8.29 (m, 1H), 8.02–8.00 (m, 2H), 7.67–7.64 (m, 1H), 7.57–7.55 (m, 2H), 7.47–7.43 (m, 2H), 7.35–7.21 (m, 7H), 6.86–6.82 (m, 4H), 6.14–6.13 (m, 1H), 4.84–4.81 (m, 1H), 4.74–4.69 (m, 1H), 4.37–4.31 (m, 1H), 3.95–3.63 (m, 11H), 3.56–3.52 (m, 1H), 3.50–3.45 (m, 1H), 3.39–3.33 (m, 1H), 2.74–2.65 (m, 1H), 2.52 (t, *J* = 6.1 Hz, 1H), 1.53–1.48 (m, 2H), 1.34–1.19 (m, 47H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.90 (t, *J* = 6.8 Hz, 3H). **^{31}P NMR** (243 MHz, CD_3CN) δ 149.89, 149.85, 149.81, 149.77, 149.74, 149.70, 149.48, 149.44, 149.40, 149.36, 149.32, 149.28. **LCMS** (ESI) calculated for $C_{69}H_{97}N_7O_8P$ $[M+H]^+$ m/z = 1182.71, found 1182.6.

Scheme 2



Scheme 2. Synthesis of 2'-C22 G phosphoramidite. (i) 1-Bromodocosane, NaH, DMF, rt to 90 °C, 24 h. (ii) DMTrCl, Pyridine, rt, overnight. (iv) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, EtOAc, 0 °C to rt, 1 h.

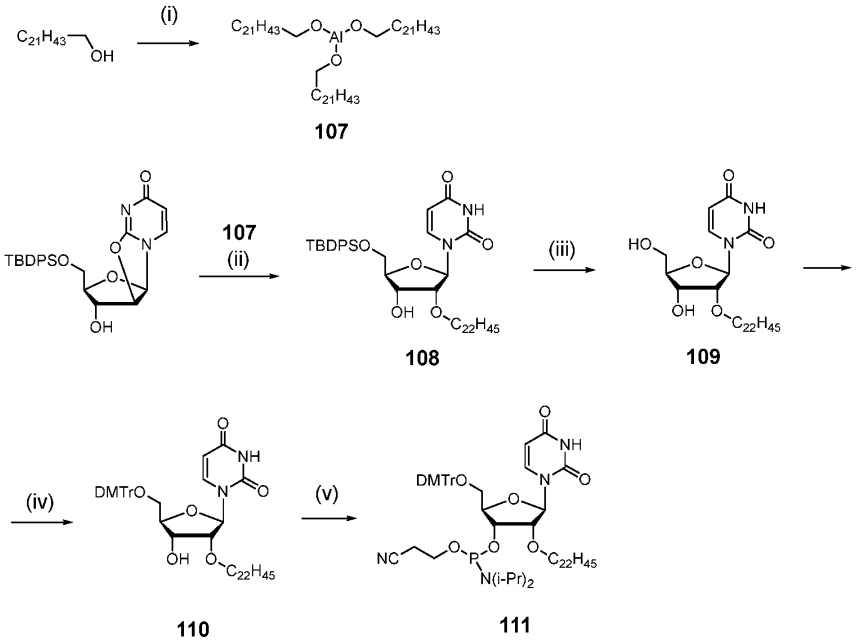
Compound 104: *N*-Isobutyrylguanosine (5 g, 14.2 mmol) and DMF (50 mL) were added into a 250 mL round-bottom flask, and then the solution was cooled to 0 °C. NaH (60% in mineral oil; 1.42 g, 35.4 mmol) was added portion-wise into the solution and the suspension was stirred at 0 °C for 30 min. To the mixture was added 1-bromodocosane (8.27 g, 21.2 mmol). The suspension was wormed up to 90 °C and stirred for 24 h. TLC indicated that *N*-isobutyrylguanosine remained, and a new major spot was detected (100% ethyl acetate, Rf = 0.4). The reaction mixture was cooled to 0 °C and quenched by addition of NH_4Cl (a lot of insoluble matters were observed). The mixture, including the insoluble matter, was poured into a 1 L separating funnel and diluted with CH_2Cl_2 and H_2O. (3 phases; organic phase, aqueous phase and emulsion phase, were observed.) The aqueous and emulsion phases were extracted with CH_2Cl_2 3 times. The collected organic phase was dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel (0–10% MeOH in CH_2Cl_2 for 30 min) to obtain a mixture of 2'-C22 (compound **104**)/3'-C22 products(104a), contained some other impurities, as a white solid (2.10 g, 22%). **LCMS** (ESI) calculated for $C_{36}H_{64}N_5O_6$ $[M+H]^+$ m/z = 662.49, found 662.6. The obtained compound **104** was used for the next reaction without any further purifications.

Compound 105: To a solution of compound **104** (2.1 g, 3.17 mmol) in pyridine (30 mL) was added 4,4'-dimethoxytriphenyl chloride (1.07 g, 3.17 mmol) and the mixture was stirred at room temperature for overnight (14 h). TLC indicated that compound **104** was consumed, and a new major spot was detected (80% ethyl acetate in hexane, Rf = 0.8). The reaction was quenched with saturated $NaHCO_3$ (aq.) and diluted with ethyl acetate. The organic layer was washed with water, brine and dried (Na_2SO_4) and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–45% ethyl acetate in hexane for 10 min and then kept 45% ethyl acetate in hexane for 30 min) to obtain compound **105** as a white form (2.13 g, 70%). **^{1}H NMR** (600 MHz, DMSO-d_6) δ 12.10 (brs, 1H), 11.64 (brs, 1H), 8.12 (s, 1H), 7.36–7.34 (m, 2H), 7.27–7.19 (m, 7H), 6.85–6.81 (m, 4H), 5.93 (d, *J* = 5.8 Hz, 1H), 5.18 (d, *J* = 5.4 Hz, 1H), 4.40 (t, *J* = 5.4 Hz, 1H), 4.26 (q, *J* = 4.8 Hz, 1H), 4.05 (ddd, *J* = 3.5, 3.5, 6.0 Hz, 1H), 3.73 (s, 6H), 3.59 (dt, *J* = 6.4, 9.7 Hz, 1H), 3.45 (dt, *J* = 6.4, 9.7 Hz, 1H), 3.30 (d, *J* = 6.0, 10.5 Hz, 1H), 3.16 (d, *J* = 3.5, 10.5 Hz, 1H), 2.76 (sept, *J* = 6.8 Hz, 1H), 1.44–1.40 (m, 2H), 1.26–1.11 (m, 44H), 0.84 (t, *J* = 6.8 Hz, 3H). **LCMS** (ESI) calculated for $C_{57}H_{82}N_5O_8$ $[M+H]^+$ m/z = 964.62, found 964.6.

Compound 106: To a solution of compound **105** (2 g, 2.07 mmol) and DIPEA (0.470 mL, 2.70 mmol) in ethyl acetate (20 mL) was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.601 mL, 2.70 mmol) at 0 °C and the mixture was stirred at room temperature for 1 h. TLC indicated that compound **105** was consumed, and a new major spot was detected (50% ethyl acetate in hexane, Rf = 0.6). The reaction mixture was washed with saturated $NaHCO_3$ (aq.), water, brine, dried (Na_2SO_4) and concentrated under vacuum. The crude residue was purified by column chromatography on silica gel (0–50% ethyl acetate in hexane for 30 min and then

kept 50% ethyl acetate in hexane for 10 min) to obtain compound **106** as a white form (1.76 g, 73%). **^{1}H NMR** (600 MHz, CD_3CN) δ 11.99 (brs, 1H), 9.16 (brs, 1H), 7.89–7.87 (m, 1H), 7.49–7.23 (m, 9H), 6.87–6.83 (m, 4H), 5.92 (t, *J* = 6.1 Hz, 1H), 4.68–4.63 (m, 1H), 4.53–4.47 (m, 1H), 4.34–4.26 (m, 1H), 3.92–3.60 (m, 11H), 3.54–3.49 (m, 1H), 3.40 (d, *J* = 4.0 Hz, 1H), 3.35 (d, *J* = 4.0 Hz, 1H), 2.73–2.65 (m, 1H), 2.57–2.47 (m, 2H), 1.53–1.47 (m, 2H), 1.34–1.18 (m, 47H), 1.15–1.06 (m, 9H), 0.90 (t, *J* = 6.7 Hz, 3H). **^{31}P NMR** (243 MHz, CD_3CN) δ 149.80, 149.76, 149.72, 149.68, 149.64, 149.60, 149.56, 149.51, 149.48, 149.44. **LCMS** (ESI) calculated for $C_{66}H_{99}N_7O_9P$ $[M+H]^+$ m/z = 1164.72, found 1164.8.

Scheme 3



Scheme 3. Synthesis of 2'-C22 U. (i) $AlMe_3$, diglyme, 100 °C, 0.5 h, 100%. (ii) diglyme, 145 °C, 48 h, 36%. (iii) Et_3N/3HF, THF, 50 °C, 14 h, 98%. (iv) DMTrCl, Py, Et_3N, 86%. (v) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, rt, 14 h, 93%.

Compound 107: 2M solution of $AlMe_3$ (50 mL, 0.10 mol) was added slowly for ca. 15 min to a stirred suspension of 1-docosanol (108 g, 0.33 mol) in anhyd. diglyme (90 mL) under Ar atmosphere in a 2-neck 1L flask fitted with a magnetic stirring bar, and an outlet with a gas bubbler over a reflux condenser. After completion of the addition, the mixture was heated to 100 °C until evolution of gas through the bubbler was complete (30 min). The mixture was cooled to 65 °C and diluted with anhyd. AcOEt (150 mL) and anhyd. ACN (150 mL). The mixture was cooled down to rt in the bath overnight, a white residue formed was filtered through a 600 mL glass filtering funnel under the cushion of Ar, and washed with a 1:1 mixture of anhyd. AcOEt and anhyd. ACN (400 mL x 2) under

the cushion of Ar. The residue was dried on the funnel in reverse flow of nitrogen, transferred to a flask and dried in high vacuum for 24 h to afford 107.4 g of the alkoxide **107** of ca. 93% purity containing ca. 7% of 1-docosanol that was used in the next step without of further purification. The product was stored under Ar atmosphere.

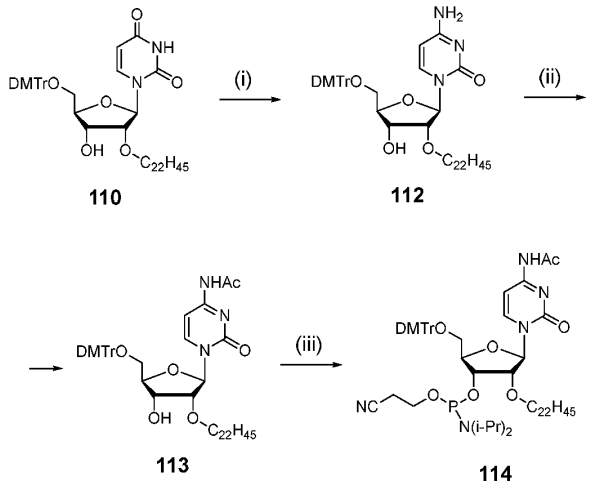
Compound 108: A mixture of 5'-TBDPS-protected anhydro-uridine (18.6 g, 40 mmol), aluminum alkoxide **107** (~93%, 47.6 g, 44 mmol) and anhyd. diglyme (60 mL) was heated to 145 °C bath temperature in a flask fitted with a magnetic stirring bar and a reflux condenser under slight positive pressure of Ar using a balloon for 48 h. The mixture was cooled down to 70 °C in the bath, diluted with AcOEt (200 mL), further cooled down 30 °C and quenched by addition of 10% H_3PO_4 (200 mL). A suspension thus formed was stirred at rt overnight, filtered through a 600 mL glass filtering funnel, and the solids were washed thoroughly with water (ca. 50 mL) and AcOEt (ca. 300 mL) mixture. Thoroughly compressed solid residue was dried in warm air to afford 25.4 g (55%) of recovered 1-docosanol. The filtrate was transferred to a separatory funnel, the organic layer was separated, washed with 1% NaCl (500 mL x 2), saturated NaCl (200 mL) and dried over anhyd. Na_2SO_4. The solvent was removed in vacuo, the residue was co-evaporated with additional portion of AcOEt (300 mL) to afford 61.8 g of crude residue. The latter was dissolved in 190 mL of AcOEt-hexanes 1:4 mixture and liquid-loaded on a standard 330 g column of silica gel. The column was eluted with isocratic 20 % AcOEt in hexanes followed by gradient of 20 to 40% of AcOEt in hexanes, the fractions containing product were pulled, evaporated in vacuum, co-evaporated twice with ACN-diethyl ether mixture, and dried in high vacuum to afford 11.4 g (36%) of pure product **108**. ^{1}H NMR (600 MHz, Acetone-d6) δ 10.08 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.80 – 7.76 (m, 2H), 7.76 - 7.73 (m, 2H), 7.53-7.44 (m, 6H); 6.00 (d, *J* = 3.0 Hz, 1H), 5.27 (d, *J* = 8.4 Hz, 1H), 4.46 (q, *J* = 5.4 Hz, 1H), 4.12 (dd, *J* = 12.0, 2.4 Hz, 1H), 4.08 – 4.04 (m, 2H), 3.98 (dd, *J* = 11.4, 2.4 Hz, 1H), 3.95 (d, *J* = 7.2 Hz, 1H), 3.78 (dt, *J* = 9.6, 6.6 Hz, 1H), 3.69 (dt, *J* = 9.6, 6.6 Hz, 1H), 1.65 – 1.59 (m, 2H), 1.43 - 1.36 (m, 2H), 1.35 - 1.25 (m, 36H), 1.13 (s, 9H), 0.89 (t, *J* = 6.6 Hz, 3H). MS (ESI+APCI), calculated for $C_{47}H_{74}N_2O_6Si$ $[M+H]^+$ exact mass m/z = 791.54, found 791.7.

Compound 109: A mixture of TBDPS-protected nucleoside **108** (2.25 g, 2.8 mmol), anhyd. THF (10 mL), and triethylamine trihydrofluoride (2 mL, 12 mmol) was heated at 50 °C under Ar atmosphere for 24 h. Heptane (40 mL) followed by water (40 mL) were added, the heating bath was removed, the mixture was stirred overnight at rt, filtered, and washed thoroughly by water-heptane mixture. The solid was dried in the flow of nitrogen for 2 h followed by warm air overnight to afford 1.52 g (98%) of **109** as a white solid. ^{1}H NMR (600 MHz, Acetone-d6) δ 10.01 (s, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 5.97 (d, *J* = 4.2 Hz, 1H), 5.60 (d, *J* = 8.4 Hz, 1H), 4.37 (s, 1H), 4.35-4.29 (m, 1H), 4.06 (t, *J* = 4.8 Hz, 1H), 4.00 (dt, *J* = 5.4, 2.4 Hz, 1H), 3.93 – 3.87 (m, 1H), 3.84 (d, *J* = 6.6 Hz, 1H), 3.83 - 3.78 (m, 1H), 3.74 - 3.63 (m, 2H), 1.64 - 1.56 (m, 2H), 1.43 - 1.23 (m, 38H), 0.89 (t, *J* = 6.6 Hz, 3H). MS (ESI+APCI), calculated for $C_{31}H_{56}N_2O_6$ $[M+H]^+$ exact mass m/z = 553.42, found 553.5.

Compound 110: Triethylamine (0.77 mL, 5.5 mmol) was added to a solution of nucleoside **109** (1.49 g, 2.7 mmol) and DMTrCl (1.86 g, 5.5 mmol) in anhyd. pyridine (10 mL) under Ar atmosphere. The mixture was stirred at rt overnight, quenched by addition of MeOH (0.2 mL), and diluted with ACN (25 mL). The solvents were evaporated in vacuo at 25 °C, the residue was co-evaporated twice with ACN at 25 °C and partitioned between AcOEt and 5% NaCl. The organic phase was separated, washed with sat. NaCl, and dried over anhyd. Na_2SO_4. The solvent was removed in vacuo to afford 3.50 g of crude product. The latter was purified by chromatography over a standard 40 g column of silicagel with isocratic 30% of AcOEt in hexanes followed by gradient of 30 to 50% of AcOEt in hexanes. Fractions contained product were pulled evaporated in vacuum, co-evaporated twice with ACN-diethyl ether mixture, and dried in high vacuum to afford 1.98 g (86%) of product **110** as a yellowish foam. ^{1}H NMR (600 MHz, Acetone-d6) δ 10.08 (s, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.52 - 7.48 (m, 2H), 7.40 – 7.33 (m, 6H), 7.29 – 7.26 (m, 1H), 6.93 (d split, *J* = 9.0 Hz, 4H), 5.96 (d, *J* = 2.4 Hz, 1H), 5.27 (d, *J* = 7.8 Hz, 1H), 4.52 – 4.48 (m, 1H), 4.12 - 4.07 (m, 2H), 3.94 (d, *J* = 7.8 Hz, 1H), 3.85 - 3.77 (m, 1H), 3.81 (s, 6H), 3.73 – 3.68 (m, 1H), 3.52 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.46 (dd, *J* = 10.8, 3.0 Hz, 1H), 1.67 – 1.59 (m, 2H), 1.43 – 1.56 (m, 2H), 1.36 – 1.23 (m, 36H), 0.89 (t, *J* = 6.6 Hz, 3H). MS (ESI+APCI), calculated for $C_{52}H_{74}N_2O_8$ $[M+H]^+$ exact mass m/z = 854.54.

Compound 111: DIPEA (0.46 mL, 2.6 mmol) followed by 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.57 mL, 2.6 mmol) were added to a solution of compound **110** (1.71 g, 2 mmol) in anhyd. DCM (10 mL) under Ar. The mixture was stirred at rt for 14 h, cooled to 0 °C, quenched by addition of sat. $NaHCO_3$ and extracted with AcOEt (25 mL). Organic phase was separated, washed with sat. NaCl, and dried over anhyd. sodium sulfate. Crude material (2.20 g) was purified over a standard 40 g flash column of silica gel that was eluted with isocratic 50% of AcOEt containing 0.3% of TEA in hexanes to afford 1.96 g (93%) of **111** as a white foam. ^{1}H NMR (500 MHz, CD3CN) δ 8.93 (s, 1H), 7.82 – 7.70 (m, 1H), 7.47 – 7.41 (m, 2H), 7.36 – 7.23 (m, 8H), 6.91 – 6.85 (m, 4H), 5.87 – 5.82 (m, 1H), 5.24 – 5.19 (m, 1H), 4.50 – 4.38 (m, 1H), 4.19 – 4.11 (m, 1H), 4.06 – 3.99 (m, 1H), 3.77 (s, 3H), 3.77 (s, 3H), 3.63 – 3.59 (m, 2H), 3.45 – 3.41 (m, 1H), 2.72 – 2.59 (m, 1H), 2.52 (t, *J* = 6.0 Hz, 1H), 1.60 – 1.50 (m, 2H), 1.42 – 1.07 (m, 51H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.90 – 0.84 (m, 3H). C13 NMR (151 MHz, CD3CN) δ 163.47, 163.43, 159.33, 159.31, 159.30, 150.84, 145.32, 145.25, 140.54, 140.51, 135.99, 135.95, 135.85, 135.77, 130.76, 130.71, 130.69, 128.63, 128.60, 128.51, 127.57, 119.12, 119.00, 113.68, 113.67, 102.11, 102.02, 88.36, 88.23, 87.22, 87.18, 82.95, 82.93, 82.80, 82.76, 81.98, 81.25, 81.22, 71.24, 71.00, 70.76, 70.66, 70.59, 70.51, 62.45, 62.00, 59.15, 59.02, 58.78, 58.64, 55.48, 55.46, 43.58, 43.54, 43.50, 43.45, 32.21, 30.01, 29.99, 29.96, 29.92, 29.85, 29.73, 29.69, 29.65, 26.32, 26.30, 24.67, 24.62, 24.57, 24.52, 24.48, 24.46, 24.41, 22.96, 20.65, 20.60, 13.98. P31 NMR (202 MHz, CD3CN) δ 149.47, 149.08.

Scheme 4



Scheme 4. Synthesis of 2'-C22 C. (i) a). TMSCl, NMP, ACN, rt, 1h. b). TFAA, NMP, ACN, 0 °C, 40 min, then p-nitrophenol, 0 °C, 3h. c). NH_4OH, H_2O, dioxane, 55 °C, 24 h, 60%. (ii) Ac_2O, DMF, rt, 48 h, 100%. (iii) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, DIPEA, DCM, rt, 14 h, 90%.

Compound 112: TMSCl (0.4 mL, 3.2 mmol) was added to a solution of **110** (1.20 g, 1.4 mmol) and NMP (1.2 mL, 11.8 mmol) in anhyd. MeCN (7 mL) under Ar atmosphere. The mixture was stirred at rt for 1 h, cooled to 0 °C, and TFAA (0.5 mL, 3.6 mmol) was added slowly dropwise via syringe. The mixture was stirred at 0 °C for 40 min, and p-nitrophenol (0.56 g, 4 mmol) was added. The mixture was stirred at 0 °C for 3 h and quenched by addition of sat. sodium bicarbonate (15 mL). The cooling bath was removed, ethyl acetate (30 mL) was added, followed by minimal amount of water to dissolve inorganic precipitates. The organic phase was separated, washed with sat. NaCl, dried over anhyd. sodium sulfate and evaporated in vacuum to afford 1.94 g of oily residue. The latter was dissolved in dioxane (15 mL), the solution was transferred to a pressure bottle, saturated ammonium hydroxide solution (2.2 mL) was added, and the bottle was heated at 55 °C with stirring for 24 h. The mixture was cooled to rt, the solvent was evaporated in vacuum and the residue (2.40 g) was chromatographed over a column of silica gel with gradient of methanol in ethyl acetate (0 to 6%). The fraction containing product were pulled, evaporated in vacuum, and the residue was treated with 5 mL of ACN that triggered extensive crystallization. The mixture was kept at 0 °C for 4 h, filtered, the crystalline residue was washed with ACN, and air-dried to afford 0.73 g (60%) of C-22 cytidine **112** as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.77 (d, *J* = 7.2 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.28 - 7.22 (m, 5H), 7.22 – 7.12 (m, 2H), 6.89 (d, *J* = 8.8 Hz, 4H), 5.80 (d, *J* = 2.8 Hz, 1H), 5.48 (d, *J* = 7.6 Hz, 1H), 4.98 (d, *J* = 6.8 Hz, 1H), 4.19 – 4.11 (m, 1H), 3.99 – 3.90 (m, 1H), 3.77 – 3.69 (m, 1H), 3.73 (s, 6H), 3.68 – 3.52 (m, 2H), 3.30 – 3.22 (m, 2H), 1.55 – 1.45 (m, 2H), 1.34

– 1.14 (m, 38H), 0.83 (t, *J* = 6.8 Hz, 3H). MS (ESI+APCI), calculated for $C_{52}H_{75}N_3O_7$ $[M+H]^+$ exact mass m/z = 854.57.

Compound 113: C-22-Cytidine **112** (0.68 g, 0.8 mmol) was dissolved in anhyd. DMF (4 mL) under Ar atmosphere, and acetic anhydride (0.09 mL, 0.9 mmol) was added. The mixture was stirred at rt for 48 h, cooled to 0 °C, quenched by addition of 5% NaCl (10 mL), and diluted with ethyl acetate (10 mL). The organic phase was separated, washed with 5% NaCl (2x20 mL), sat. sodium bicarbonate, sat. NaCl, and dried over anhyd. sodium sulfate. The solvent was removed in vacuum and the residue was co-evaporated twice with ACN-diethyl ether mixture to afford 0.72 g (100%) of C-22 N(Ac)-cytidine **113** as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 10.90 (s, 1H), 8.28 (d, *J* = 7.6 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 7.28 - 7.21 (m, 5H), 7.00 (d, *J* = 7.2 Hz, 1H), 6.92 - 6.86 (m, 4H), 5.79 (d, *J* = 1.2 Hz, 1H), 5.05 (d, *J* = 7.2 Hz, 1H), 4.25 – 4.18 (m, 1H), 4.05 – 3.99 (m, 1H), 3.78 (dd, *J* = 4.8, 1.2 Hz, 1H), 3.77 – 3.69 (m, 1H), 3.74 (s, 6H), 3.65 – 3.57 (m, 1H), 3.37 – 3.27 (m, 2H), 2.08 (s, 3H), 1.57 – 1.48 (m, 2H), 1.35 – 1.14 (m, 38H), 0.83 (t, *J* = 6.8 Hz, 3H). MS (ESI+APCI), calculated for $C_{54}H_{77}N_3O_8$ $[M+H]^+$ exact mass m/z = 896.58.

Compound 114: DIPEA (0.16 mL, 0.9 mmol) followed by 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (0.20 mL, 0.9 mmol) were added to a solution of compound 113 (0.63 g, 0.7 mmol) in anhyd. DCM (5 mL) under Ar. The mixture was stirred at rt for 14 h, cooled to 0 °C, quenched by addition of sat. $NaHCO_3$ and extracted with AcOEt (15 mL). Organic phase was separated, washed with sat. NaCl, and dried over anhyd. sodium sulfate. Crude material (0.82 g) was purified over a standard 24 g flash column of silica gel that was eluted with isocratic 70% of AcOEt containing 0.3% of TEA in hexanes followed by gradient 70 to 100% of AcOEt containing 0.3% of TEA in hexanes to afford 0.69 g (90%) of **114** as a white foam. ^{1}H NMR (600 MHz, DMSO) δ 10.94 (s, 1H), 8.46 – 8.34 (m, 1H), 7.46 – 7.36 (m, 2H), 7.36 – 7.20 (m, 7H), 6.98 (t, J = 8.4 Hz, 1H), 6.89 (t, J = 8.6 Hz, 4H), 5.85 (d, J = 13.8 Hz, 1H), 4.55 – 4.34 (m, 1H), 4.20 – 4.11 (m, 1H), 4.06 – 3.83 (m, 2H), 3.75 (s, 3H), 3.74 (s, 3H), 3.68 – 3.64 (m, 1H), 3.50 – 3.44 (m, 2H), 2.77 – 2.69 (m, 1H), 2.66 – 2.59 (m, 1H), 2.10 (s, 3H), 1.56 – 1.49 (m, 2H), 1.26 – 1.16 (m, 51H), 0.96 (d, J = 6.7 Hz, 3H), 0.85 – 0.82 (m, 3H). P31 NMR (243 MHz, DMSO) δ 149.23, 147.94.

Example 2. dsRNA Synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA Design

siRNAs targeting the human adrenoceptor beta 1 (ADRB1) gene (human: GenBank NM_000684.3, NCBI GeneID: 153) were designed using custom R and Python scripts. The human ADRB1 REFSEQ NM_000684.3 mRNA, has a length of 3039 bases.

siRNAs comprising a GalNAc conjugate targeting ligand were designed and synthesized as described below. siRNAs comprising an unsaturated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, were designed and synthesized as described above.

Detailed lists of the modified ADRB1 sense and antisense strand nucleotide sequences comprising an unsarurated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, are shown in Table 3, and the corresponding unmodified ADRB1 sense and antisense nucleotide sequences are shown in Table 2.

Detailed lists of the modified ADRB1 sense and antisense strand nucleotide sequences comprising a GalNAc conjugate targeting ligand are shown in Table 5, and the corresponding unmodified ADRB1 sense and antisense nucleotide sequences are shown in Table 4.
It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

siRNA Synthesis

siRNAs comprising a GalNAc conjugate targeting ligand were designed, synthesized, and prepared using methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 µmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 µL of Methylamine (40% aqueous) at room temperature in 96 well plates for

approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for *in vitro* screening assays.

Example 3. *In vitro* Screening Methods

Cell culture and 384-well transfections

Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO_2 in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of complete growth media without antibiotic containing ~1.5 x10^4 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM, 1 nM, and 0.1 nM final duplex concentration.

In vitro Dual-Luciferase and Endogenous Screening Assays

Hepa1-6 cells cells were transfected by adding 50 µL of siRNA duplexes and 75 ng of a plasmid, comprising human ADRB1 target sequence, per well along with 100 µL of Opti-MEM plus 0.5 µL of Lipofectamine 2000 per well (Invitrogen, Carlsbad CA. cat # 13778-150) and then incubated at room temperature for 15 minutes. The mixture was then added to the cells which are re-

suspended in 35 µL of fresh complete media. The transfected cells were incubated at 37°C in an atmosphere of 5% CO2. Single-dose experiments were performed at 10 nM or 50 nM.

Twenty-four hours after the siRNAs and psiCHECK2 plasmid are transfected; Firefly (transfection control) and Renilla (fused to ADRB1 target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 75 µL of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 75 µL of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenches the firefly luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (MUC5B) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done with n=4.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

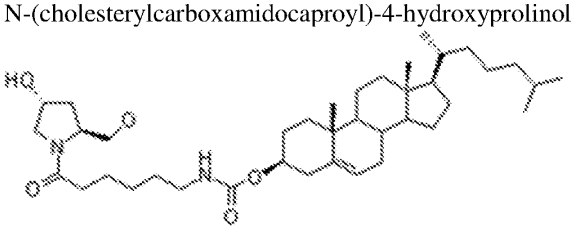
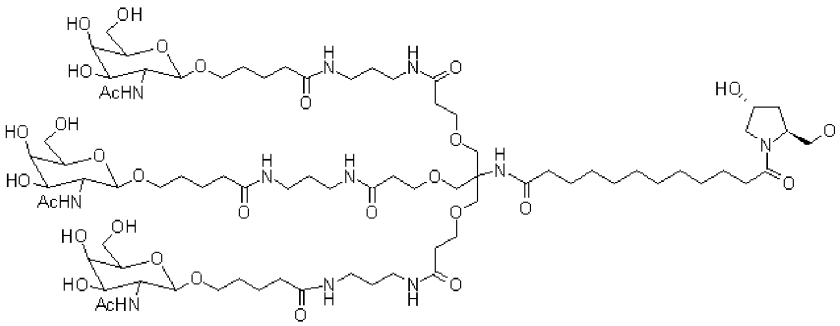
Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human AGT, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche). To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were

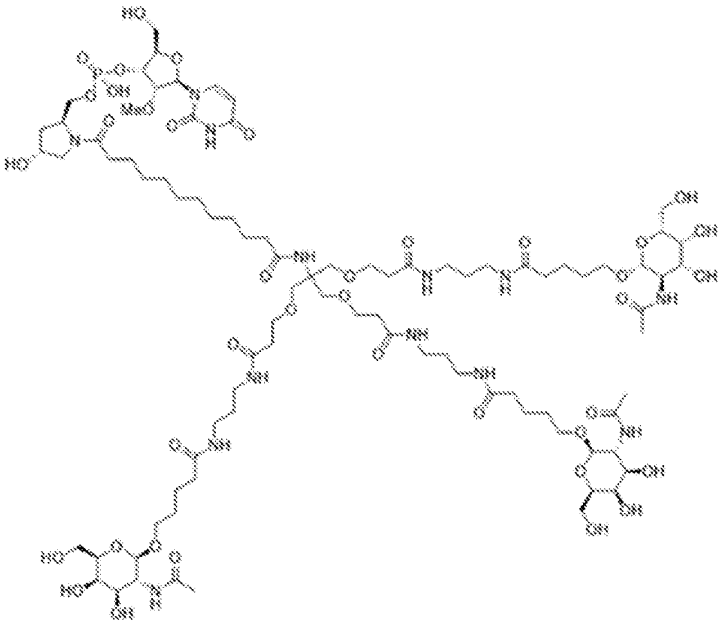
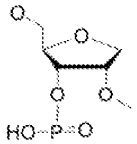
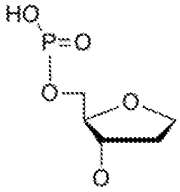
calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: 5'-cuuAcGcuGAGuAcuucGAdTsdT-3' and antisense 5'-UCGAAGuACUcAGCGuAAGdTsdT-3'.

The results of the dual-luciferase assays of the agents listed in Tables 4 and 5 are provided in Table 6.

Table 1. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds; and it is understood that when the nucleotide contains a 2'-fluoro modification, then the fluoro replaces the hydroxy at that position in the parent nucleotide (*i.e.*, it is a 2'-deoxy-2'-fluoronucleotide). It is to be further understood that the nucleotide abbreviations in the table omit the 3'-phosphate (*i.e.*, they are 3'-OH) when placed at the 3'-terminal position of an oligonucleotide.

Abbreviation	**Nucleotide(s)**
A	Adenosine-3'-phosphate
Ab	beta-L-adenosine-3`-phosphate
Abs	beta-L-adenosine-3`-phosphorothioate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cb	beta-L-cytidine-3`-phosphate
Cbs	beta-L-cytidine-3'-phosphorothioate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gb	beta-L-guanosine-3`-phosphate
Gbs	beta-L-guanosine-3`-phosphorothioate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine -3'-phosphorothioate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'- phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'- phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'- phosphorothioate

Abbreviation	Nucleotide(s)
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol) 
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol (Hyp-(GalNAc-alkyl)3) (2S,4R)-1-[29-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-14,14-bis[[3-[[3-[[5-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-1-oxopentyl]amino]propyl]amino]-3-oxopropoxy]methyl]-1,12,19,25-tetraoxo-16-oxa-13,20,24-triazanonacos-1-yl]-4-hydroxy-2-hydroxymethylpyrrolidine 

Abbreviation	Nucleotide(s)
uL96	2'-O-methyluridine-3'-phosphate ((2S,4R)-1-[29-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-14,14-bis[[3-[[3-[[5-[[2-(acetylamino)-2-deoxy-β-D-galactopyranosyl]oxy]-1-oxopentyl]amino]propyl]amino]-3-oxopropoxy]methyl]-1,12,19,25-tetraoxo-16-oxa-13,20,24-triazanonacos-1-yl]-4-hydroxy-2-pyrrolidinyl)methyl ester 
Y34	2-hydroxymethyl-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose) 
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate) 
(Agn)	Adenosine-glycol nucleic acid (GNA) S-Isomer
(Cgn)	Cytidine-glycol nucleic acid (GNA) S-Isomer
(Ggn)	Guanosine-glycol nucleic acid (GNA) S-Isomer
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphonate
dA	2`-deoxyadenosine-3`-phosphate
dAs	2`-deoxyadenosine-3`-phosphorothioate
dC	2`-deoxycytidine-3`-phosphate
dCs	2`-deoxycytidine-3`-phosphorothioate

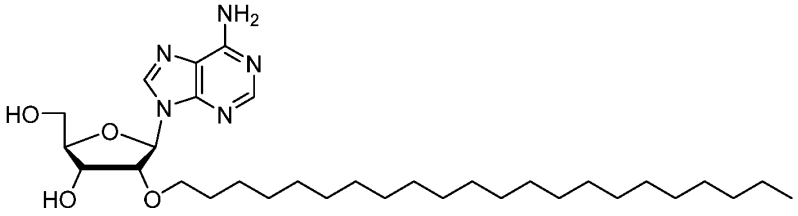
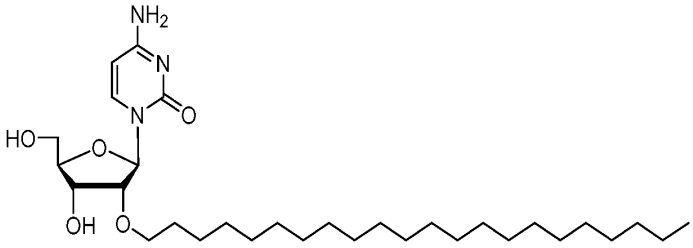
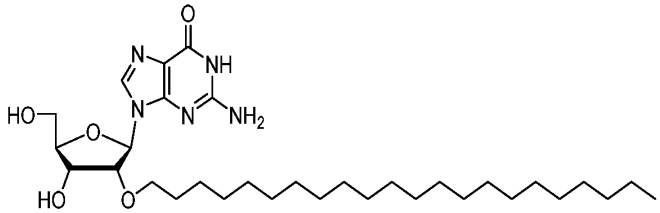
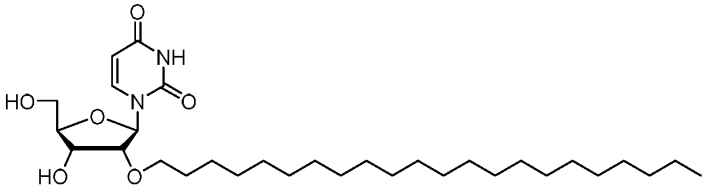
Abbreviation	Nucleotide(s)
dG	2`-deoxyguanosine-3`-phosphate
dGs	2`-deoxyguanosine-3`-phosphorothioate
dT	2`-deoxythimidine -3`-phosphate
dTs	2`-deoxythimidine-3`-phosphorothioate
dU	2`-deoxyuridine
dUs	2`-deoxyuridine-3`-phosphorothioate
(C2p)	cytidine-2`-phosphate
(G2p)	guanosine-2`-phosphate
(U2p)	uridine-2`-phosphate
(A2p)	adenosine-2`-phosphate
(Chd)	2'-O-hexadecyl-cytidine-3'-phosphate
(Ahd)	2'-O-hexadecyl-adenosine-3'-phosphate
(Ghd)	2'-O-hexadecyl-guanosine-3'-phosphate
(Uhd)	2'-O-hexadecyl-uridine-3'-phosphate
Ada	 (2*R*,3*S*,4*S*,5*R*)-5-(6-amino-9*H*-purin-9-yl)-4-(docosyloxy)-2-(hydroxymethyl)tetrahydrofuran-3-ol
Cda	 4-amino-1-((2*R*,3*R*,4*R*,5*R*)-3-(docosyloxy)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidin-2(1*H*)-one
Gda	 2-amino-9-((2*R*,3*S*,4*S*,5*R*)-3-(docosyloxy)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1,9-dihydro-6*H*-purin-6-one
Uda	 1-((2*R*,3*R*,4*R*,5*R*)-3-(docosyloxy)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)pyrimidine-2,4(1*H*,3*H*)-dione

Table 2. Unmodified Sense and Antisense Strand Sequences of ADRB1 dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	Seq ID NO:	Range in NM_000684.3
AD-1646600	UAAAUUCUACUUCCUCUUGUA		UACAAGAGGAAGUAGAAUUUACA		2932-2954
AD-1646601	AUUCAAACUCUACUUCUGUUA		UAACAGAAGUAGAGUUUGAAUUU		2740-2762
AD-1646602	UCCUUUAUCAUGGUACUGUAA		UUACAGUACCAUGAUAAAGGAGU		2811-2833
AD-1646603	AAGAAAUGUUAAGCUCUUCUA		UAGAAGAGCUUAACAUUUCUUGA		2301-2323
AD-1646604	CUUUUUAUACUCCUUUAUCAA		UUGAUAAAGGAGUAUAAAAAGGU		2801-2823
AD-1646605	AUUGGGAAAAUACCUUUUUAA		UUAAAAAGGUAUUUUCCCAAUGA		2788-2810
AD-1646606	AAGACAUAUUUCCUUUUGCUA		UAGCAAAAGGAAAUAUGUCUUGA		2184-2206
AD-1646607	CUACUUCUGUUGUCUAGUAUA		UAUACUAGACAACAGAAGUAGAG		2749-2771
AD-1646608	AGUGUUAGGAAUUACAAAAUA		UAUUUUGUAAUUCCUAACACUGU		2643-2665
AD-1646609	AUCACGUUUCAAGAAAUGUUA		UAACAUUUCUUGAAACGUGAUUU		2291-2313
AD-1646610	GUGGUUCAAAAUGCCAUUUUA		UAAAAUGGCAUUUUGAACCACUC		2618-2640
AD-1646611	CUUGUUUAUAUUAAACAGCUA		UAGCUGUUUAAUAUAAACAAGUC		2441-2463
AD-1646612	GUGUUUACUUAAGACCGAUAA		UUAUCGGUCUUAAGUAAACACAG		1756-1778
AD-1646613	UUCCAGAGAAAUUUCAUUUUA		UAAAAUGAAAUUUCUCUGGAAAG		2204-2226
AD-1646614	UGUAACUGUAUCCAUAUUAUA		UAUAAUAUGGAUACAGUUACAGU		2827-2849
AD-1646615	UGCUGUUAUGAAAGCAAAGAA		UUCUUUGCUUUCAUAACAGCAGA		2244-2266
AD-1646616	UAGUAUGUUAUUGAGCUAAUA		UAUUAGCUCAAUAACAUACUAGA		2763-2785
AD-1646617	UUUACCAAGUAUUUAUACUCA		UGAGUAUAAAUACUUGGUAAAAC		2956-2978
AD-1646618	CCUCUUCGUCUUCUUCAACUA		UAGUUGAAGAAGACGAAGAGGCG		1336-1358
AD-1646619	GUGCAAUAAAAUACCAAUGAAA		UUUCAUUGGUAUUUAUUGCACAU		3012-3034
AD-1646620	CUUGUGUGUUUUACCAAGUAA		UUACUUGGUAAAACACACAAGAG		2947-2969
AD-1646621	CACCAACCUCUUCAUCAUGUA		UACAUGAUGAAGAGGUUGGUGAG		541-563
AD-1646622	AGUAAUGAUUUCUGCUGUUAA		UUAACAGCAGAAAUCAUUACUUA		2232-2254
AD-1646623	GGUGGUUUUUGACACUCUCUA		UAGAGAGUGUCAAAAACCACCUG		2022-2044
AD-1646624	UGUGACAUGUGACUCUGUCAA		UUGACAGAGUCACAUGUCACAGA		2510-2532
AD-1646625	UGCCAUUUUUGCACAGUGUUA		UAACACUGUGCAAAAAUGGCAUU		2629-2651
AD-1646626	UAACUACUGUGUGAGGAAUUA		UAAUUCCUCACACAGUAGUUAGU		2985-3007
AD-1646627	ACUUGUGUGUAAAUUCUACUA		UAGUAGAAUUUACACACAAGUGC		2923-2945
AD-1646628	GAUAGAAAGACUUGUUUAUAA		UUAUAAACAAGUCUUUCUAUCUG		2431-2453

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	Seq ID NO:	Range in NM_000684.3
AD-1646629	AAGCUCUUCUUGGAACAAGCA		UGCUUGUUCCAAGAAGAGCUUAA		2311-2333
AD-1646630	CCACAGAAGAUGUUACUUGCA		UGCAAGUAACAUCUUCUGUGGAU		2663-2685
AD-1646631	UCUGUCAAUUGAAGACAGGAA		UUCCUGUCUUCAAUUGACAGAGU		2523-2545
AD-1646632	GAAACAGUUCAGAUUACUGCA		UGCAGUAAUCUGAACUGUUUCUC		2565-2587
AD-1646633	AUUGAGCUAAUGAUUCAUUGA		UCAAUGAAUCAUUAGCUCAAUAA		2772-2794
AD-1646634	CCUUUUGCUUUCCAGAGAAAA		UUUUCUCUGGAAAGCAAAAGGAA		2195-2217
AD-1646635	GUGAUGCAUCUUUAGAUUUUA		UAAAAUCUAAAGAUGCAUCACAC		1990-2012
AD-1646636	AAUCCUCGUCUGAAUCAUCCA		UGGAUGAUUCAGACGAGGAUUGU		1797-1819
AD-1646637	AAAGUUAGCACUUGUGUGUAA		UUACACACAAGUGCUAACUUUCA		2914-2936
AD-1646638	AAUUACAAAAUCCACAGAAGA		UCUUCUGUGGAUUUUGUAAUUCC		2652-2674
AD-1646639	AUGACAGUUUGUCAAGACAUA		UAUGUCUUGACAAACUGUCAUUC		2171-2193
AD-1646640	GGGCAGAUCUUAAAUAAAAUA		UAUUUUAUUUAAGAUCUGCCCAG		2722-2744
AD-1646641	GAUUAAAAUCGAUCAUCGUGA		UCACGAUGAUCGAUUUUAAUCCC		2098-2120
AD-1646642	UUGCUGAUGUUCCUUGUUGUA		UACAACAAGGAACAUCAGCAAGC		1884-1906
AD-1646643	CUACCUCACACUGUGCAUUUA		UAAAUGCACAGUGUGAGGUAGAA		2403-2425
AD-1646644	GUAUCAAUAUUAGUUGGAAGA		UCUUCCAACUAAUAUUGAUACAU		2468-2490
AD-1646645	AGAUUACUGCACAUGUGGAUA		UAUCCACAUGUGCAGUAAUCUGA		2575-2597
AD-1646646	GAGGGAUUUCUACCUCACACA		UGUGUGAGGUAGAAAUCCCUCAG		2394-2416
AD-1646647	UGAAGACAGGACAUUAAAAGA		UCUUUUAAUGUCCUGUCUUCAAU		2532-2554
AD-1646648	GAGGAGAUCUGUGUUUACUUA		UAAGUAAACACAGAUCUCCUCGU		1746-1768
AD-1646649	ACUCUGGUGCAACUAACUACA		UGUAGUUAGUUGCACCAGAGUAU		2972-2994
AD-1646650	CACCUUGCUUUCCUUGUGUAA		UUACACAAGGAAAGCAAGGUGGG		2333-2355
AD-1646651	UCCGUAGUCUCCUUCUACGUA		UACGUAGAAGGAGACUACGGACG		950-972
AD-1646652	UUGCUGGUGAAAGUUAGCACA		UGUGCUAACUUUCACCAGCAAAU		2905-2927
AD-1646653	CUCCUUCUUCUGCGAGCUGUA		UACAGCUCGCAGAAGAAGGAGCC		646-668
AD-1646654	GGAAUUGGUCCAUGUGCAAUA		UAUUGCACAUGGACCAAUUCCUC		2999-3021
AD-1646655	UGAAUCAUCCGAGGCAAAGAA		UUCUUUGCCUCGGAUGAUUCAGA		1807-1829
AD-1646656	AAAGCAAAGAGAAAGGAUGGA		UCCAUCCUUUCUCUUUGCUUUCA		2254-2276
AD-1646657	UCUUUUGUGUGUGCGUGUGAA		UUCACACGCACACACAAAAGAAG		1974-1996
AD-1646658	GCAUUUGCACAGCAGAUAGAA		UUCUAUCUGCUGUGCAAAUGCAC		2417-2439
AD-1646659	AGGAAAGUUUGGGAAGGGAUA		UAUCCCUUCCCAAACUUUCCUUU		1853-1875

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	Seq ID NO:	Range in NM_000684.3
AD-1646660	AGCCUCUCUCUGUGACAUGUA		UACAUGUCACAGAGAGGCUCU		2500-2522
AD-1646661	GGGCGUCUUCACGCUCUGCUA		UAGCAGAGCGUGAAGACGCCCAU		1255-1277
AD-1646662	AGAAAGGAUGGAGGCAAAAUA		UAUUUUGCCUCCAUCCUUUCUCU		2263-2285
AD-1646663	GACAUUAAAAGAGAGCGAGAA		UUCUCGCUCUCUUUUAAUGUCCU		2541-2563
AD-1646664	CGGGAACAGGAACACACUACA		UGUAGUGUGUUCCUGUUCCCGGG		2134-2156
AD-1646665	AGAGAGGAGAAUGACAGUUUA		UAAACUGUCAUUCUCCUCUCUCU		2161-2183
AD-1646666	CUGCGACUUCGUCACCAACCA		UGGUUGGUGACGAAGUCGCAGCA		910-932
AD-1646667	GCCCUUCUUCCUGGCCAACGA		UCGUUGGCCAGGAAGAAGGGCAG		1279-1301
AD-1646668	UAAGACCGAUAGCAGGUGAAA		UUUCACCUGCUAUCGGUCUUAAG		1765-1787
AD-1646669	GGUGAAGAAGAUCGACAGCUA		UAGCUGUCGAUCUUCUUCACCUG		1027-1049
AD-1646670	CACGUGAAUUUGCUGGUGAAA		UUUCACCAGCAAAUUCACGUGGG		2896-2918
AD-1646671	AGCGAGAGAGAAACAGUUA		UAACUGUUUCUCUCUCUCGCUCU		2554-2576
AD-1646672	GAGGCAAAGAGAAAAGCCACA		UGUGGCUUUUCUCUUUGCCUCGG		1817-1839
AD-1646673	GCCCAUCCUCAUGCACUGGUA		UACCAGUGCAUGAGGAUGGGCAG		841-863
AD-1646674	ACCCUGUGUGUCAUUGCCCUA		UAGGGCAAUGACACACAGGGUCU		707-729
AD-1646675	UAGCAGGUGAACUCGAAGCCA		UGGCUUCGAGUUCACCUGCUAUC		1774-1796
AD-1646676	GCGCUCAUCGUGCUGCUCAUA		UAUGAGCAGCACGAUGAGCGCCA		461-483
AD-1646677	CUGUGCAUCAUGGCCUUCGUA		UACGAAGGCCAUGAUGCACAGGG		974-996
AD-1646678	CCCAUCCCUUUCCCGGGAACA		UGUUCCCGGGAAAGGGAUGGGAG		2121-2143
AD-1646679	CCUCGGAAUCCAAGGUGUAGA		UCUACACCUUGGAUUCCGAGGCG		1680-1702
AD-1646680	GAAGCCCACAAUCCUCGUCUA		UAGACGAGGAUUGUGGGCUUCGA		1788-1810
AD-1646681	AUGGCCUUCGUGUACCUGCGA		UCGCAGGUACACGAAGGCCAUGA		983-1005
AD-1646682	UUAUGUCCAAGUGCCCACGUA		UACGUGGGCACUUGGACAUAAAA		2881-2903
AD-1646683	GACACUCUCUGAGAGGACCGA		UCGGUCCUCUCAGAGAGUGUCAA		2032-2054
AD-1646684	GACGACGACGAUGUCGUCGGA		UCCGACGACACUCGUCGUCGUCGU		1556-1578
AD-1646685	AUCGUGGCUCCCAUCCCUUUA		UAAAGGGAUGGGAGCCACGAUGA		2112-2134
AD-1646686	CUGGUGUCCUUCCUGCCCAUA		UAUGGGCAGGAAGGACACCAGGG		827-849
AD-1646687	AAGGGAGAAGCAUUAGGAGGA		UCCUCCCUAAUGCUUCUCCCUUCC		2077-2099
AD-1646688	CGCUGUCUCAGCAGUGGACAA		UUGUCCACUGCUGAGACAGCGGC		420-442
AD-1646689	UACGCCAACUCGGCCUUCAAA		UUUGAAGGCCGAGUUGGCGUAGC		1364-1386
AD-1646690	CUCGCCAUCACCUCGCCCUUA		UAAGGGCGAGGUGAUGGCGAGGU		737-759

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	Seq ID NO:	Range in NM_000684.3
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AD-1646692	AUCGCCUCGUCCGUAGUCUCA		UGAGACUACGGACGAGGCGAUGG		941-963
AD-1646693	CGGGCAAUGUGCUGGUGAUCA		UGAUCACCAGCACAUUGCCCGCC		486-508
AD-1646694	CUUCUUCAACUGGCUGGGCUA		UAGCCCAGCCAGUUGAAGAAGAC		1345-1367
AD-1646695	CUUGUGUAGGGCAAACCCGCA		UGCGGGUUUGCCCUACACAAGGA		2345-2367
AD-1646696	GGCCAACGUGGUGAAGGCCUA		UAGGCCUUCACCACGUUGGCCAG		1291-1313
AD-1646697	GGAGUACGGCUCCUUCUUCUA		UAGAAGAAGGAGCCGUACUCCCA		637-659
AD-1646698	GCCCAGAAGCAGGUGAAGAAA		UUUCUUCACCUGCUUCUGGGCCU		1016-1038
AD-1646699	CAGAAGGCGCUCAAGACGCUA		UAGCGUCUUGAGCGCCUUCUGCU		1223-1245
AD-1646700	UUCCGCUACCAGAGCCUGCUA		UAGCAGGCUCUGGUAGCGGAAGG		755-777
AD-1646701	CUUCCGCAAGGCCUUCCAGGA		UCCUGGAAGGCCUUGCGGAAGUC		1411-1433
AD-1646702	GAGUGGAAGAUGGGUGGGUUA		UAACCCACCCAUCUUCCACUCCG		2052-2074
AD-1646703	GGCCAGCAUCGAGACCCUGUA		UACAGGGUCUCGAUGCUGGCCGU		694-716
AD-1646704	CUGCUGGCUGCCCUUCUUCCA		UGGAAGAAGGGCAGCCAGCAGAG		1270-1292
AD-1646705	GACCGCUACCUCGCCAUCACA		UGUGAUGGCGAGGUAGCGGUCCA		728-750
AD-1646706	CGUGGCCAUCGCCAAGACGCA		UGCGUCUUGGCGAUGGCCACGAU		505-527
AD-1646707	AUGGGUCUGCUGAUGGCGCUA		UAGCGCCAUCAGCAGACCCAUGC		446-468
AD-1646708	GGGCAUCAUCAUGGGCGUCUA		UAGACGCCCAUGAUGAUGCCCAG		1243-1265
AD-1646709	GAACACACUACCAGCCAGAGA		UCUCUGGCUGGUAGUGUGUUCCU		2143-2165
AD-1646710	AUUGCCCUGGACCGCUACCUA		UAGGUAGCGGUCCAGGGCAAUGA		719-741
AD-1646711	AUCUCGGCCCUGGUGUCCUUA		UAAGGACACCAGGGCCGAGAUGG		818-840
AD-1646712	AGCUGCGAGCGCCGUUUCCUA		UAGGAAACGCGGCGCUCGCAGCUGU		1043-1065
AD-1646713	CAUCAUCUACUGCCGCAGCCA		UGGCUGCGGCAGUAGAUGAUGGG		1387-1409
AD-1646714	GCAGACGCUCACCAACCUCUA		UAGAGGUUGGUGAGCGUCUGCAG		532-554
AD-1646715	CUUCCAGGGACUGCUCUGCUA		UAGCAGAGCAGUCCCUGGAAGGC		1423-1445
AD-1646716	CUUCAUCAUGUCCCUGGCCAA		UUGGCCAGGGACAUGAUGAAGAG		550-572
AD-1646717	AAACAUGCUGAAGUCCCGGCA		UGCCGGGACUUCAGCAUGUUUCU		3-23
AD-1646718	AAGGCCUUCCACCGCGAGCUA		UAGCUCGCGGUGGAAGGCCUUCA		1304-1326
AD-1646719	CAGGCGCAGAGCCUCUCUCUA		UAGAGAGAGGCUCUGCGCCUGGU		2491-2513
AD-1646720	CGAGCCCGGUAACCUGUCGUA		UACGACAGGUUACCGGGCUCGGA		298-320
AD-1646721	CUGUGGACCUCAGUGGACGUA		UACGUCCACUGAGGUCCACAGCU		662-684

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	Seq ID NO:	Range in NM_000684.3
AD-1646722	GCCACGGACCGUUGCACAAAA		UUUUGUGCAACGGUCCGUGGCUU		1832-1854
AD-1646723	ACCUCGCCCUUCCGCUACCAA		UUGGUAGCGGAAGGGCGAGGUGA		746-768
AD-1646724	UCAAGACGCUGGGCAUCAUCA		UGAUGAUGCCCAGCGUCUUGAGC		1233-1255
AD-1646725	GGAGAGUGGCUUGCUGAUGUA		UACAUCAGCAAGCCACUCUCCCA		1874-1896
AD-1646726	CUGCUCAUCGUGGCGGGCAAA		UUUGCCCGCCACGAUGAGCAGCA		473-495
AD-1646727	ACCAGCCAGAGAGAGGAGAAA		UUUCUCCUCUCUCUGGCUGGUAG		2152-2174
AD-1646728	GCUGGUGAUCGUGGCCAUCGA		UCGAUGGCCACGAUCACCAGCAC		496-518
AD-1646729	AAGUCCCGGCGGCUCUUCCAA		UUGGAAGAGCCGCCGGGACUUCA		13-33
AD-1646730	GGUCAGGCUGAGGGAUUUCUA		UAGAAAUCCCUCAGCCUGACCAC		2385-2407
AD-1646731	UCAGUGGACGUGCUGUGCGUA		UACGCACAGCACGUCCACUGAGG		671-693
AD-1646732	UACCUGCGGGUGUUCCGCGAA		UUCGCGGAACACCCGCAGGUACA		995-1017
AD-1646733	UUUCUGUUUGUGGUCCGGCCA		UGGCCGGACCACAAACAGAAAAA		1953-1975
AD-1646734	ACCAACCGGGCCUACGCCAUA		UAUGGCGUAGGCCCGGUUGGUGA		923-945

Table 3. Modified Sense and Antisense Strand Sequences of ADRB1 dsRNA Agents Comprising anUnsaturated C_{22} Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646600	usasaau(Uda)CfuAfCfUfuccucuugsusa		VPusAfscaaGfaGfGfaaguAfgAfauuuascsa		UGUAAAUUCUACUUCCUCUUGUG	
AD-1646601	asususca(Ada)AfcUfCfUfacuucugususa		VPusAfsacaGfaAfGfuagaGfuUfugaaususu		AAAUUCAAACUCUACUUCUGUUG	
AD-1646602	uscscuu(Uda)AfuCfAfUfgguacugusasa		VPusUfsacaGfuAfCfcaugAfuAfaaggasgsu		ACUCCUUUAUCAUGGUACUGUAA	
AD-1646603	asasgaa(Ada)UfgUfUfAfagcucuucsusa		VPusAfsgaaGfaGfCfuuaaCfaUfuucuusgsa		UCAAGAAAUGUUAAGCUCUUCUU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646604	csusuuu(Uda)AfuAfCfUfccuuuaucsasa		VPusUfsgauAfaAfGfgaguAfuAfaaaagsgsu		ACCUUUUUAUACUCCUUUAUCAU	
AD-1646605	asusugg(Gda)AfaAfAfUfaccuuuuusasa		VPusUfsaaaAfaGfGfuauuUfuCfccaausgsa		UCAUUGGGAAAAUACCUUUUUAU	
AD-1646606	asasgac(Ada)UfaUfUfUfccuuuugcsusa		VPusAfsgcaAfaAfGfgaaaUfaUfgucuusgsa		UCAAGACAUAUUUCCUUUUGCUU	
AD-1646607	csusacu(Uda)CfuGfUfUfUfgucuaguassasg		VPusAfsuacUfaGfAfcaacAfgAfaguagsasg		CUCUACUUCUGUUGUCUAGUAUG	
AD-1646608	asgsugu(Uda)AfgGfAfAfUfuacaaaasusa		VPusAfsuuuUfgUfAfauucCfuAfacacusgsu		ACAGUGUUAGGAAUUACAAAAUC	
AD-1646609	asuscac(Gda)UfuUfCfAfagaaaugussusu		VPusAfsacaUfuUfCfuugaAfaCfgugauusu		AAAUCACGUUUCAAGAAAUGUUA	
AD-1646610	gsusggu(Uda)CfaAfAfAfUfugccauuussusc		VPusAfsaaaUfgGfCfauuuUfgAfaccacsusc		GAGUGGUUCAAAAUGCCAUUUUU	
AD-1646611	csusugu(Uda)UfaUfAfUfUfuaaacagcsusa		VPusAfsgcuGfuUfUfUfaauaUfaAfacaagsusc		GACUUGUUUAUAUUAAACAGCUU	
AD-1646612	gsusguu(Uda)AfcUfUfUfAfagaccgausasa		VPusUfsaucGfgUfCfuuaaGfuAfaacacsasg		CUGUGUUUACUUAAGACCGAUAG	
AD-1646613	ususcca(Gda)AfgAfAfAfAfuuucauuussusa		VPusAfsaaaUfgAfAfauuuCfuCfuggaasasg		CUUUCCAGAGAAAUUUCAUUUUA	
AD-1646614	usgsuaa(Cda)UfgUfAfUfUfccauauuasusa		VPusAfsuaaUfaUfUfGfgauaCfaGfuuacasgsu		ACUGUAACUGUAUCCAUAUUAUA	
AD-1646615	usgscug(Uda)UfaUfGfGfAfaagcaaagsasa		VPusUfscuuUfgCfUfuucaUfaAfcagcasgsa		UCUGCUGUUAUGAAAGCAAAGAG	
AD-1646616	usasgua(Uda)GfuUfAfUfUfugagcuaassusa		VPusAfsuuaGfcUfCfaauaAfcAfuacuasgsa		UCUAGUAUGUUAUUGAGCUAAUG	
AD-1646617	ususuac(Cda)AfaGfUfUfAfuuuauacussasc		VPusGfsaguAfuAfAfauacUfuGfguaaasasc		GUUUUACCAAGUAUUUAUACUCU	
AD-1646618	cscsucu(Uda)CfgUfCfUfucuucaacsusa		VPusAfsguuGfaAfGfaagaCfgAfagaggscsg		CGCCUCUUCGUCUUCUUCAACUG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646619	gsusgca(Ada)UfaAfAfUfaccaaugas asa		VPusUfsucaUfuGfGfuauuUfaUfugcac sasu		AUGUGCAAUAAAUACCAAUG AAG	
AD-1646620	csusugu(Gda)UfgUfUfUfUfuaccaagus asa		VPusUfsacuUfgGfUfaaaaCfaCfacaags asg		CUCUUGUGUGUUUUACCAAG UAU	
AD-1646621	csascca(Ada)CfcUfCfUfucaucaugs usa		VPusAfscauGfaUfGfaagaGfgUfuggug sasg		CUCACCAACCUCUUCAUCAU GUC	
AD-1646622	asgsuaa(Uda)GfaUfUfUfUfcugcuguus asa		VPusUfsaacAfgCfAfgaaaUfcAfuuacus usa		UAAGUAAUGAUUUCUGCUGU UAU	
AD-1646623	gsgsugg(Uda)UfuUfUfUfGfacacucucs usa		VPusAfsgagAfgUfGfucaaAfaAfccaccs usg		CAGGUGGUUUUUGACACUCU CUG	
AD-1646624	usgsuga(Cda)AfuGfUfUfGfacucugucs asa		VPusUfsgacAfgAfGfucacAfuGfucaca sgsa		UCUGUGACAUGUGACUCUGU CAA	
AD-1646625	usgscca(Uda)UfuUfUfUfGfcacagugus usa		VPusAfsacaCfuGfUfgcaaAfaAfuggcas usu		AAUGCCAUUUUUGCACAGUG UUA	
AD-1646626	usasacu(Ada)CfuGfUfUfGfugaggaaus usa		VPusAfsauuCfcUfUfCfacacAfgUfaguuas gsu		ACUAACUACUGUGUGAGGAA UUG	
AD-1646627	ascsuug(Uda)GfuGfUfUfAfaauucuacs usa		VPusAfsguaGfaAfUfuuacAfcAfcaagu sgsc		GCACUUGUGUGUAAAUUCUA CUU	
AD-1646628	gsasuag(Ada)AfaGfAfCfuuguuuaus asa		VPusUfsauaAfaCfAfagucUfuUfcuauc susg		CAGAUAGAAAGACUUGUUUA UAU	
AD-1646629	asasgcu(Cda)UfuCfUfUfUfggaacaags csa		VPusGfscuuGfuUfUfCfcaagAfaGfagcuu sasa		UUAAGCUCUUCUUUGGAACAA GCC	
AD-1646630	cscsaca(Gda)AfaGfAfUfguuacuugs csa		VPusGfscaaGfuAfAfcaucUfuCfugugg sasu		AUCCACAGAAGAUGUUACUU GCA	
AD-1646631	uscsugu(Cda)AfaUfUfUfGfaagacaggs asa		VPusUfsccuGfuCfUfucaaUfuGfacagas gsu		ACUCUGUCAAUUGAAGACAG GAC	
AD-1646632	gsasaac(Ada)GfuUfCfAfgauuacugs csa		VPusGfscagUfaAfUfcugaAfcUfguuuc susc		GAGAAACAGUUCAGAUUACU GCA	
AD-1646633	asusuga(Gda)CfuAfAfUfUfgauucauus gsa		VPusCfsaauGfaAfUfcauuAfgCfucaaus asa		UUAUUGAGCUAAUGAUUCAU UGG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646634	cscsuuu(Uda)GfcUfUfUfUfccagagaas asa		VPusUfsuucUfcUfUfGfgaaaGfcAfaaagg sasa		UUCCUUUUGCUUUCCAGAGA AAU	
AD-1646635	gsusgau(Gda)CfaUfCfUfUfuuagauuus usa		VPusAfsaaaUfcUfAfaagaUfgCfaucacs asc		GUGUGAUGCAUCUUUAGAUU UUU	
AD-1646636	asasucc(Uda)CfgUfCfUfUfgaaucaucs csa		VPusGfsgauGfaUfUfUfcagaCfgAfggauu sgsu		ACAAUCCUCGUCUGAAUCAU CCG	
AD-1646637	asasagu(Uda)AfgCfAfCfUfuugugugus asa		VPusUfsacaCfaCfAfagugCfuAfacuuus csa		UGAAAGUUAGCACUUGUGUG UAA	
AD-1646638	asasuua(Cda)AfaAfAfUfUfccacagaas gsa		VPusCfsuucUfgUfUfGfgauuUfuGfuaauu scsc		GGAAUUACAAAAUCCACAGA AGA	
AD-1646639	asusgac(Ada)GfuUfUfUfGfucaagacas usa		VPusAfsuguCfuUfUfGfacaaAfcUfgucau susc		GAAUGACAGUUUGUCAAGAC AUA	
AD-1646640	gsgsgca(Gda)AfuCfUfUfUfaaauaaaas usa		VPusAfsuuuUfaUfUfUfuaagAfuCfugccc sasg		CUGGGCAGAUCUUAAAUAAA AUU	
AD-1646641	gsasuua(Ada)AfaUfCfCfGfaucaucgus gsa		VPusCfsacgAfuGfAfUfcgaUfuUfuaauc scsc		GGGAUUAAAAUCGAUCAUCG UGG	
AD-1646642	ususgcu(Gda)AfuGfUfUfUfccuuguug susa		VPusAfscaaCfaAfGfgaacAfuCfagcaas gsc		GCUUGCUGAUGUUCCUUGUU GUU	
AD-1646643	csusacc(Uda)CfaCfAfCfUfugugcauus usa		VPusAfsaauGfcAfCfagugUfgAfgguag sasa		UUCUACCUCACACUGUGCAU UUG	
AD-1646644	gsusauc(Ada)AfuAfUfUfUfaguuggaas gsa		VPusCfsuucCfaAfCfuaauAfuUfgauacs asu		AUGUAUCAAUAUUAGUUGGA AGG	
AD-1646645	asgsauu(Ada)CfuGfCfCfAfcauguggas usa		VPusAfsuccAfcAfUfUfgugcAfgUfaaucu sgsa		UCAGAUUACUGCACAUGUGG AUA	
AD-1646646	gsasggg(Ada)UfuUfCfUfUfaccucacas csa		VPusGfsuguGfaGfGfuagaAfaUfcccuc sasg		CUGAGGGAUUUCUACCUCAC ACU	
AD-1646647	usgsaag(Ada)CfaGfGfGfAfcauuaaaas gsa		VPusCfsuuuUfaAfUfUfguccUfgUfcuuca sasu		AUUGAAGACAGGACAUUAAA AGA	
AD-1646648	gsasgga(Gda)AfuCfUfUfGfuguuuacus usa		VPusAfsaguAfaAfCfacagAfuCfuccucs gsu		ACGAGGAGAUCUGUGUUUAC UUA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646649	ascsucu(Gda)GfuGfCfAfacuaacuas csa		VPusGfsuagUfuAfGfuugcAfcCfagagu sasu		AUACUCUGGUGCAACUAACU ACU	
AD-1646650	csasccu(Uda)GfcUfUfUfUfccuugugus asa		VPusUfsacaCfaAfGfgaaaGfcAfaggugs gsg		CCCACCUUGCUUUCCUUGUG UAG	
AD-1646651	uscscgu(Ada)GfuCfUfCfcuucuacgs usa		VPusAfscguAfgAfAfggagAfcUfacgga scsg		CGUCCGUAGUCUCCUUCUAC GUG	
AD-1646652	ususgcu(Gda)GfuGfAfAfaguuagcas csa		VPusGfsugcUfaAfCfuuucAfcCfagcaas asu		AUUUGCUGGUGAAAGUUAGC ACU	
AD-1646653	csusccu(Uda)CfuUfCfUfgcgagcugs usa		VPusAfscagCfuCfGfcagaAfgAfaggag scsc		GGCUCCUUCUUCUGCGAGCU GUG	
AD-1646654	gsgsaau(Uda)GfgUfCfCfaugugcaas usa		VPusAfsuugCfaCfAfuggaCfcAfauucc susc		GAGGAAUUGGUCCAUGUGCA AUA	
AD-1646655	usgsaau(Cda)AfuCfCfGfaggcaaags asa		VPusUfscuuUfgCfCfucggAfuGfauuca sgsa		UCUGAAUCAUCCGAGGCAAA GAG	
AD-1646656	asasagc(Ada)AfaGfAfGfaaaggaugs gsa		VPusCfscauCfcUfUfucucUfuUfgcuuu scsa		UGAAAGCAAAGAGAAAGGAU GGA	
AD-1646657	uscsuuu(Uda)GfuGfUfGfugcgugug sasa		VPusUfscacAfcGfCfacacAfcAfaaagas asg		CUUCUUUUGUGUGUGCGUGU GAU	
AD-1646658	gscsauu(Uda)GfcAfCfAfgcagauags asa		VPusUfscuaUfcUfGfcuguGfcAfaaugc sasc		GUGCAUUUGCACAGCAGAUA GAA	
AD-1646659	asgsgaa(Ada)GfuUfUfUfGfggaagggas usa		VPusAfsuccCfuUfCfccaaAfcUfuuccus usu		AAAGGAAAGUUUGGGAAGGG AUG	
AD-1646660	asgsccu(Cda)UfcUfCfUfgugacaugs usa		VPusAfscauGfuCfAfcagaGfaGfaggcu scsu		AGAGCCUCUCUCUGUGACAU GUG	
AD-1646661	gsgsgcg(Uda)CfuUfCfAfcgcucugcs usa		VPusAfsgcaGfaGfGfCfgugaAfgAfcgccc sasu		AUGGGCGUCUUCACGCUCUG CUG	
AD-1646662	asgsaaa(Gda)GfaUfGfGfGfaggcaaaas usa		VPusAfsuuuUfgCfCfuccaUfcCfuuucu scsu		AGAGAAAGGAUGGAGGCAAA AUA	
AD-1646663	gsascau(Uda)AfaAfAfGfagagcgags asa		VPusUfscucGfcUfCfucuuUfuAfauguc scsu		AGGACAUUAAAAGAGAGGCGA GAG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646664	csgsgga(Ada)CfaGfGfAfacacacuas csa		VPusGfsuagUfgUfGfuuccUfgUfucccg sgsg		CCCGGGAACAGGAACACACU ACC	
AD-1646665	asgsaga(Gda)GfaGfAfAfugacaguus usa		VPusAfsaacUfgUfCfauucUfcCfucucus csu		AGAGAGAGGAGAAUGACAGU UUG	
AD-1646666	csusgcg(Ada)CfuUfCfGfucaccaacs csa		VPusGfsguuGfgUfGfacgaAfgUfcgcag scsa		UGCUGCGACUUCGUCACCAA CCG	
AD-1646667	gscsccu(Uda)CfuUfCfCfuggccaacs gsa		VPusCfsguuGfgCfCfaggaAfgAfagggc sasg		CUGCCCUUCUUCCUGGCCAA CGU	
AD-1646668	usasaga(Cda)CfgAfUfAfgcaggugas asa		VPusUfsucaCfcUfGfcuauCfgGfucuua sasg		CUUAAGACCGAUAGCAGGUG AAC	
AD-1646669	gsgsuga(Ada)GfaAfGfAfucgacagcs usa		VPusAfsgcuGfuCfGfaucuUfcUfucacc susg		CAGGUGAAGAAGAUCGACAG CUG	
AD-1646670	csascgu(Gda)AfaUfUfUfgcuggugas asa		VPusUfsucaCfcAfGfcaaaUfuCfacgugs gsg		CCCACGUGAAUUUGCUGGUG AAA	
AD-1646671	asgscga(Gda)AfgAfGfAfgaaacagus usa		VPusAfsacuGfuUfUfcucuCfuCfucgcu scsu		AGAGCGAGAGAGAAACAG UUC	
AD-1646672	gsasggc(Ada)AfaGfAfGfaaaagccas csa		VPusGfsuggCfuUfUfucucUfuUfgccuc sgsg		CCGAGGCAAAGAGAAAAGCC ACG	
AD-1646673	gscscca(Uda)CfcUfCfAfugcacuggs usa		VPusAfsccaGfuGfCfaugaGfgAfugggc sasg		CUGCCCAUCCUCAUGCACUG GUG	
AD-1646674	ascsccu(Gda)UfgUfGfUfcauugcccs usa		VPusAfsgggCfaAfUfgacaCfaCfagggu scsu		AGACCCUGUGUGUCAUUGCC CUG	
AD-1646675	usasgca(Gda)GfuGfAfAfcucgaagcs csa		VPusGfsgcuUfcGfAfguucAfcCfugcua susc		GAUAGCAGGUGAACUCGAAG CCC	
AD-1646676	gscsgcu(Cda)AfuCfGfUfgcugcucas usa		VPusAfsugaGfcAfGfcacgAfuGfagcgc scsa		UGGCGCUCAUCGUGCUGCUC AUC	
AD-1646677	csusgug(Cda)AfuCfAfUfggccuucgs usa		VPusAfscgaAfgGfCfcaugAfuGfcacag sgsg		CCCUGUGCAUCAUGGCCUUC GUG	
AD-1646678	cscscau(Cda)CfcUfUfUfcccgggaasc sa		VPusGfsuucCfcGfGfgaaaGfgGfauggg sasg		CUCCCAUCCCUUUCCCGGGA ACA	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646679	cscsucg(Gda)AfaUfCfCfCfaagguguas gsa		VPusCfsuacAfcCfUfuggaUfuCfcgagg scsg		CGCCUCGGAAUCCAAGGUGU AGG	
AD-1646680	gsasagc(Cda)CfaCfAfAfuccucgucs usa		VPusAfsgacGfaGfGfauugUfgGfgcuuc sgsa		UCGAAGCCCACAAUCCUCGU CUG	
AD-1646681	asusggc(Cda)UfuCfGfUfguaccugcs gsa		VPusCfsgcaGfgUfAfcacgAfaGfgccaus gsa		UCAUGGCCUUCGUGUACCUG CGG	
AD-1646682	ususaug(Uda)CfcAfAfGfugcccacgs usa		VPusAfscguGfgGfCfacuuGfgAfcauaa sasa		UUUUAUGUCCAAGUGCCCAC GUG	
AD-1646683	gsascac(Uda)CfuCfUfCfagaggaccs gsa		VPusCfsgguCfcUfCfucagAfgAfguguc sasa		UUGACACUCUCUGAGAGGAC CGG	
AD-1646684	gsascga(Cda)GfaCfGfAfugucgucgs gsa		VPusCfscgaCfgAfCfaucgUfcGfucgucs gsu		ACGACGACGACGAUGUCGUC GGG	
AD-1646685	asuscgu(Gda)GfcUfCfCfcfcauccccuus usa		VPusAfsaagGfgAfUfgggaGfcCfacgau sgsa		UCAUCGUGGGCUCCCCAUCCCU UUC	
AD-1646686	csusggu(Gda)UfcCfUfUfUfccugcccas usa		VPusAfsuggGfcAfGfgaagGfaCfaccag sgsg		CCCUGGUGUCCUUCCUGCCC AUC	
AD-1646687	asasggg(Ada)GfaAfGfCfauuaggags gsa		VPusCfscucCfuAfAfugcuUfcUfcccuu scsc		GGAAGGGAGAAGCAUUAGGA GGG	
AD-1646688	csgscug(Uda)CfuCfAfGfcaguggacs asa		VPusUfsgucCfaCfUfgcugAfgAfcagcg sgsc		GCCGCUGUCUCAGCAGUGGA CAG	
AD-1646689	usascgc(Cda)AfaCfUfUfCfggccuucas asa		VPusUfsugaAfgGfCfcgagUfuGfgcgua sgsc		GCUACGCCAACUCGGCCUUC AAC	
AD-1646690	csuscgc(Cda)AfuCfAfCfcucgcccus usa		VPusAfsaggGfcGfAfggugAfuGfgcgag sgsu		ACCUCGCCAUCACCUCGCCC UUC	
AD-1646691	gsusccg(Gda)CfcUfUfUfCfuuuugugus gsa		VPusCfsacaCfaAfAfagaaGfgCfcggacs csa		UGGUCCGGCCUUCUUUUGUG UGU	
AD-1646692	asuscgc(Cda)UfcGfUfUfCfcguagucus csa		VPusGfsagaCfuAfUfCfggacGfaGfgcgau sgsg		CCAUCGCCUCGUCCGUAGUC UCC	
AD-1646693	csgsggc(Ada)AfuGfUfUfGfcuggugaus csa		VPusGfsaucAfcCfAfgcacAfuUfgccccgs csc		GGCGGGCAAUGUGCUGGUGA UCG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646694	csusucu(Uda)CfaAfCfUfggcugggcsusa		VPusAfsgccCfaGfCfcaguUfgAfagaagsasc		GUCUUCUUCAACUGGCUGGGCUA	
AD-1646695	csusugu(Gda)UfaGfGfGfcaaacccgscsa		VPusGfscggGfuUfUfgcccUfaCfacaagsgsa		UCCUUGUGUAGGGCAAACCCGCU	
AD-1646696	gsgscca(Ada)CfgUfGfGfugaaggccsusa		VPusAfsggcCfuUfCfaccaCfgUfuggccsasg		CUGGCCAACGUGGUGAAGGCCUU	
AD-1646697	gsgsagu(Ada)CfgGfCfUfccuucuucsusa		VPusAfsgaaGfaAfGfgagcCfgUfacuccscsa		UGGGAGUACGGCUCCUUCUUCUG	
AD-1646698	gscscca(Gda)AfaGfCfAfggugaagasasa		VPusUfsucuUfcAfCfcugcUfuCfugggcscsu		AGGCCCAGAAGCAGGUGAAGAAG	
AD-1646699	csasgaa(Gda)GfcGfCfUfcaagacgcsusa		VPusAfsgcgUfcUfUfgagcGfcCfuucugscsu		AGCAGAAGGCGCUCAAGACGCUG	
AD-1646700	ususccg(Cda)UfaCfCfAfgagccugcsusa		VPusAfsgcaGfgCfUfcuggUfaGfcggaasgsg		CCUUCCGCUACCAGAGCCUGCUG	
AD-1646701	csusucc(Gda)CfaAfGfGfccuuccagsgsa		VPusCfscugGfaAfGfgccuUfgCfggaagsusc		GACUUCCGCAAGGCCUUCCAGGG	
AD-1646702	gsasgug(Gda)AfaGfAfUfgggugggususa		VPusAfsaccCfaCfCfcaucUfuCfcacucscsg		CGGAGUGGGAAGAUGGGUGGGUUA	
AD-1646703	gsgscca(Gda)CfaUfCfGfagacccugsusa		VPusAfscagGfgUfCfucgaUfgCfuggccsgsu		ACGGCCAGCAUCGAGACCCUGUG	
AD-1646704	csusgcu(Gda)GfcUfGfCfccuucuucsasg		VPusGfsgaaGfaAfGfggcaGfcCfagcagscsa		CUCUGCUGGCUGCCCUUCUUCCU	
AD-1646705	gsasccg(Cda)UfaCfCfUfcgccaucascsa		VPusGfsugaUfgGfCfgaggUfaGfcggucscsa		UGGACCGCUACCUCGCCAUCACC	
AD-1646706	csgsugg(Cda)CfaUfCfGfccaagacgscsa		VPusGfscguCfuUfGfgcgaUfgGfccacgsasu		AUCGUGGCCAUCGCCAAGACGCC	
AD-1646707	asusggg(Uda)CfuGfCfUfgauggcgcsusa		VPusAfsgcgCfcAfUfcagcAfgAfcccausgsc		GCAUGGGUCUGCUGAUGGCGCUC	
AD-1646708	gsgsgca(Uda)CfaUfCfAfugggcgucsusa		VPusAfsgacGfcCfCfaugaUfgAfugcccsasg		CUGGGCAUCAUCAUGGGCGUCUU	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646709	gsasaca(Cda)AfcUfAfCfcagccagasg sa		VPusCfsucuGfgCfUfgguaGfuGfuguuc scsu		AGGAACACACUACCAGCCAG AGA	
AD-1646710	asusugc(Cda)CfuGfGfAfccgcuaccs usa		VPusAfsgguAfgCfGfguccAfgGfgcaau sgsa		UCAUUGCCCUGGACCGCUAC CUC	
AD-1646711	asuscuc(Gda)GfcCfCfUfgguguccus usa		VPusAfsaggAfcAfCfcaggGfcCfgagau sgsg		CCAUCUCGGCCCUGGUGUCC UUC	
AD-1646712	asgscug(Cda)GfaGfCfGfccguuuccs usa		VPusAfsggaAfaCfGfgcgcUfcGfcagcu sgsu		ACAGCUGCGAGCGCCGUUUC CUC	
AD-1646713	csasuca(Uda)CfuAfCfUfgccgcagcs csa		VPusGfsgcuGfcGfGfcaguAfgAfugaug sgsg		CCCAUCAUCUACUGCCGCAG CCC	
AD-1646714	gscsaga(Cda)GfcUfCfAfccaaccucsu sa		VPusAfsgagGfuUfGfgugaGfcGfucugc sasg		CUGCAGACGCUCACCAACCU CUU	
AD-1646715	csusucc(Ada)GfgGfAfCfugcucugcs usa		VPusAfsgcaGfaGfCfagucCfcUfggaags gsc		GCCUUCCAGGGACUGCUCUG CUG	
AD-1646716	csusuca(Uda)CfaUfGfUfcccuggccs asa		VPusUfsggcCfaGfGfgacaUfgAfugaag sasg		CUCUUCAUCAUGUCCCUGGC CAG	
AD-1646717	asasaca(Uda)GfcUfGfAfagucccggs csa		VPusGfsccgGfgAfCfuucaGfcAfuguuu scsu		AGAAACAUGCUGAAGUCCCG GCG	
AD-1646718	asasggc(Cda)UfuCfCfAfccgcgagcs usa		VPusAfsgcuCfgCfGfguggAfaGfgccuu scsa		UGAAGGCCUUCCACCGCGAG CUG	
AD-1646719	csasggc(Gda)CfaGfAfGfccucucucs usa		VPusAfsgagAfgAfGfgcucUfgCfgccug sgsu		ACCAGGCGCAGAGGCCUCUCU CUG	
AD-1646720	csgsagc(Cda)CfgGfUfAfaccugucgs usa		VPusAfscgaCfaGfGfuuacCfgGfgcucg sgsa		UCCGAGCCCGGUAACCUGUC GUC	
AD-1646721	csusgug(Gda)AfcCfUfCfaguggacgs usa		VPusAfscguCfcAfCfugagGfuCfcacags csu		AGCUGUGGACCUCAGUGGAC GUG	
AD-1646722	gscscac(Gda)GfaCfCfGfuugcacaasa sa		VPusUfsuugUfgCfAfacggUfcCfguggc susu		AAGCCACGGACCGUUGCACA AAA	
AD-1646723	ascscuc(Gda)CfcCfUfUfccgcuaccsa sa		VPusUfsgguAfgCfGfgaagGfgCfgaggu sgsa		UCACCUCGCCCUUCCGCUAC CAG	

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1646724	uscsaag(Ada)CfgCfUfGfggcaucaus csa		VPusGfsaugAfuGfCfccagCfgUfcuuga sgsc		GCUCAAGACGCUGGGCAUCA UCA	
AD-1646725	gsgsaga(Gda)UfgGfCfUfugcugaugs usa		VPusAfscauCfaGfCfaagcCfaCfucuccs csa		UGGGAGAGUGGGCUUGCUGAU GUU	
AD-1646726	csusgcu(Cda)AfuCfGfUfggcgggcas asa		VPusUfsugccCfcGfCfcacgAfuGfagcags csa		UGCUGCUCAUCGUGGCGGGC AAU	
AD-1646727	ascscag(Cda)CfaGfAfGfagaggagas asa		VPusUfsucuCfcUfCfucucUfgGfcuggu sasg		CUACCAGCCAGAGAGAGGAG AAU	
AD-1646728	gscsugg(Uda)GfaUfCfGfuggccaucs gsa		VPusCfsgauGfgCfCfacgaUfcAfccagcs asc		GUGCUGGUGAUCGUGGCCAU CGC	
AD-1646729	asasguc(Cda)CfgGfCfGfgcucuuccs asa		VPusUfsggaAfgAfGfccgcCfgGfgacuu scsa		UGAAGUCCCGGCGGCUCUUC CAG	
AD-1646730	gsgsuca(Gda)GfcUfGfAfgggauuucs usa		VPusAfsgaaAfuCfCfcucaGfcCfugaccs asc		GUGGUCAGGCUGAGGGAUUU CUA	
AD-1646731	uscsagu(Gda)GfaCfGfUfgcugugcgs usa		VPusAfscgcAfcAfGfcacgUfcCfacugas gsg		CCUCAGUGGACGUGCUGUGC GUG	
AD-1646732	usasccu(Gda)CfgGfGfUfguuccgcgs asa		VPusUfscgcGfgAfAfcaccCfgCfagguas csa		UGUACCUGCGGGUGUUCCGC GAG	
AD-1646733	ususucu(Gda)UfuUfGfUfgguccggcs csa		VPusGfsgccGfgAfCfcacaAfaCfagaaas asa		UUUUUCUGUUUGUGGUCCGG CCU	
AD-1646734	ascscaa(Cda)CfgGfGfCfcuacgccasu sa		VPusAfsuggCfgUfAfggccCfgGfuuggu sgsa		UCACCAACCGGGCCUACGCC AUC	

Table 4. Unmodified Sense and Antisense Strand Sequences of ADRB1 dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000684.3
AD-1637961	AAACAUGCUGAAGUCCCGGCU		AGCCGGGACUUCAGCAUGUUUCU		3-23
AD-1637971	AAGUCCCGGCGGCUCUUCCAU		AUGGAAGAGCCGCCGGGACUUCA		13-33
AD-1638021	CGAGCCCGGUAACCUGUCGUU		AACGACAGGUUACCGGGCUCGGA		298-320
AD-1638051	CGCUGUCUCAGCAGUGGACAU		AUGUCCACUGCUGAGACAGCGGC		420-442
AD-1638077	AUGGGUCUGCUGAUGGCGCUU		AAGCGCCAUCAGCAGACCCAUGC		446-468
AD-1638092	GCGCUCAUCGUGCUGCUCAUU		AAUGAGCAGCACGAUGAGCGCCA		461-483
AD-1638104	CUGCUCAUCGUGGCGGGCAAU		AUUGCCCGCCACGAUGAGCAGCA		473-495
AD-1638117	CGGGCAAUGUGCUGGUGAUCU		AGAUCACCAGCACAUUGCCCGCC		486-508
AD-1638127	GCUGGUGAUCGUGGCCAUCGU		ACGAUGGCCACGAUCACCAGCAC		496-518
AD-1638136	CGUGGCCAUCGCCAAGACGCU		AGCGUCUUGGCGAUGGCCACGAU		505-527
AD-1638154	GCAGACGCUCACCAACCUCUU		AAGAGGUUGGUGAGCGUCUGCAG		532-554
AD-1638163	CACCAACCUCUUCAUCAUGUU		AACAUGAUGAAGAGGUUGGUGAG		541-563
AD-1638172	CUUCAUCAUGUCCCUGGCCAU		AUGGCCAGGGACAUGAUGAAGAG		550-572
AD-1638196	GGAGUACGGCUCCUUCUUCUU		AAGAAGAAGGAGCCGUACUCCCA		637-659
AD-1638205	CUCCUUCUUCUGCGAGCUGUU		AACAGCUCGCAGAAGAAGGAGCC		646-668
AD-1638221	CUGUGGACCUCAGUGGACGUU		AACGUCCACUGAGGUCCACAGCU		662-684
AD-1638230	UCAGUGGACGUGCUGUGCGUU		AACGCACAGCACGUCCACUGAGG		671-693
AD-1638253	GGCCAGCAUCGAGACCCUGUU		AACAGGGUCUCGAUGCUGGCCGU		694-716
AD-1638266	ACCCUGUGUGUCAUUGCCCUU		AAGGGCAAUGACACACAGGGUCU		707-729
AD-1638278	AUUGCCCUGGACCGCUACCUU		AAGGUAGCGGUCCAGGGCAAUGA		719-741
AD-1638287	GACCGCUACCUCGCCAUCACU		AGUGAUGGCGAGGUAGCGGUCCA		728-750
AD-1638296	CUCGCCAUCACCUCGCCCUUU		AAAGGGCGAGGUGAUGGCGAGGU		737-759
AD-1638305	ACCUCGCCCUUCCGCUACCAU		AUGGUAGCGGAAGGGCGAGGUGA		746-768
AD-1638314	UUCCGCUACCAGAGCCUGCUU		AAGCAGGCUCUGGUAGCGGAAGG		755-777
AD-1638347	AUCUCGGCCCUGGUGUCCUUU		AAAGGACACCAGGGCCGAGAUGG		818-840
AD-1638356	CUGGUGUCCUUCCUGCCCAUU		AAUGGGCAGGAAGGACACCAGGG		827-849
AD-1638370	GCCCAUCCUCAUGCACUGGUU		AACCAGUGCAUGAGGAUGGGCAG		841-863
AD-1638393	CUGCGACUUCGUCACCAACCU		AGGUUGGUGACGAAGUCGCAGCA		910-932

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000684.3
AD-1638406	ACCAACCGGGCCUACGCCAUU		AAUGGCGUAGGCCCGGUUGGUGA		923-945
AD-1638422	AUCGCCUCCGUCCGUAGUCUCU		AGAGACUACGGACGAGGCGAUGG		941-963
AD-1638431	UCCGUAGUCUCCUUCUACGUU		AACGUAGAAGGAGACUACGGACG		950-972
AD-1638435	CUGUGCAUCAUGGCCUUCGUU		AACGAAGGCCAUGAUGCACAGGG		974-996
AD-1638444	AUGGCCUUCGUGUACCUGCGU		ACGCAGGUACACGAAGGCCAUGA		983-1005
AD-1638456	UACCUGCGGGUGUUCCGCGAU		AUCGCGGAACACCCGCAGGUACA		995-1017
AD-1638474	GCCCAGAAGCAGGUGAAGAAU		AUUCUUCACCUGCUUCUGGGCCU		1016-1038
AD-1638485	GGUGAAGAAGAUCGACAGCUU		AAGCUGUCGAUCUUCUUCACCUG		1027-1049
AD-1638500	AGCUGCGAGCGCCGUUUCCUU		AAGGAAACGGCGCUCGCAGCUGU		1043-1065
AD-1638529	CAGAAGGCGCUCAAGACGCUU		AAGCGUCUUGAGCGCCUUCUGCU		1223-1245
AD-1638539	UCAAGACGCUGGGCAUCAUCU		AGAUGAUGCCCAGCGUCUUGAGC		1233-1255
AD-1638549	GGGCAUCAUCAUGGGCGUCUU		AAGACGCCCAUGAUGAUGCCCAG		1243-1265
AD-1638561	GGGCGUCUUCACGCUCUGCUU		AAGCAGAGCGUGAAGACGCCCAU		1255-1277
AD-1638576	CUGCUGGCUGCCCUUCUUCCU		AGGAAGAAGGGCAGCCAGCAGAG		1270-1292
AD-1638585	GCCCUUCUUCCUGGCCAACGU		ACGUUGGCCAGGAAGAAGGGCAG		1279-1301
AD-1638597	GGCCAACGUGGUGAAGGCCUU		AAGGCCUUCACCACGUUGGCCAG		1291-1313
AD-1638610	AAGGCCUUCCACCGCGAGCUU		AAGCUCGCGGUGGAAGGCCUUCA		1304-1326
AD-1638631	CCUCUUCGUCUUCUUCAACUU		AAGUUGAAGAAGACGAAGAGGCG		1336-1358
AD-1638640	CUUCUUCAACUGGCUGGGCUU		AAGCCCAGCCAGUUGAAGAAGAC		1345-1367
AD-1638659	UACGCCAACUCGGCCUUCAAU		AUUGAAGGCCGAGUUGGCGUAGC		1364-1386
AD-1638662	CAUCAUCUACUGCCGCAGCCU		AGGCUGCGGCAGUAGAUGAUGGG		1387-1409
AD-1638666	CUUCCGCAAGGCCUUCCAGGU		ACCUGGAAGGCCUUGCGGAAGUC		1411-1433
AD-1638678	CUUCCAGGGACUGCUCUGCUU		AAGCAGAGCAGUCCCUGGAAGGC		1423-1445
AD-1638705	GACGACGACGAUGUCGUCGGU		ACCGACGACAUCGUCGUCGUCGU		1556-1578
AD-1638732	CCUCGGAAUCCAAGGUGUAGU		ACUACACCUUGGAUUCCGAGGCG		1680-1702
AD-1638752	GAGGAGAUCUGUGUUUACUUU		AAAGUAAACACAGAUCUCCUCGU		1746-1768
AD-1638762	GUGUUUACUUAAGACCGAUAU		AUAUCGGUCUUAAGUAAACACAG		1756-1778
AD-1638771	UAAGACCGAUAGCAGGUGAAU		AUUCACCUGCUAUCGGUCUUAAG		1765-1787
AD-1638780	UAGCAGGUGAACUCGAAGCCU		AGGCUUCGAGUUCACCUGCUAUC		1774-1796
AD-1638794	GAAGCCCACAAUCCUCGUCUU		AAGACGAGGAUUGUGGGCUUCGA		1788-1810

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000684.3
AD-1638803	AAUCCUCGUCUGAAUCAUCCU		AGGAUGAUUCAGACGAGGAUUGU		1797-1819
AD-1638813	UGAAUCAUCCGAGGCAAAGAU		AUCUUUGCCUCGGAUGAUUCAGA		1807-1829
AD-1638823	GAGGCAAAGAGAAAAGCCACU		AGUGGCUUUUCUCUUUGCCUCGG		1817-1839
AD-1638838	GCCACGGACCGUUGCACAAAU		AUUUGUGCAACGGUCCGUGGCUU		1832-1854
AD-1638859	AGGAAAGUUUGGGAAGGGAUU		AAUCCCUUCCCAAACUUUCCUUU		1853-1875
AD-1638876	GGAGAGUGGCUUGCUGAUGUU		AACAUCAGCAAGCCACUCUCCCA		1874-1896
AD-1638886	UUGCUGAUGUUCCUUGUUGUU		AACAACAAGGAACAUCAGCAAGC		1884-1906
AD-1638890	UUUCUGUUUGUGGUCCGGCCU		AGGCCGGACCACACAAACAGAAAAA		1953-1975
AD-1638902	GUCCGGCCUUCUUUUGUGUGU		ACACACAAAAGAAGGCCGGACCA		1965-1987
AD-1638911	UCUUUUGUGUGUGCGUGUGAU		AUCACACGCACACACAAAAGAAG		1974-1996
AD-1638927	GUGAUGCAUCUUUAGAUUUUU		AAAAAUCUAAAGAUGCAUCACAC		1990-2012
AD-1638933	GGUGGUUUUUUGACACUCUCUU		AAGAGAGUGUCAAAAACCACCUG		2022-2044
AD-1638943	GACACUCUCUGAGAGGACCGU		ACGGUCCUCUCUCAGAGAGUGUCAA		2032-2054
AD-1638961	GAGUGGAAGAUGGGUGGGUUU		AAACCCACCCAUCUUCCACUCCG		2052-2074
AD-1638968	AAGGGAGAAGCAUUAGGAGGU		ACCUCCUAAUGCUUCUCCCUUCC		2077-2099
AD-1638971	GAUUAAAAAUCGAUCAUCGUGU		ACACGAUGAUCGAUUUUUAAUCCC		2098-2120
AD-1638985	AUCGUGGCUCCCCAUCCCUUUU		AAAAGGGAUGGGAGCCACGAUGA		2112-2134
AD-1638994	CCCAUCCCUUUCCCGGGAACU		AGUUCCCGGGAAAGGGAUGGGAG		2121-2143
AD-1639007	CGGGAACAGGAACACACUACU		AGUAGUGUGUUCCUGUUCCCGGG		2134-2156
AD-1639016	GAACACACUACCAGCCAGAGU		ACUCUGGCUGGUAGUGUGUUCCU		2143-2165
AD-1639025	ACCAGCCAGAGAGAGGAGAAU		AUUCUCCUCUCUCUGGCUGGUAG		2152-2174
AD-1639034	AGAGAGGAGAAUGACAGUUUU		AAAACUGUCAUUCUCCUCUCUCU		2161-2183
AD-1639044	AUGACAGUUUGUCAAGACAUU		AAUGUCUUGACACAAACUGUCAUUC		2171-2193
AD-1639057	AAGACAUAUUUCCUUUUGCUU		AAGCAAAAGGAAAUAUGUCUUGA		2184-2206
AD-1639068	CCUUUUGCUUUUCCAGAGAAU		AUUUCUCUGGAAAGCAAAAGGAA		2195-2217
AD-1639077	UUCCAGAGAAAUUUUCAUUUUU		AAAAAUGAAAUUUUCUCUGGAAAG		2204-2226
AD-1639087	AGUAAUGAUUUCUGCUGUUAU		AUAACAGCAGAAAUCAUUACUUA		2232-2254
AD-1639099	UGCUGUUAUGAAAGCAAAGAU		AUCUUUGCUUUUCAUAAACAGCAGA		2244-2266
AD-1639108	AAAGCAAAGAGAAAGGAUGGU		ACCAUCCUUUUCUCUUUGCUUUUCA		2254-2276
AD-1639116	AGAAAGGAUGGAGGCAAAAUU		AAUUUUGCCUCCCAUCCUUUUCUCU		2263-2285

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000684.3
AD-1639123	AUCACGUUUCAAGAAAUGUUU		AAACAUUUCUUGAAACGUGAUUU		2291-2313
AD-1639133	AAGAAAUGUUAAGCUCUUCUU		AAGAAGAGCUUAACAUUUCUUGA		2301-2323
AD-1639143	AAGCUCUUCUUGGAACAAGCU		AGCUUGUUCCAAGAAGAGCUUAA		2311-2333
AD-1639145	CACCUUGCUUUCCUUGUGUAU		AUACACAAGGAAAGCAAGGUGGG		2333-2355
AD-1639157	CUUGUGUAGGGCAAACCCGCU		AGCGGGUUUGCCCUACACAAGGA		2345-2367
AD-1639174	GGUCAGGCUGAGGGAUUUCUU		AAGAAAUCCCUCAGCCUGACCAC		2385-2407
AD-1639183	GAGGGAUUUCUACCUCACACU		AGUGUGAGGUAGAAAUCCCUCAG		2394-2416
AD-1639192	CUACCUCACACUGUGCAUUUU		AAAAUGCACAGUGUGAGGUAGAA		2403-2425
AD-1639206	GCAUUUGCACAGCAGAUAGAU		AUCUAUCUGCUGUGCAAAUGCAC		2417-2439
AD-1639220	GAUAGAAAGACUUGUUUAUAU		AUAUAAACAAGUCUUUCUAUCUG		2431-2453
AD-1639226	CUUGUUUAUAUUAAACAGCUU		AAGCUGUUUAAUAUAAACAAGUC		2441-2463
AD-1639240	GUAUCAAUAUUAGUUGGAAGU		ACUUCCAACUAAUAUUGAUACAU		2468-2490
AD-1639263	CAGGCGCAGAGCCUCUCUCUU		AAGAGAGAGGCUCUGCGCCUGGU		2491-2513
AD-1639272	AGCCUCUCUGUGACAUGUU		AACAUGUCACAGAGAGAGGCUCU		2500-2522
AD-1639282	UGUGACAUGUGACUCUGUCAU		AUGACAGAGUCACAUGUCACAGA		2510-2532
AD-1639295	UCUGUCAAUUGAAGACAGGAU		AUCCUGUCUUCAAUUGACAGAGU		2523-2545
AD-1639304	UGAAGACAGGACAUUAAAAGU		ACUUUUAAUGUCCUGUCUUCAAU		2532-2554
AD-1639313	GACAUUAAAAGAGAGCGAGAU		AUCUCGCUCUCUUUUAAUGUCCU		2541-2563
AD-1639321	AGCGAGAGAGAAACAGUUU		AAACUGUUUCUCUCUCUCGCUCU		2554-2576
AD-1639332	GAAACAGUUCAGAUUACUGCU		AGCAGUAAUCUGAACUGUUUCUC		2565-2587
AD-1639342	AGAUUACUGCACAUGUGGAUU		AAUCCACAUGUGCAGUAAUCUGA		2575-2597
AD-1639362	GUGGUUCAAAAUGCCAUUUUU		AAAAAUGGCAUUUUGAACCACUC		2618-2640
AD-1639373	UGCCAUUUUUGCACAGUGUUU		AAACACUGUGCAAAAAUGGCAUU		2629-2651
AD-1639387	AGUGUUAGGAAUUACAAAAUU		AAUUUUGUAAUUCCUAACACUGU		2643-2665
AD-1639396	AAUUACAAAAUCCACAGAAGU		ACUUCUGUGGAUUUUGUAAUUCC		2652-2674
AD-1639407	CCACAGAAGAUGUUACUUUGCU		AGCAAGUAACAUCUUCUGUGGAU		2663-2685
AD-1639429	GGGCAGAUCUUAAAUAAAAUU		AAUUUUAUUUAAGAUCUGCCCAG		2722-2744
AD-1639437	AUUCAAACUCUACUUCUGUUU		AAACAGAAGUAGAGUUUGAAUUU		2740-2762
AD-1639446	CUACUUCUGUUGUCUAGUAUU		AAUACUAGACAACAGAAGUAGAG		2749-2771
AD-1639460	UAGUAUGUUAUUGAGCUAAUU		AAUUAGCUCAAUAACAUACUAGA		2763-2785

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in NM_000684.3
AD-1639469	AUUGAGCUAAUGAUUCAUUGU		ACAAUGAAUCAUUAGCUCAAUAA		2772-2794
AD-1639485	AUUGGGAAAAUACCUUUUUAU		AUAAAAAGGUAUUUUCCCAAUGA		2788-2810
AD-1639495	CUUUUUAUACUCCUUUAUCAU		AUGAUAAAGGAGUAUAAAAAGGU		2801-2823
AD-1639505	UCCUUUAUCAUGGUACUGUAU		AUACAGUACCAUGAUAAAGGAGU		2811-2833
AD-1639521	UGUAACUGUAUCCAUAUUAUU		AAUAAUAUGGAUACAGUUACAGU		2827-2849
AD-1639526	UUAUGUCCAAGUGCCCACGUU		AACGUGGGCACUUGGACAUAAAA		2881-2903
AD-1639541	CACGUGAAUUUGCUGGUGAAU		AUUCACCAGCAAAUUCACGUGGG		2896-2918
AD-1639550	UUGCUGGUGAAAGUUAGCACU		AGUGCUAACUUUCACCAGCAAAU		2905-2927
AD-1639559	AAAGUUAGCACUUGUGUGUAU		AUACACACAAGUGCUAACUUUCA		2914-2936
AD-1639568	ACUUGUGUGUAAAUUCUACUU		AAGUAGAAUUUACACACAAGUGC		2923-2945
AD-1639577	UAAAUUCUACUUCCUCUUGUU		AACAAGAGGAAGUAGAAUUUACA		2932-2954
AD-1639591	CUUGUGUGUUUUACCAAGUAU		AUACUUGGUAAAACACACAAGAG		2947-2969
AD-1639599	UUUACCAAGUAUUUAUACUCU		AGAGUAUAAAUACUUGGUAAAAC		2956-2978
AD-1639615	ACUCUGGUGCAACUAACUACU		AGUAGUUAGUUGCACCAGAGUAU		2972-2994
AD-1639628	UAACUACUGUGUGAGGAAUUU		AAAUUCCUCACACAGUAGUUAGU		2985-3007
AD-1639642	GGAAUUGGUCCAUGUGCAAUU		AAUUGCACAUGGACCAAUUCCUC		2999-3021
AD-1639655	GUGCAAUAAAUACCAAUGAAU		AUUCAUUGGUAUUUAUUGCACAU		3012-3034

Table 5. Modified Sense and Antisense Strand Sequences of ADRB1 dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1637961	asasacauGfcUfGfAfaguccc ggcuL96		asGfsccgGfgAfCfuucaGfcAfuguuuscsu		AGAAACAUGCUGAAGUCCCGGCG	
AD-1637971	asasguccCfgGfCfGfgcucuucc auL96		asUfsggaAfgAfGfccgcCfgGfgacuuscsa		UGAAGUCCCGGCGGCUCUUCCAG	
AD-1638021	csgsagccCfgGfUfAfaccugucg uL96		asAfscgaCfaGfGfuuacCfgGfgcucgsgsa		UCCGAGCCCGGUAACCUGUCGUC	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1638051	csgscuguCfuCfAfGfcaguggaca uL96		asUfsgucCfaCfUfgcugAfgAfcagc ggsgsc		GCCGCUGUCUCAGCAGUGGAC AG	
AD-1638077	asusggguCfuGfCfUfgauggcgcu uL96		asAfsgcgCfcAfUfcagcAfgAfcccau sgsc		GCAUGGGUCUGCUGAUGGCGC UC	
AD-1638092	gscsgcucAfuCfGfUfgcugcucau uL96		asAfsugaGfcAfGfcacgAfuGfagcg cscsa		UGGCGCUCAUCGUGCUGCUCA UC	
AD-1638104	csusgcucAfuCfGfUfggcgggcaa uL96		asUfsugcCfcGfCfcacgAfuGfagcag scsa		UGCUGCUCAUCGUGGCGGGCA AU	
AD-1638117	csgsggcaAfuGfUfUfGfcuggugauc uL96		asGfsaucAfcCfAfgcacAfuUfgcccg scsc		GGCGGGCAAUGUGCUGGUGA UCG	
AD-1638127	gscsugguGfaUfCfGfuggccaucg uL96		asCfsgauGfgCfCfacgaUfcAfccagc sasc		GUGCUGGUGAUCGUGGCCAUC GC	
AD-1638136	csgsuggcCfaUfCfGfccaagacgcu L96		asGfscguCfuUfGfgcgaUfgGfccac gsasu		AUCGUGGCCAUCGCCAAGACG CC	
AD-1638154	gscsagacGfcUfCfAfccaaccucuu L96		asAfsgagGfuUfGfgugaGfcGfucug csasg		CUGCAGACGCUCACCAACCUC UU	
AD-1638163	csasccaaCfcUfCfUfucaucauguu L96		asAfscauGfaUfGfaagaGfgUfuggu gsasg		CUCACCAACCUCUUCAUCAUG UC	
AD-1638172	csusucauCfaUfGfUfcccuggcca uL96		asUfsggcCfaGfGfgacaUfgAfugaa gsasg		CUCUUCAUCAUGUCCCUGGCC AG	
AD-1638196	gsgsaguaCfgGfCfUfccuucucucu uL96		asAfsgaaGfaAfGfgagcCfgUfacucc scsa		UGGGAGUACGGCUCCUUCUUC UG	
AD-1638205	csusccuuCfuUfCfUfgcgagcugu uL96		asAfscagCfuCfGfcagaAfgAfaggag scsc		GGCUCCUUCUUCUGCGAGCUG UG	
AD-1638221	csusguggAfcCfUfCfaguggacgu uL96		asAfscguCfcAfCfugagGfuCfcacag scsu		AGCUGUGGACCUCAGUGGACG UG	
AD-1638230	uscsagugGfaCfGfUfUfgcugugcgu uL96		asAfscgcAfcAfGfcacgUfcCfacuga sgsg		CCUCAGUGGACGUGCUGUGCG UG	
AD-1638253	gsgsccagCfaUfCfCfGfagacccugu uL96		asAfscagGfgUfCfucgaUfgCfuggc csgsu		ACGGCCAGCAUCGAGACCCUG UG	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1638266	ascsccugUfgUfGfUfcauugcccu uL96		asAfsgggCfaAfUfgacaCfaCfagggu scsu		AGACCCUGUGUGGUCAUUGCCC UG	
AD-1638278	asusugcccCfuGfGfAfccgcuaccu uL96		asAfsgguAfgCfGfguccAfgGfgcaa usgsa		UCAUUGCCCUGGACCGCUACC UC	
AD-1638287	gsascCfgcUfaCfCfUfcgccaucacu L96		asGfsugaUfgGfCfgaggUfaGfcggu cscsa		UGGACCGCUACCUCGCCAUCA CC	
AD-1638296	csuscgccAfuCfAfCfcucgcccuu uL96		asAfsaggGfcGfAfggugAfuGfgcga ggsgsu		ACCUCGCCAUCACCUCGCCCU UC	
AD-1638305	ascscucgCfcCfUfUfccgcuaccau L96		asUfsgguAfgCfGfgaagGfgCfgagg usgsa		UCACCUCGCCCUUCCGCUACC AG	
AD-1638314	ususccgcUfaCfCfAfgagccugcu uL96		asAfsgcaGfgCfUfcuggUfaGfcgga asgsg		CCUUCCGCUACCAGAGCCUGC UG	
AD-1638347	asuscucgGfcCfCfUfgguguccuu uL96		asAfsaggAfcAfCfcaggGfcCfgagau sgsg		CCAUCUCGGCCCUGGUGUCCU UC	
AD-1638356	csusggugUfcCfUfUfUfccugcccau uL96		asAfsuggGfcAfGfgaagGfaCfaccag sgsg		CCCUGGUGUCCUUCCUGCCCA UC	
AD-1638370	gscsccauCfcUfCfAfugcacuggu uL96		asAfsccaGfuGfCfaugaGfgAfuggg csasg		CUGCCCAUCCUCAUGCACUGG UG	
AD-1638393	csusgcgaCfuUfCfGfucaccaaccu L96		asGfsguuGfgUfGfacgaAfgUfcgca gscsa		UGCUGCGACUUCGUCACCAAC CG	
AD-1638406	ascscaacCfgGfGfCfCfcuacgccauu L96		asAfsuggCfgUfAfggccCfgGfuugg usgsa		UCACCAACCGGGCCUACGCCA UC	
AD-1638422	asuscgccUfcGfUfCfCfcguagucuc uL96		asGfsagaCfuAfCfggacGfaGfgcgau sgsg		CCAUCGCCUCGUCCGUAGUCU CC	
AD-1638431	uscscguaGfuCfUfCfCfcuucuacgu uL96		asAfscguAfgAfAfggagAfcUfacgg ascsg		CGUCCGUAGUCUCCUUCUACG UG	
AD-1638435	csusgugcAfuCfAfUfUfggccuucgu uL96		asAfscgaAfgGfCfCfcaugAfuGfcacag sgsg		CCCUGUGCAUCAUGGCCUUCG UG	
AD-1638444	asusggccUfuCfGfUfUfguaccugcg uL96		asCfsgcaGfgUfAfUfcacgAfaGfgccau sgsa		UCAUGGCCUUCGUGUACCUGC GG	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1638456	usasccugCfgGfGfUfguuccgcga uL96		asUfscgcGfgAfAfcaccCfgCfaggua scsa		UGUACCUGCGGGUGUUCCGCG AG	
AD-1638474	gscsccagAfaGfCfAfggugaagaa uL96		asUfsucuUfcAfCfcugcUfuCfuggg cscsu		AGGCCCAGAAGCAGGUGAAG AAG	
AD-1638485	gsgsugaaGfaAfGfAfucgacagcu uL96		asAfsgcuGfuCfGfaucuUfcUfucacc susg		CAGGUGAAGAAGAUCGACAG CUG	
AD-1638500	asgscugcGfaGfCfGfccguuuccu uL96		asAfsggaAfaCfGfgcgcUfcGfcagcu sgsu		ACAGCUGCGAGCGCCGUUUCC UC	
AD-1638529	csasgaagGfcGfCfUfcaagacgcuu L96		asAfsgcgUfcUfUfgagcGfcCfuucu gscsu		AGCAGAAGGCGCUCAAGACGC UG	
AD-1638539	uscsaagaCfgCfUfGfggcaucauc uL96		asGfsaugAfuGfCfccagCfgUfcuug asgsc		GCUCAAGACGCUGGGCAUCAU CA	
AD-1638549	gsgsgcauCfaUfCfAfuggcgucu uL96		asAfsgacGfcCfCfaugaUfgAfugccc sasg		CUGGGCAUCAUCAUGGGCGUC UU	
AD-1638561	gsgsgcguCfuUfCfAfcgcucugcu uL96		asAfsgcaGfaGfCfgugaAfgAfcgccc sasu		AUGGGCGUCUUCACGCUCUGC UG	
AD-1638576	csusgcugGfcUfGfCfccuucuucc uL96		asGfsgaaGfaAfGfggcaGfcCfagcag sasg		CUCUGCUGGCUGCCCUUCUUC CU	
AD-1638585	gscsccuuCfuUfCfCfuggccaacg uL96		asCfsguuGfgCfCfaggaAfgAfaggg csasg		CUGCCCUUCUUCCUGGCCAAC GU	
AD-1638597	gsgsccaaCfgUfGfGfugaaggccu uL96		asAfsggcCfuUfCfaccaCfgUfuggcc sasg		CUGGCCAACGUGGUGAAGGCC UU	
AD-1638610	asasggccUfuCfCfAfccgcgagcu uL96		asAfsgcuCfgCfGfguggAfaGfgccu uscsa		UGAAGGCCUUCCACCGCGAGC UG	
AD-1638631	cscsucuuCfgUfCfUfucuucaacu uL96		asAfsguuGfaAfGfaagaCfgAfagag gscsg		CGCCUCUUCGUCUUCUUCAAC UG	
AD-1638640	csusucuuCfaAfCfUfggcuggggcu uL96		asAfsgccCfaGfCfcaguUfgAfagaag sasc		GUCUUCUUCAACUGGCUGGGC UA	
AD-1638659	usascgccAfaCfUfCfggccuucaau L96		asUfsugaAfgGfCfcgagUfuGfgcgu asgsc		GCUACGCCAACUCGGCCUUCA AC	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1638662	csasucauCfuAfCfUfgccgcagccu L96		asGfsgcuGfcGfGfcaguAfgAfugau ggsgg		CCCAUCAUCUACUGCCGGCAGC CC	
AD-1638666	csusuccgCfaAfGfGfGfccuuccagg uL96		asCfscugGfaAfGfgccuUfgCfggaa gsusc		GACUUCCGCAAGGCCUUCCAG GG	
AD-1638678	csusuccaGfgGfAfCfugcucugcu uL96		asAfsgcaGfaGfCfagucCfcUfggaag sgsc		GCCUUCCAGGGACUGCUCUGC UG	
AD-1638705	gsascgacGfaCfGfAfugucgucgg uL96		asCfscgaCfgAfCfaucgUfcGfucguc sgsu		ACGACGACGACGAUGUCGUCG GG	
AD-1638732	cscsucggAfaUfCfCfaagguguag uL96		asCfsuacAfcCfUfuggaUfuCfcgagg scsg		CGCCUCGGAAUCCAAGGUGUA GG	
AD-1638752	gsasggagAfuCfUfGfuguuuacuu uL96		asAfsaguAfaAfCfacagAfuCfuccuc sgsu		ACGAGGAGAUCUGUGUUUAC UUA	
AD-1638762	gsusguuuAfcUfUfUfAfagaccgaua uL96		asUfsaucGfgUfCfuuaaGfuAfaacac sasg		CUGUGUUUACUUAAGACCGA UAG	
AD-1638771	usasgacCfgAfUfAfgcaggugaa uL96		asUfsucaCfcUfGfcuauCfgGfucuua sasg		CUUAAGACCGAUAGCAGGUG AAC	
AD-1638780	usasgcagGfuGfAfAfcucgaagcc uL96		asGfsgcuUfcGfAfguucAfcCfugcu asusc		GAUAGCAGGUGAACUCGAAG CCC	
AD-1638794	gsasagccCfaCfAfAfuccucgucu uL96		asAfsgacGfaGfGfauugUfgGfgcuu csgsa		UCGAAGCCCACAAUCCUCGUC UG	
AD-1638803	asasuccuCfgUfCfCfUfgaaucauccu L96		asGfsgauGfaUfUfcagaCfgAfggau usgsu		ACAAUCCUCGUCUGAAUCAUC CG	
AD-1638813	usgsaaucAfuCfCfCfGfaggcaaaga uL96		asUfscuuUfgCfCfucggAfuGfauuc asgsa		UCUGAAUCAUCCGAGGCAAAG AG	
AD-1638823	gsasggcaAfaGfAfGfGfaaaagccacu L96		asGfsuggCfuUfUfucucUfuUfgccu csgsg		CCGAGGCAAAGAGAAAAGCCA CG	
AD-1638838	gscscacgGfaCfCfCfGfuugcacaaau L96		asUfsuugUfgCfAfacggUfcCfgugg csusu		AAGCCACGGACCGUUGCACAA AA	
AD-1638859	asgsgaaaGfuUfUfGfggaagggau uL96		asAfsuccCfuUfCfccaaAfcUfuuccu susu		AAAGGAAAGUUUGGGAAGGG AUG	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1638876	gsgsagagUfgGfCfUfugcugaugu uL96		asAfscauCfaGfCfaagcCfaCfucucc scsa		UGGGAGAGUGGCUUGCUGAU GUU	
AD-1638886	ususgcugAfuGfUfUfUfccuuguugu uL96		asAfscaaCfaAfGfgaacAfuCfagcaa sgsc		GCUUGCUGAUGUUCCUUGUU GUU	
AD-1638890	ususucugUfuUfGfUfgguccggcc uL96		asGfsgccGfgAfCfcacaAfaCfagaaa sasa		UUUUUCUGUUUGUGGUCCGG CCU	
AD-1638902	gsusccggCfcUfUfUfCfuuuugugug uL96		asCfsacaCfaAfAfagaaGfgCfcggac scsa		UGGUCCGGCCUUCUUUUGUGU GU	
AD-1638911	uscsuuuuGfuGfUfGfugcguguga uL96		asUfscacAfcGfCfacacAfcAfaaaga sasg		CUUCUUUUGUGUGUGCGUGU GAU	
AD-1638927	gsusgaugCfaUfCfUfuuagauuuu uL96		asAfsaaaUfcUfAfaagaUfgCfaucac sasc		GUGUGAUGCAUCUUUAGAUU UUU	
AD-1638933	gsgsugguUfuUfUfGfacacucucu uL96		asAfsgagAfgUfGfucaaAfaAfccacc susg		CAGGUGGUUUUUGACACUCUC UG	
AD-1638943	gsascacuCfuCfUfGfagaggaccg uL96		asCfsgguCfcUfCfucagAfgAfgugu csasa		UUGACACUCUCUGAGAGGACC GG	
AD-1638961	gsasguggAfaGfAfUfgggugggu uuL96		asAfsaccCfaCfCfcaucUfuCfcacuc scsg		CGGAGUGGAAGAUGGGUGGG UUA	
AD-1638968	asasgggaGfaAfGfCfauuaggagg uL96		asCfscucCfuAfAfugcuUfcUfcccuu scsc		GGAAGGGAGAAGCAUUAGGA GGG	
AD-1638971	gsasuuaaAfaUfCfCfGfaucaucgug uL96		asCfsacgAfuGfAfucgaUfuUfuaau csCsc		GGGAUUAAAAUCGAUCAUCG UGG	
AD-1638985	asuscgugGfcUfCfCfCfcaucccuuu uL96		asAfsaagGfgAfUfgggaGfcCfacga usgsa		UCAUCGUGGGCUCCCCAUCCCUU UC	
AD-1638994	cscscaucCfcUfUfUfCfcccgggaacu L96		asGfsuucCfcGfGfgaaaGfgGfaugg gsasg		CUCCCAUCCCUUUCCCGGGAA CA	
AD-1639007	csgsggaaCfaGfGfAfacacacuacu L96		asGfsuagUfgUfGfuuccUfgUfuccc ggsgsg		CCCGGGAACAGGAACACACUA CC	
AD-1639016	gsasacacAfcUfAfCfcagccagagu L96		asCfsucuGfgCfUfgguaGfuGfuguu cscsu		AGGAACACACUACCAGCCAGA GA	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1639025	ascscagcCfaGfAfGfagaggagaauL96		asUfsucuCfcUfCfucucUfgGfcugusasg		CUACCAGCCAGAGAGGAGGAGAAU	
AD-1639034	asgsagagGfaGfAfAfugacaguuuuL96		asAfsaacUfgUfCfauucUfcCfucucuscsu		AGAGAGAGGAGAAUGACAGUUUG	
AD-1639044	asusgacaGfuUfUfGfucaagacauuL96		asAfsuguCfuUfGfacaaAfcUfgucaususc		GAAUGACAGUUUGUCAAGACAUA	
AD-1639057	asasgacaUfaUfUfUfccuuuugcuuL96		asAfsgcaAfaAfGfgaaaUfaUfgucusgsa		UCAAGACAUAUUUCCUUUUGCUU	
AD-1639068	cscsuuuuGfcUfUfUfUfccagagaaauL96		asUfsuucUfcUfGfgaaaGfcAfaaaggsasa		UUCCUUUUGCUUUCCAGAGAAAU	
AD-1639077	ususccagAfgAfAfAfauuucauuuuuL96		asAfsaaaUfgAfAfAfauuuCfuCfuggaasasg		CUUUCCAGAGAAAUUUCAUUUUA	
AD-1639087	asgsuaauGfaUfUfUfUfcugcuguuauL96		asUfsaacAfgCfAfgaaaUfcAfuuacususa		UAAGUAAUGAUUUCUGCUGUUAU	
AD-1639099	usgscuguUfaUfUfGfAfaagcaaagauL96		asUfscuuUfgCfUfuucaUfaAfcagcasgsa		UCUGCUGUUAUGAAAGCAAAGAG	
AD-1639108	asasagcaAfaGfAfGfGfaaaggauggauL96		asCfscauCfcUfUfUfucucUfuUfgcuuuscsa		UGAAAGCAAAGAGAAAGGAUGGA	
AD-1639116	asgsaaagGfaUfGfGfGfaggcaaaauuL96		asAfsuuuUfgCfCfuccaUfcCfuuucuscsu		AGAGAAAGGAUGGAGGCAAAAUA	
AD-1639123	asuscacgUfuUfUfCfAfagaaauguuuL96		asAfsacaUfuUfCfuugaAfaCfguguasusu		AAAUCACGUUUCAAGAAAUGUUA	
AD-1639133	asasgaaaUfgUfUfUfAfagcucuucuuL96		asAfsgaaGfaGfCfuuaaCfaUfuucuusgsa		UCAAGAAAUGUUAAGCUCUUCUU	
AD-1639143	asasgcucUfuCfUfUfUfggaacaagcuL96		asGfscuuGfuUfCfcaagAfaGfagcusasa		UUAAGCUCUUCUUGGAACAAGCC	
AD-1639145	csasccuuGfcUfUfUfUfccuuguguauL96		asUfsacaCfaAfGfgaaaGfcAfaggugsgsg		CCCACCUUGCUUUCCUUGUGUAG	
AD-1639157	csusugugUfaGfGfGfcaaacccgcuL96		asGfscggGfuUfUfgcccUfaCfacaagsgsa		UCCUUGUGUAGGGCAAACCCGCU	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1639174	gsgsucagGfcUfGfAfgggauuucu uL96		asAfsgaaAfuCfCfCfcucaGfcCfugacc sasc		GUGGUCAGGCUGAGGGAUUU CUA	
AD-1639183	gsasgggaUfuUfCfUfaccucacac uL96		asGfsuguGfaGfGfuagaAfaUfcccu csasg		CUGAGGGAUUUCUACCUCACA CU	
AD-1639192	csusaccuCfaCfAfCfugugcauuu uL96		asAfsaauGfcAfCfagugUfgAfggua gsasa		UUCUACCUCACACUGUGCAUU UG	
AD-1639206	gscsauuuGfcAfCfAfgcagauaga uL96		asUfscuaUfcUfGfcuguGfcAfaaug csasc		GUGCAUUUGCACAGCAGAUA GAA	
AD-1639220	gsasuagaAfaGfAfCfuuguuuaua uL96		asUfsauaAfaCfAfagucUfuUfcuauc susg		CAGAUAGAAAGACUUGUUUA UAU	
AD-1639226	csusuguuUfaUfAfUfuaaacagcu uL96		asAfsgcuGfuUfUfaauaUfaAfacaag susc		GACUUGUUUAUAUUAAACAG CUU	
AD-1639240	gsusaucaAfuAfUfUfaguuggaag uL96		asCfsuucCfaAfCfuaauAfuUfgauac sasu		AUGUAUCAAUAUUAGUUGGA AGG	
AD-1639263	csasggcgCfaGfAfGfccucucucu uL96		asAfsgagAfgAfGfgcucUfgCfgccu ggsu		ACCAGGCGCAGAGGCCUCUCUC UG	
AD-1639272	asgsccucUfcUfCfUfgugacaugu uL96		asAfscauGfuCfAfcagaGfaGfaggcu scsu		AGAGCCUCUCUCUGUGACAUG UG	
AD-1639282	usgsugacAfuGfUfGfacucuguca uL96		asUfsgacAfgAfGfucacAfuGfucaca sgsa		UCUGUGACAUGUGACUCUGUC AA	
AD-1639295	uscsugucAfaUfUfUfGfaagacagga uL96		asUfsccuGfuCfUfucaaUfuGfacaga sgsu		ACUCUGUCAAUUGAAGACAG GAC	
AD-1639304	usgsaagaCfaGfGfGfAfcauuaaaag uL96		asCfsuuuUfaAfUfgucuUfgUfcuuc asasu		AUUGAAGACAGGACAUUAAA AGA	
AD-1639313	gsascauuAfaAfAfGfgagcgaga uL96		asUfscucGfcUfCfucuuUfuAfaugu cscsu		AGGACAUUAAAGAGAGCGA GAG	
AD-1639321	asgscgagAfgAfGfAfgaaacaguu uL96		asAfsacuGfuUfUfcucuCfuCfucgc uscsu		AGAGCGAGAGAGAAACAG UUC	
AD-1639332	gsasaacaGfuUfCfAfgauuacugc uL96		asGfscagUfaAfUfcugaAfcUfguuu csusc		GAGAAACAGUUCAGAUUACU GCA	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
AD-1639342	asgsauuaCfuGfCfAfcauguggauuL96		asAfsuccAfcAfUfgugcAfgUfaaucusgsa		UCAGAUUACUGCACAUGUGGAUA	
AD-1639362	gsusgguuCfaAfAfAfugccauuuuuL96		asAfsaaaUfgGfCfauuuUfgAfaccacsusc		GAGUGGUUCAAAAUGCCAUUUUU	
AD-1639373	usgsccauUfuUfUfGfcacaguguuuL96		asAfsacaCfuGfUfgcaaAfaAfuggcausu		AAUGCCAUUUUUGCACAGUGUUA	
AD-1639387	asgsuguuAfgGfAfAfuuacaaaauuL96		asAfsuuuUfgUfAfauucCfuAfacacusgsu		ACAGUGUUAGGAAUUACAAAAUC	
AD-1639396	asasuuacAfaAfAfAfUfccacagaaguL96		asCfsuucUfgUfGfgauuUfuGfuaauuscsc		GGAAUUACAAAAUCCACAGAAGA	
AD-1639407	cscsacagAfaGfAfUfguuacuugcL96		asGfscaaGfuAfAfcauuUfuCfugugcsasu		AUCCACAGAAGAUGUUACUUGCA	
AD-1639429	gsgsgcagAfuCfUfUfUfaaauaaaauuL96		asAfsuuuUfaUfUfuaagAfuCfugcccsasg		CUGGGCAGAUCUUAAAUAAAAUU	
AD-1639437	asusucaaAfcUfCfUfUfacuucuguuuL96		asAfsacaGfaAfGfuagaGfuUfugaaususu		AAAUUCAAACUCUACUUCUGUUG	
AD-1639446	csusacuuCfuGfUfUfUfgucuaguauuL96		asAfsuacUfaGfAfcaacAfgAfaguagsasg		CUCUACUUCUGUUGUCUAGUAUG	
AD-1639460	usasguauGfuUfAfUfugagcuaauuL96		asAfsuuaGfcUfCfaauaAfcAfuacuasgsa		UCUAGUAUGUUAUUGAGCUAAUG	
AD-1639469	asusugagCfuAfAfUfUfgauucauugsasa		asCfsaauGfaAfUfUfcauuAfgCfucaauUGG		UUAUUGAGCUAAUGAUUCAU	
AD-1639485	asusugggAfaAfAfUfUfaccuuuuuaL96		asUfsaaaAfaGfGfuauuUfuCfccaauUAU		UCAUUGGGAAAAUACCUUUU	
AD-1639495	csusuuuuAfuAfCfUfUfccuuuaucaL96		asUfsgauAfaAfGfgaguAfuAfaaaaggsgsu		ACCUUUUUAUACUCCUUUAUCAU	
AD-1639505	uscscuuuAfuCfAfUfUfgguacuguaL96		asUfsacaGfuAfUfCfcaugAfuAfaaggasgsu		ACUCCUUUAUCAUGGUACUGUAA	
AD-1639521	usgsuaacUfgUfAfUfUfccauauuauL96		asAfsuaaUfaUfUfGfgauaCfaGfuuacasgsu		ACUGUAACUGUAUCCAUAUUAUA	

Duplex Name	oligoSeq	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA Target Sequence 5' to 3'	SEQ ID NO:
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AD-1639541	csascgugAfaUfUfUfUfgcuggugaa uL96		asUfsucaCfcAfGfcaaaUfuCfacgug sgsg		CCCACGUGAAUUUGCUGGUGA AA	
AD-1639550	ususgcugGfuGfAfAfaguuagcac uL96		asGfsugcUfaAfCfuuucAfcCfagcaa sasu		AUUUGCUGGUGAAAGUUAGC ACU	
AD-1639559	asasaguuAfgCfAfCfuugugugua uL96		asUfsacaCfaCfAfagugCfuAfacuuu scsa		UGAAAGUUAGCACUUGUGUG UAA	
AD-1639568	ascsuuguGfuGfUfAfaauucuacu uL96		asAfsguaGfaAfUfuuacAfcAfcaag usgsc		GCACUUGUGUGUAAAUUCUA CUU	
AD-1639577	usasaauuCfuAfCfUfuccucuugu uL96		asAfscaaGfaGfGfaaguAfgAfauuu ascsa		UGUAAAUUCUACUUCCUCUUG UG	
AD-1639591	csusugugUfgUfUfUfuaccaagua uL96		asUfsacuUfgGfUfaaaaCfaCfacaag sasg		CUCUUGUGUGUUUUACCAAG UAU	
AD-1639599	ususuaccAfaGfUfAfuuuauacuc uL96		asGfsaguAfuAfAfauacUfuGfguaa asasc		GUUUUACCAAGUAUUUAUAC UCU	
AD-1639615	ascsucugGfuGfCfAfacuaacuac uL96		asGfsuagUfuAfGfuugcAfcCfagag usasu		AUACUCUGGUGCAACUAACUA CU	
AD-1639628	usasacuaCfuGfUfGfugaggaauu uL96		asAfsauuCfcUfCfacacAfgUfaguua sgsu		ACUAACUACUGUGUGAGGAA UUG	
AD-1639642	gsgsaauuGfgUfCfCfaugugcaau uL96		asAfsuugCfaCfAfuggaCfcAfauucc susc		GAGGAAUUGGUCCAUGUGCA AUA	
AD-1639655	gsusgcaaUfaAfAfUfaccaaugaa uL96		asUfsucaUfuGfGfuauuUfaUfugca csasu		AUGUGCAAUAAAUACCAAUG AAG	

Table 6. Reporter Screen for Human ADRB1 in Hepa1-6 Cells

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1639655.1	36.084	2.929
AD-1639642.1	42.211	1.351
AD-1639628.1	34.874	1.542
AD-1639615.1	35.502	2.252
AD-1639599.1	29.675	2.190
AD-1639591.1	40.543	1.627
AD-1639577.1	31.585	2.243
AD-1639568.1	32.152	2.750
AD-1639559.1	30.767	2.016
AD-1639550.1	30.100	1.316
AD-1639541.1	54.998	0.655
AD-1639526.1	60.064	6.239
AD-1639521.1	38.648	2.606
AD-1639505.1	33.810	2.120
AD-1639495.1	30.011	3.407
AD-1639485.1	33.311	1.239
AD-1639469.1	30.608	3.449
AD-1639460.1	28.107	1.807
AD-1639446.1	32.926	1.836
AD-1639437.1	35.594	0.996
AD-1639429.1	36.858	0.515
AD-1639407.1	27.111	1.887
AD-1639396.1	26.091	0.591
AD-1639387.1	23.177	1.067
AD-1639373.1	28.514	1.069
AD-1639362.1	23.057	1.017
AD-1639342.1	23.441	1.056
AD-1639332.1	39.829	1.991
AD-1639321.1	42.864	3.218
AD-1639313.1	27.956	1.076
AD-1639304.1	27.695	1.370

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1639295.1	46.853	1.375
AD-1639282.1	46.448	3.809
AD-1639272.1	51.909	4.028
AD-1639263.1	52.664	2.318
AD-1639240.1	28.741	1.724
AD-1639226.1	28.551	1.140
AD-1639220.1	30.348	1.401
AD-1639206.1	50.474	3.902
AD-1639192.1	55.729	3.739
AD-1639183.1	53.680	1.718
AD-1639174.1	71.248	4.148
AD-1639157.1	58.249	1.688
AD-1639145.1	59.585	1.125
AD-1639143.1	57.108	3.631
AD-1639133.1	39.929	1.668
AD-1639123.1	38.089	0.975
AD-1639116.1	53.771	2.538
AD-1639108.1	54.713	1.465
AD-1639099.1	33.124	1.104
AD-1639087.1	17.831	0.919
AD-1639077.1	21.644	1.218
AD-1639068.1	34.199	1.527
AD-1639057.1	30.434	0.612
AD-1639044.1	21.603	1.077
AD-1639034.1	44.290	0.840
AD-1639025.1	54.566	3.721
AD-1639016.1	77.977	2.892
AD-1639007.1	66.117	4.314
AD-1638994.1	82.085	6.808
AD-1638985.1	94.681	4.373
AD-1638971.1	39.182	3.350
AD-1638968.1	82.886	5.744

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1638961.1	71.673	6.710
AD-1638943.1	50.576	1.865
AD-1638933.1	25.070	1.640
AD-1638927.1	29.333	1.531
AD-1638911.1	34.023	1.142
AD-1638902.1	87.193	1.563
AD-1638890.1	87.995	4.106
AD-1638886.1	33.282	1.122
AD-1638876.1	69.635	4.698
AD-1638859.1	40.500	3.358
AD-1638838.1	52.523	2.748
AD-1638823.1	50.377	1.268
AD-1638813.1	45.052	3.079
AD-1638803.1	62.258	2.853
AD-1638794.1	54.294	1.919
AD-1638780.1	47.039	2.699
AD-1638771.1	50.779	4.235
AD-1638762.1	34.324	2.813
AD-1638752.1	32.510	2.607
AD-1638732.1	63.193	3.476
AD-1638705.1	87.981	8.265
AD-1638678.1	82.539	8.707
AD-1638666.1	79.422	6.876
AD-1638662.1	86.689	6.919
AD-1638659.1	73.164	6.691
AD-1638640.1	67.176	5.555
AD-1638631.1	60.178	2.869
AD-1638610.1	82.033	7.435
AD-1638597.1	67.660	4.963
AD-1638585.1	73.917	3.520
AD-1638576.1	57.774	6.457
AD-1638561.1	76.720	5.989

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1638549.1	72.374	3.388
AD-1638539.1	76.908	6.768
AD-1638529.1	62.888	5.852
AD-1638500.1	55.562	4.231
AD-1638485.1	64.806	4.092
AD-1638474.1	74.497	3.329
AD-1638456.1	82.647	10.910
AD-1638444.1	65.271	7.489
AD-1638435.1	59.200	4.952
AD-1638431.1	68.084	4.786
AD-1638422.1	81.550	7.527
AD-1638406.1	77.835	6.358
AD-1638393.1	65.579	4.797
AD-1638370.1	67.546	2.641
AD-1638356.1	70.953	2.786
AD-1638347.1	76.982	7.255
AD-1638314.1	72.999	5.056
AD-1638305.1	76.084	6.443
AD-1638296.1	63.672	3.251
AD-1638287.1	59.750	4.653
AD-1638278.1	70.698	6.732
AD-1638266.1	41.929	6.152
AD-1638253.1	72.481	3.612
AD-1638230.1	63.118	6.706
AD-1638221.1	75.254	6.239
AD-1638205.1	62.400	3.774
AD-1638196.1	69.535	5.021
AD-1638172.1	83.596	6.016
AD-1638163.1	59.876	3.051
AD-1638154.1	48.625	3.199
AD-1638136.1	77.997	3.698
AD-1638127.1	81.215	3.826

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1638117.1	65.762	5.370
AD-1638104.1	94.098	7.149
AD-1638092.1	62.706	1.669
AD-1638077.1	62.318	4.690
AD-1638051.1	90.974	6.574
AD-1638021.1	98.145	5.195
AD-1637971.1	90.755	4.262
AD-1637961.1	57.582	1.207
Positive Control	1.420	0.166

Example 4: SOD1 mRNA Knockdown in Mouse Cardiac and Skeletal Muscle

dsRNA single strands comprising one or more lipophilic moieties (*e.g.*, any compound or chemical moiety having an affinity for lipids, *e.g.*, a C_{22} hydrocarbon chain) conjugated at various positions on the sense strand targeting mouse Superoxide Dismutase 1 (SOD1) or mouse myostatin (MTSN) were synthesized on a solid support followed by hybridization of complementary strands to form a duplex.

The modified nucleotide sequences of the sense strands used in this Example are provided in Table 8A and the modified nucleotide sequences of the duplexes used in this study are provided in Table 8B.

The effect of the agents in Table 8B was examined *in vivo* in wild type C57BL/6 mice (6-8 weeks old) as summarized in Table 7. Briefly, animals (n=3) were intravenously administered a single 5 mg/kg or 20 mg/kg dose of the agent on Day 0. On Day 14, animals were sacrificed and livers, heart, and quadriceps were collected.

For SOD1 analysis, RNA was isolated from powdered quadricep and heart with the PerkinElmer Chemagic system according to the supplier's guidelines. Resulting RNA was used to generate cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368813). RT-qPCR was performed using the LightCycler® 480 Probes Master in the LightCycler® 480 Instrument system using gene specific TaqMan assays for each target mouse-specific SOD1 (ThermoFisher Scientific, Mm01344233_g1) and Gapdh (ThermoFisher Scientific, Mm99999915_g1) were used for RT-qPCR analysis. Each sample was analyzed in duplicate by RT-qPCR. Data were analyzed using the ΔΔCt method normalizing to control animals treated with 1X PBS alone.

For MTSN analysis, heart and quadricep powder were homogenized and lysed in 750 µL of QIAzol Lysis Reagent for 5 minutes x2 at 25 Hz using a TissueLyser. Chloroform (150 µL) was thoroughly mixed with each sample and lysates were centrifugated at full speed for 15 minutes at 4 °C. Total RNA was isolated from the supernatant using the RNeasy® 96 Universal Tissue Kit. Resulting RNA (1500 ng) was used for cDNA synthesis using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor. For qPCR reactions, two µl of cDNA and 10µl Fast Advanced Mastermix (ThermoFisher Scientific A44359) were added to 1x house- keeping probe and 1x target probe per well. Real time PCR was done in a QuantStudio5 Real Time PCR system (ThermoFisher Scientific). The mean level of SOD1 mRNA was normalized to the mean level of GAPDH mRNA for each sample. Group mean values were then normalized to the mean value for the PBS treated group, to obtain the relative level of SOD1 mRNA expression.

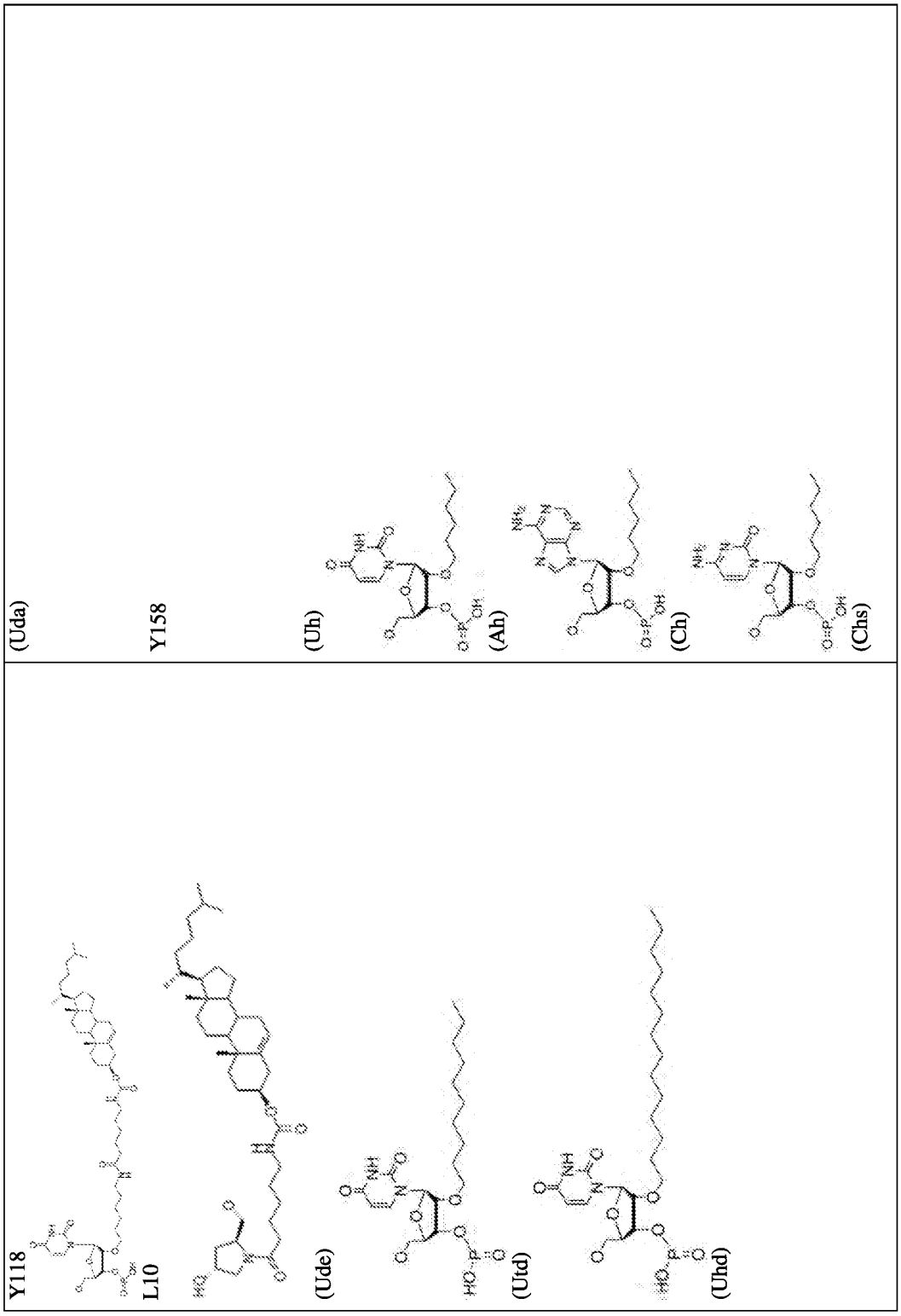
The results are shown in FIG. 1 and demonstrate that an increase in chain length of the lipophilic moiety on the sense strand at position 6 shows a strong positive correlation with SOD1 knockdown in the heart and skeletal muscle. These data also demonstrate that the most potent knockdown was observed in muscle tissue *via* intramuscular administration of a C_{22} hydrocarbon chain conjugated to the sense strand at position 6 of the dsRNA agent as evidenced by the potent inhibition of AD-1427062.

Table 7. Study Design

Group	Duplex ID	# Animals	Delivery Route	Dose (mg/kg)
1	PBS	3	IV	5
2	AD-463791	3	IV	5
3	AD-1427060	3	IV	5
4	AD-224940	3	IV	5
5	AD-454741	3	IV	5
6	AD-454740	3	IV	5
7	AD-401824	3	IV	5
8	AD-1427061	3	IV	5
9	AD-413635	3	IV	5
10	AD-1427062	3	IV	5
11	AD-1427062	3	IV	20
12	AD-1427063	3	IV	5
13	AD-1321428	3	IV	5
14	AD-1321423	3	IV	5
15	AD-1321429	3	IV	5

Table 8A. siRNA Sense Strands Synthesized for *in vivo* Studies

Duplex ID	Strand	Target	Oligonucleotide Sequence 5' to 3'	Molecular weight	Molecular weight found
A-899929	sense	SOD1	csasuuuuAfaUfCfCfucacucuasasa	6833.579	6830.02
A-2219787	sense	SOD1	csasuuuY118AfaUfCfCfucacucuasasa	7444.543	7440.52
A-444399	sense	SOD1	csasuuuuAfaUfCfCfucacucuaaaL10	7506.365	7502.51
A-637445	sense	SOD1	csasuuu(Ude)AfaUfCfCfucacucuasasa	6959.826	6956.16
A-637447	sense	SOD1	csasuuu(Utd)AfaUfCfCfucacucuasasa	7015.926	7012.22
A-637448	sense	SOD1	csasuuu(Uhd)AfaUfCfCfucacucuasasa	7043.976	7040.25
A-2356923	sense	SOD1	csasuuu(Uod)AfaUfCfCfucacucuasasa	7072.031	7068.28
A-637449	sense	SOD1	csasuuu(Uol)AfaUfCfCfucacucuasasa	7070.026	7066.26
A-2578434	sense	SOD1	csasuuu(Uda)AfaUfCfCfucacucuasasa	7128.132	7124.35
A-2248662	sense	SOD1	csasuuuY158AfaUfCfCfucacucuasasa	7121.081	7117.25
A-2219771	sense	SOD1	csasuuu(Uh)AfaUfCfCfucac(Uh)cuasasa	6973.84	6970.17
A-2219766	sense	SOD1	csasuuu(Uh)AfaUfCfCfucac(Uh)cuasas(Ah)	7043.978	7040.25
A-2219772	sense	SOD1	(Chs)asuuu(Uh)AfaUfCfCfucac(Uh)(Ch)uasas(Ah)	7184.246	7180.41
A-3340763	sense	SOD1	ususggg(Cda)AfaAfGfGfuggaaaugsasa	7468.423	7464.473
A-3013336	sense	Mstn	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7385.31


Y118
L10
(Ude)
(Utd)
(Uhd)
(Uda)
Y158
(Uh)
(Ah)
(Ch)
(Chs)

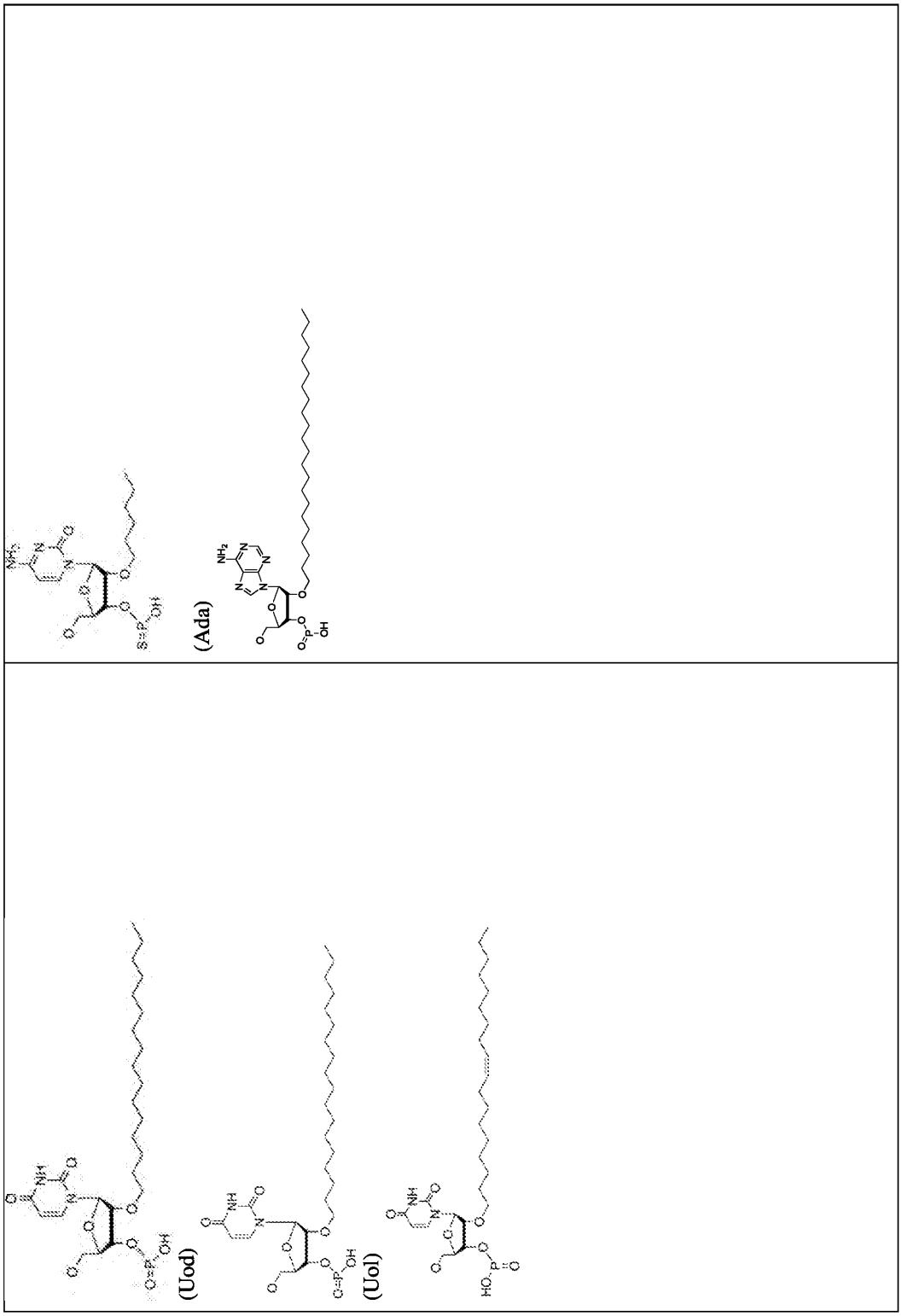
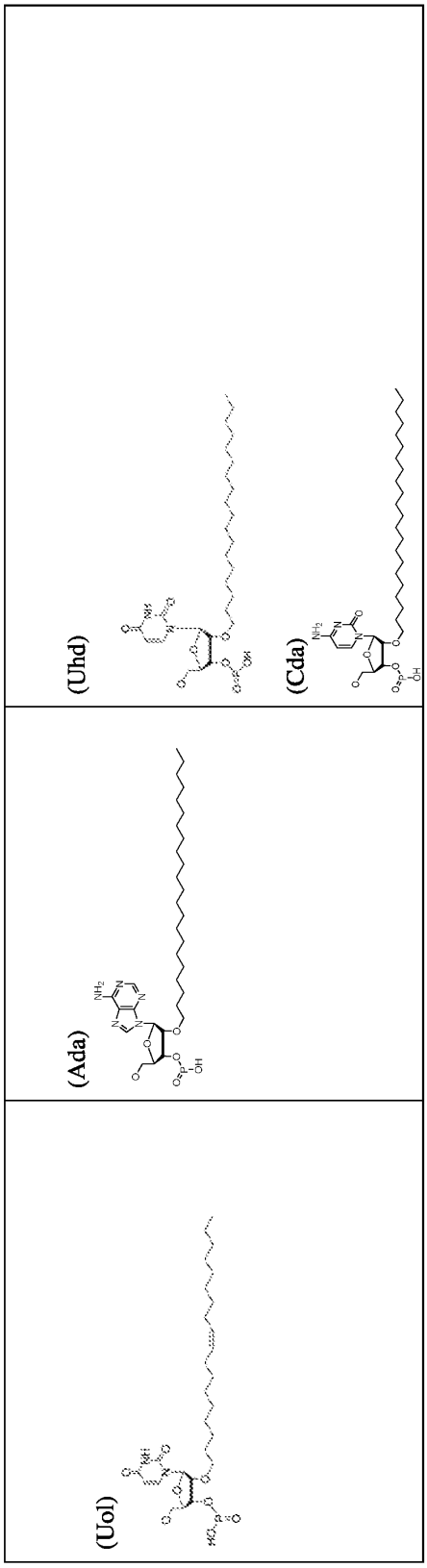

(Ada)
(Uod)
(Uol)

Table 8B: siRNA Duplexes Synthesized for *in vivo* Studies

Duplex ID	Oligo ID	Strand	Target	Sequence 5' to 3'	Molecular Weight	Molecular Weight Found
AD-463791	A-899929	sense	SOD1	csasuuuuAfaUfCfCfucacucuasasa	6833.579	6830.02
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1427060	A-2219787	sense	SOD1	csasuuuY118AfaUfCfCfucacucuasasa	7444.543	7440.52
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-224940	A-444399	sense	SOD1	csasuuuuAfaUfCfCfucacucuaaaL10	7506.365	7502.52
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-454741	A-637445	sense	SOD1	csasuuu(Ude)AfaUfCfCfucacucuasasa	6959.826	6956.16
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-454740	A-637447	sense	SOD1	csasuuu(Utd)AfaUfCfCfucacucuasasa	7015.926	7012.22
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-401824	A-637448	sense	SOD1	csasuuu(Uhd)AfaUfCfCfucacucuasasa	7043.976	7040.25
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1427061	A-2356923	sense	SOD1	csasuuu(Uod)AfaUfCfCfucacucuasasa	7072.031	7068.28
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-413635	A-637449	sense	SOD1	csasuuu(Uol)AfaUfCfCfucacucuasasa	7070.026	7066.27
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1427062	A-2578434	sense	SOD1	csasuuu(Uda)AfaUfCfCfucacucuasasa	7128.132	7124.34
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1427063	A-2248662	sense	SOD1	csasuuuY158AfaUfCfCfucacucuasasa	7121.081	7117.24
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1321428	A-2219771	sense	SOD1	csasuuu(Uh)AfaUfCfCfucac(Uh)cuasasa	6973.84	6970.17
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1321423	A-2219766	sense	SOD1	csasuuu(Uh)AfaUfCfCfucac(Uh)cuasas(Ah)	7043.978	7040.25
	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1321429	A-2219772	sense	SOD1	(Chs)asuuu(Uh)AfaUfCfCfucac(Uh)(Ch)uasas(Ah)	7184.246	7180.41

	A-444402	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.15
AD-1640773	A-3013336	sense	Mstn	asusggc(Ada)AfaGfAfAfAfcaaauaausasa	7387.441	7385.31
	A-135655	antis	Mstn	VPusUfsauuAfuUfUfguucUfuUfgccaususa	7533.956	7530.85
AD-1812376	A-3340763	sense	SOD1	ususggg(Cda)AfaAfGfGfuggaaaugsasa		
	A-859296	antis	SOD1	VPusUfscauUfuCfCfaccuUfuGfcccaasgsu		
AD-1615344	A-2894724	sense	MALAT1	asascga(Cda)UfgGfAfGfuaugauuasasa	7373.359	7369.449
	A-1850572	antis	MALAT1	VPusUfsuaaucauacucCfaGfucguususc	7593.999	7590.091
AD-1615345	A-2225899	sense	MALAT1	cscsgcugCfuAfUfUfagaaugcasusa	6967.675	6964.065
	A-2894725	antis	MALAT1	VPusAfsugcauucuaauAfg(Cda)agcggsgsa	8104.808	8100.55


(Uhd)
(Cda)
(Ada)
(Uol)

Example 5: MALAT1 and SOD1 mRNA Knockdown in Mouse Cardiac and Skeletal Muscle

MALAT1 (metastasis associated lung adenocarcinoma transcript 1) and SOD1 (superoxide dismutase type 1) gene silencing in cardiac and skeletal muscle was studied with dsRNA agents comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand.

dsRNA single strands comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, targeting mouse MALAT1 or mouse SOD1 were synthesized on a solid support followed by hybridization of complementary strands to form a duplex.

The modified nucleotide sequences of the duplexes used in this study are provided in Table 7.

The effect of these was examined *in vivo* in wild type C57BL/6 mice (6-8 weeks old) as summarized in Table 9. Animals (n=3) were intravenously administered a single 1 mg/kg, 5 mg/kg, or 20 mg/kg dose of the agent on Day 0. On Day 14 or Day 28, animals were sacrificed and tissues (*e.g.,* livers, heart, and quadriceps) were collected and the level of MALAT1 mRNA or SOD1 mRNA was determined by quantitative RT-PCR.

The results of the effect of AD-1615344 and AD-1615345 on MALAT1 expression in skeletal muscle tissue are shown in FIGS. 2A and 2B, respectively, and demonstrate that at Day 28 post-dose the duplex AD-1615344, comprising a C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, potently inhibits MALAT1 expression in skeletal muscle tissue.

Table 9. Study Design

Group	N	siRNA	I.V. dose	sac	tissues
1	3	PBS	NA	D14	Quad, heart, liver, kidney
2		AD-1615344	1 mg/kg		Quad, heart
3		AD-1615345			
4		AD-1615344	5 mg/kg		
5		AD-1615345			
6		AD-1427062			Quad, heart, liver, kidney
7		AD-1615344	20 mg/kg		Quad, heart
8		AD-1615345			
9		PBS	NA	D28	Quad, heart, liver, kidney
10		AD-1615344	1 mg/kg		Quad, heart
11		AD-1615345			
12		AD-1615344	5 mg/kg		
13		AD-1615345			
14		AD-1427062			Quad, heart, liver, kidney
15		AD-1615344	20 mg/kg		Quad, heart

Group	N	siRNA	I.V. dose	sac	tissues
16		AD-1615345			

Example 6: MSTN1 mRNA Knockdown in Mouse Skeletal Muscle and SOD1 mRNA Knockdown in Mouse Skeletal Muscle, Mouse Cardiac Muscle, and Mouse Adipose Tissue

The effect of route of administration on mouse myostatin (MSTN1) gene knockdown in mouse skeletal muscle and the effect of route of administration on mouse superoxide dismutase 1 (SOD1) knockdown in mouse skeletal muscle, mouse cardiac muscle, and mouse adipose tissue by dsRNA agents comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand were examined.

Briefly, a single 5 mg/kg dose of AD-1640773, targeting the mouse MSTN1 gene, or AD-1427062, targeting the mouse SOD1 gene, was administered to wild type C57BL/6 mice (6-8 weeks old) intravenously (IV), subcutaneously (SQ), or intramuscularly (IM) at Day 0. For the IM groups, the dsRNA agent was only administered in the left quadricep. A single 5 mg/kg dose of AD-1427062 was also administered to wild type C57BL/6 mice (6-8 weeks old) intraperitoneally (IP). PBS was intravenously administered as a control. At Day 21, animals were sacrificed, tissue including, quadricep muscle tissue from both quadriceps (left quadricep, IM injected (IM (I) and right quadricep, IM distal, not injected (IM-(D)), cardiac muscle tissue, and gonadal adipose tissue, was collected, and the level of MSTN1 or SOD1 mRNA was determined by quantitative RT-PCR.

The results of these analyses are provided in FIGS. 3A-3E.

In FIG. 3A, the data demonstrate that, although IM administration of AD-1427062 shows slightly better knockdown of SOD1 mRNA in the injected skeletal muscle, this observed knockdown is comparable to the knockdown of SOD1 mRNA observed in the distal muscle tissue and to the knockdown of SOD1 mRNA in skeletal muscle observed following administration of AD-1640773 intravenously, subcutaneously, or intraperitoneally.

FIG. 3B demonstrates that the route of administration of AD-1427062 does not affect the ability of dsRNA agents comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand to potently knockdown SOD1 mRNA in cardiac muscle tissue.

Although the basal level of myostatin is extremely variable and all routes of administration of AD-1640773 knocked down MTSN mRNA levels, FIG. 3C demonstrates that IM administration of AD-1640773 provides the most potent knockdown of MTSN mRNA in skeletal muscle tissue.

FIGS. 3D and 3E demonstrate that the route of administration of AD-1427062 does not affect the ability of dsRNA agents comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand to potently knockdown SOD1 mRNA in adipose tissue.

Example 7: Dose Response of SOD1 mRNA Knockdown in Mouse Adipose Tissue Intravenously (IV) Administered SOD1 siRNA at Days 14 and 28 Post-Dose.

The effect of dsRNA agents targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, were examined for knockdown in brown, gonadal, and subcutaneous adipose tissues.

Specifically, an exemplary duplex targeting SOD1, AD-1427062, was examined *in vivo* in wild type C57BL/6 mice (6-8 weeks old) as summarized in Table 10. Briefly, animals (n=3) were intravenously administered a single 5 mg/kg, 2 or 0.5 mg/kg dose of the dsRNA agent on Day 0. On Days 14 and 28, animals were sacrificed, and brown, gonadal, and subcutaneous adipose tissues were collected.

For SOD1 analysis, RNA was isolated by lysing the tissues in 1 mL of Qiazol in the TissueLyser II two cycles of 20 Hz for 2 minutes. Total RNA was then isolated by using the RNeasy Kit from Qiagen following manufacturer's instructions. The optional DNAse digestion step included in the kit was also performed. Resulting RNA was used to generate cDNA using ThermoFisher Scientific Superscript IV cDNA reverse transcription kit (ThermoFisher Scientific, Cat. No.11756050). In particular, twenty µl of a ready to use master mix and 11 µl of H2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on thermalcycler at 25ºC for 10 min, followed by 42ºC for 10 minutes and inactivation step at 85ºC for 5 minutes. RT-qPCR was performed by using two µl of cDNA and 10µl Fast Advanced Mastermix (ThermoFisher Scientific A44359) are added to 1x house-keeping probe (GAPDH, ThermoFisher Scientific, Mm99999915_g1) and 1x target probe (SOD1, Mm01344233_g1) per well. Real time PCR was done in a QuantStudio5 Real Time PCR system (ThermoFisher Scientific). The mean level of SOD1 mRNA was normalized to the mean level of GAPDH mRNA for each sample. Group mean values were then normalized to the mean value for the PBS treated group, to obtain the relative level of SOD1 mRNA expression.

The results are shown in Figures 4A-C and demonstrate that a C22 hydrocarbon chain conjugated to the sense strand at position 6 of the dsRNA agent AD-1427062 shows a strong positive correlation with SOD1 knockdown in all adipose tissues analyzed up to day 28 after intravenous injection of a dose as low as 0.5 mg/kg.

Table 10. Study Design

Group	Duplex ID	# Animals	Delivery Route	Dose (mg/kg)	Day
1	PBS	3	IV	NA	14
2	AD-1427062			5	
3				2	
4				0.5	
5	PBS			NA	28

Group	Duplex ID	# Animals	Delivery Route	Dose (mg/kg)	Day
6	AD-1427062			5	
7				2	
8				0.5	

Example 8: Dose Response of SOD1 mRNA Knockdown in Non-Human Primates (*Macaca fascicularis*) Adipose Tissue Intravenously Administered SOD1 siRNA at Day 30 Post-Dose.

The effect of dsRNA agents targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, were examined for knockdown in brown aortic, uterine, subcutaneous and hind limb adipose tissues.

Specifically, an exemplary duplex targeting SOD1, AD-1812376, was examined *in vivo* in *Macaca fascicularis* as summarized in Table 11. Briefly, animals (n=3) were intravenously administered a single 3 mg/kg dose of the agent on Day 0. On Day 30, animals were sacrificed, and brown aortic, uterine, subcutaneous and hind limb adipose tissues were collected.

For SOD1 analysis, RNA was isolated by lysing the tissues in 1 mL of Qiazol in the TissueLyser II two cycles of 20 Hz for 2 minutes. Total RNA was then isolated by using the RNeasy Kit from Qiagen following manufacturer's instructions. Resulting RNA was used to generate cDNA using ThermoFisher Scientific Superscript IV cDNA reverse transcription kit (ThermoFisher Scientific, Cat. No.11756050):

Twenty µl of a ready to use master mix and 11 µl of H2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on thermalcycler at 25ºC for 10 min, followed by 42ºC for 10 min and inactivation step at 85 ºC for 5 min. RT-qPCR was performed by using two µl of cDNA and 10µl Fast Advanced Mastermix (ThermoFisher Scientific A44359) are added to 1x house- keeping probe (GAPDH, ThermoFisher Scientific, Mf04392546_g1) and 1x target probe (SOD1, Mf04363557_m1,) per well. Real time PCR was done in a QuantStudio5 Real Time PCR system (ThermoFisher Scientific). The mean level of SOD1 mRNA was normalized to the mean level of GAPDH mRNA for each sample. Group mean values were then normalized to the mean value for the PBS treated group, to obtain the relative level of SOD1 mRNA expression.

The results are shown in Figure 5 and demonstrate that a C22 hydrocarbon chain conjugated to the sense strand at position 6 of the dsRNA agent AD-1812376 shows a strong positive correlation with SOD1 knockdown in all adipose tissues analyzed at day 30 post-intravenous injection of SOD siRNA.

Table 11. Study Design

Group	Duplex ID	# Animals	Delivery Route	Dose (mg/kg)	Day
1	PBS	3	IV	NA	30

Group	Duplex ID	# Animals	Delivery Route	Dose (mg/kg)	Day
2	AD-1812376	3	IV	3	30

Example 9: Effect of dsRNA Agents Targeting Leptin and Comprising One or More C_{22} Hydrocarbon Chains Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand on mRNA Knockdown in Adipose Tissues

Leptin gene silencing in adipose tissue was studied with dsRNA agents comprising one or more C22 hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand. Leptin is a secreted protein highly enriched in adipose tissue. Knockdown of leptin in adipose tissue will improve metabolic syndrome in mice.

dsRNA agents comprising one or more C22 hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, targeting human leptin were synthesized as described above.

The effect of these dsRNA agents was examined *in vivo* in female and male lean mice (20 weeks old) that were fed with either chow diet or high fat diet, as summarized in Table 12. The modified sense and antisense strand nucleotide sequence of AD-1888031 and AD-1888032 are provided in Table 13.

On study day 0, mice were weighed and then lightly anesthetized under isoflurane and blood was collected *via* retroorbital collection and processed to serum. Mice were then subcutaneously administered a single 5 mg/kg dose of AD-1888031 or AD-1888032, or PBS control in 10 µl of a solution having a concentration of 0.5mg/mL. The modified sense and antisense strand nucleotide sequence of AD-1888031 and AD-1888032 are provided in Table 13.

Body weights and blood were then collected at Day 8, Day 14, and Day 21 post-dose to evaluate circulating leptin for each mouse. All serum was collected in the fed state.

Table 12. Study Design

Group	Sex	Diet	Treatment	Subcutaneous Dose (mg/kg)	Serum Collection
1	Female	Chow	PBS	NA	Day 0, 8, 14, 21
2	Female	Chow	AD-1888031	5	
3	Female	Chow	AD-1888032	5	
4	Male	Chow	PBS	NA	
5	Male	Chow	AD-1888031	5	
6	Male	Chow	AD-1888032	5	
7	Male	HFD	AD-1888031	5	
8	Male	HFD	AD-1888032	5	

Table 13.

Duplex ID	Strand	Target	Sequence 5' to 3'	Molecular Weight	Molecular Weight Found
AD-1888031	sense	LEP	ususgaa(Gda)UfgUfAfGfuuuuauacsasa	7289.23	7285.35
	antis	LEP	VPusUfsguaUfaAfAfacuaCfaCfuucaasgsc	7651.03	7647.12
AD-1888032	sense	LEP	gsusgac(Uda)GfgUfUfUfuguuucuasusa	7235.11	7231.28
	antis	LEP	VPusAfsuagAfaAfCfaaaaCfcAfgucacscsa	7719.17	7715.22

Mouse serum leptin levels were measured using the Mouse Serum Leptin Elisa (Crystal Chem, Catalog # 90030) following the manufacturer's protocol. All serum samples were measured in duplicate using 5mL of serum. Serum samples from HFD mice was diluted 1:10 before adding 5□L to the ELISA plate to ensure signal was within the range of the standard curve.

Changes in leptin levels were graphed in GraphPad Prism (Version 9.4.1 (681)) using the group average of serum leptin levels (Figure 6A, 7A, and 8A) or percent change in serum leptin (Figures 6B, 7B, and 8B) for each timepoint +/- standard error. Percent change was calculated with the serum concentration of leptin divided by the Day 0 Serum leptin concentration for each respective mouse. Individual serum leptin concentrations, group average leptin concentrations, and group leptin concentration standard error are listed in Table 14, Table 15, and Table 16, respectively. Individual percent change in serum leptin concentrations, group average percent change in serum leptin concentrations, and group standard error of leptin concentration are listed in Table 17, Table 18, and Table 19, respectively.

The data demonstrate that a dose of 5 mg/kg of AD-1888031.1 and AD-1888032.1, comprising a C22 hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, was able to reduce circulating leptin by about 50% at two weeks in lean female mice (FIGS. 6A and 6B), and by about 65% at two weeks in lean male mice (FIGS. 7A and 7B). Both female and male mice showed minimal recovery of leptin expression at week 3.

The effect of C22-conjugated dsRNA agents on male mice fed with high fat diet was also evaluated and shown in FIGS. 8A and 8B. A dose of 5 mg/kg of AD-1888031.1 and AD-1888032.1 dramatically reduced circulating leptin in high fat diet mice by about 90%, with only slight recovery of leptin expression at Day 21.

The levels of inhibition of the dsRNA agents increased from female mice, to male mice, to high fat diet male mice, suggesting that body weight may be a driving factor for gene silencing. For example, the heavier the mice (*i.e.*, more body weight), the more dsRNA agents each animal would have received, and the more inhibition on leptin expression. In general, each lean female animal would have received about 0.11 mg dsRNA agent, while each lean male animal and each high fat diet male animal would have received about 0.14 mg and about 0.23 mg dsRNA agent, respectively.

In conclusion, the C22-conjugated dsRNA agents, *e.g.*, AD-1888031.1 and AD-1888032.1, comprising a C22 hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, were able to target leptin, an adipocyte specific gene, and potently and durably inhibit leptin expression levels in lean female mice, lean male mice, and male mice fed with a high fat diet. Moreover, a partial reduction in leptin levels may also prevent leptin resistance in mice and, thus, improve metabolism and reduce body weight.

Table 14. Individual Mouse Serum Concentrations

Serum Leptin (ng/mL)					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	3.57	4.81	3.84	4.29
	1002	2.01	1.53	1.77	3.42
	1003	6.66	7.08	6.76	8.84
Lean Female Leptin AD-1888031	2001	3.24	1.50	1.24	2.00
	2002	2.60	1.16	1.29	1.89
	2003	8.91	5.77	4.22	4.33
Lean Female Leptin AD-1888032	3001	3.03	1.96	1.47	2.18
	3002	2.23	0.97	0.88	1.28
	3003	2.67	1.88	1.44	1.61
Lean Male Leptin PBS	4001	2.77	3.59	4.09	6.11
	4002	3.50	4.30	3.19	4.78
	4003	4.60	6.22	6.13	6.94
Lean Male Leptin AD-1888031	5001	5.94	2.37	1.88	2.18
	5002	3.60	1.91	1.44	2.04
	5003	4.20	2.06	1.65	1.34
Lean Male Leptin AD-1888032	6001	2.46	0.96	1.02	1.47
	6002	8.34	2.75	2.20	1.80
	6003	3.63	0.79	1.27	2.00
HFD Male Leptin AD-1888031	7001	91.58	3.05	4.04	7.05
	7002	116.72	3.56	4.12	6.77
	7003	92.96	5.76	5.65	6.68
HFD Male Leptin AD-1888032	8001	81.84	2.94	3.54	4.40
	8002	83.77	4.50	3.91	6.28
	8003	129.14	3.01	3.96	4.03

Table 15. Group Average Serum Leptin Concentrations

Group Average Serum Leptin (ng/mL)					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	4.08	4.47	4.12	5.52

Group Average Serum Leptin (ng/mL)					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
	1002				
	1003				
Lean Female Leptin AD-1888031	2001	4.92	2.81	2.25	2.74
	2002				
	2003				
Lean Female Leptin AD-1888032	3001	2.65	1.60	1.26	1.69
	3002				
	3003				
Lean Male Leptin PBS	4001	3.62	4.70	4.47	5.94
	4002				
	4003				
Lean Male Leptin AD-1888031	5001	4.58	2.11	1.66	1.85
	5002				
	5003				
Lean Male Leptin AD-1888032	6001	4.81	1.50	1.50	1.76
	6002				
	6003				
HFD Male Leptin AD-1888031	7001	100.42	4.12	4.60	6.83
	7002				
	7003				
HFD Male Leptin AD-1888032	8001	98.25	3.49	3.80	4.90
	8002				
	8003				

Table 16. Group Standard Error for Serum Leptin

Group Standard Error Serum Leptin (ng/mL)					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	2.363317	2.789439	2.508842	2.909304
	1002				
	1003				
Lean Female Leptin AD-1888031	2001	3.474091	2.568692	1.707754	1.377894
	2002				
	2003				
Lean Female Leptin AD-1888032	3001	0.40135	0.552431	0.329123	0.457212
	3002				
	3003				
Lean Male Leptin PBS	4001	0.924664	1.358248	1.50584	1.094218
	4002				
	4003				
Lean Male Leptin AD-1888031	5001	1.219495	0.2369	0.215962	0.448158

Group Standard Error Serum Leptin (ng/mL)					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
	5002				
	5003				
Lean Male Leptin AD-1888032	6001	3.116211	1.083027	0.620133	0.265945
	6002				
	6003				
HFD Male Leptin AD-1888031	7001	14.12996	1.442328	0.906329	0.191559
	7002				
	7003				
HFD Male Leptin AD-1888032	8001	26.76696	0.881409	0.231078	1.203625
	8002				
	8003				

Table 17. Percent Change in Serum Leptin for each Individual Animal

Percent Change in Serum Leptin					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	100	134.86	107.66	104.39
	1002	100	76.06	87.67	149.70
	1003	100	106.38	101.55	145.50
Lean Female Leptin AD-1888031	2001	100	46.47	38.31	51.48
	2002	100	44.60	49.66	63.56
	2003	100	64.76	47.40	52.33
Lean Female Leptin AD-1888032	3001	100	64.70	48.36	67.88
	3002	100	43.38	39.63	51.34
	3003	100	70.39	53.90	52.89
Lean Male Leptin PBS	4001	100	129.83	147.70	206.10
	4002	100	122.84	91.31	115.28
	4003	100	135.04	133.18	130.02
Lean Male Leptin AD-1888031	5001	100	39.95	31.56	38.11
	5002	100	53.15	40.17	56.38
	5003	100	48.98	39.20	41.13
Lean Male Leptin AD-1888032	6001	100	39.24	41.63	63.01
	6002	100	32.95	26.38	25.46
	6003	100	21.90	35.13	61.44
HFD Male Leptin AD-1888031	7001	100	3.07	5.15	7.69
	7002	100	1.49	3.05	5.80
	7003	100	5.02	5.52	7.19
HFD Male Leptin AD-1888032	8001	100	3.59	4.96	5.38
	8002	100	4.58	4.31	7.49
	8003	100	2.12	2.94	3.12

Table 18. Group Average Percent Change in Serum Leptin

Group Average Percent Change in Serum Leptin					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	100	105.77	98.96	133.20
	1002				
	1003				
Lean Female Leptin AD-1888031	2001	100	51.94	45.12	55.79
	2002				
	2003				
Lean Female Leptin AD-1888032	3001	100	59.49	47.30	57.37
	3002				
	3003				
Lean Male Leptin PBS	4001	100	129.23	124.06	150.47
	4002				
	4003				
Lean Male Leptin AD-1888031	5001	100	47.36	36.98	45.20
	5002				
	5003				
Lean Male Leptin AD-1888032	6001	100	31.36	34.38	49.97
	6002				
	6003				
HFD Male Leptin AD-1888031	7001	100	3.19	4.57	6.89
	7002				
	7003				
HFD Male Leptin AD-1888032	8001	100	3.43	4.07	5.33
	8002				
	8003				

Table 19. Group Standard Error in Percent Change of Serum Leptin

Group Standard Error Percent Change in Serum Leptin					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
Lean Female PBS	1001	0	29.40431	10.24501	25.03372
	1002				
	1003				
Lean Female Leptin AD-1888031	2001	0	11.13863	6.006439	6.74159
	2002				
	2003				
Lean Female Leptin AD-1888032	3001	0	14.23971	7.195551	9.134996
	3002				
	3003				
Lean Male Leptin PBS	4001	0	6.122133	29.28125	48.74046
	4002				

Group Standard Error Percent Change in Serum Leptin					
Treatment Group	**Animal ID**	**Day 0**	**Day 8**	**Day 14**	**Day 21**
	4003				
Lean Male Leptin AD-1888031	5001	0	6.748633	4.713475	9.792716
	5002				
	5003				
Lean Male Leptin AD-1888032	6001	0	8.775138	7.657442	21.24019
	6002				
	6003				
HFD Male Leptin AD-1888031	7001	0	1.765579	1.328912	0.982239
	7002				
	7003				
HFD Male Leptin AD-1888032	8001	0	1.234817	1.030463	2.186293
	8002				
	8003				

Example 10: *In vivo* Dose Response and Duration of Intravenously Administered Lipid Conjugated dsRNA Agents Targeting Myostatin

The effect of dsRNA agents targeting myostatin (MSTN1) on the mRNA levels of MTSN1 in skeletal and cardiac muscle was examined. The design of this study is shown in Table 20.

Briefly, female C57BL/6 mice, 6-8 weeks of age (N=3 per group), were intravenously administered a single 1 mg/kg, 2.5 mg/kg or 5 mg/kg dose of a dsRNA agent targeting MSTN1. The modified nucleotide sequences of the sense and antisense strands of the dsRNA agents are provided in Table 21.

On Day 14 or Day 56 post-dose,quadriceps, heart, lungs, liver and other tissues were collected, immediately flash-frozen in liquid nitrogen and stored at -80°C. Tissues were ground with a tissue grinder (Geno/Grinder 2010 SPEX Sample Prep) at 1250 rpm for 1.5 minute. RNA extraction and purification were conducted per protocols as described in Qiagen's RNeasy 96 Universal Tissue Handbook. Resulting RNA (1500 ng) was used for cDNA synthesis with the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit. Levels of target mRNA were quantified by RT-qPCR using a Roche LightCycler® 480. Relative change in target mRNA expression was determined with ΔΔCt method by doubly normalizing to GAPDH and the average of controls.

The results, shown in Figures 9A-9B, demonstrate that dsRNA agents comprising one or more lipophilic moieties (*e.g.,* any compound or chemical moiety having an affinity for lipids, *e.g.,* a C_{22} hydrocarbon chain) conjugated at various positions on the sense or antisense strand inhibit MSTN1 in muscle tissue.

Table 20. Study design

Group	siRNA		N	I.V. Dose	sac	Collection
1	PBS		3	NA	D14	Quad, heart
2	AD-1640773			5 mg/kg		
3	AD-1684752					
4	AD-1684750					
5	AD-1684751					
6	AD-1640773			2.5 mg/kg		
7	AD-1684750					
8	AD-1684751					
9	AD-1640773			1 mg/kg		
10	AD-1684750					
11	AD-1684751					
12	PBS			NA	D56	Quad, heart
13	AD-68685			5 mg/kg		
14	AD-1640773					
15	AD-1640773			2.5 mg/kg		
16	AD-1640773			1 mg/kg		

Table 21

Duplex ID	Strand	Target	Sequence 5' to 3'	Molecular Weight	Molecular Weight Found
AD-1640773	sense	MSTN	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7383.514
	antis	MSTN	VPusUfsauuAfuUfUfguucUfuUfgccaususa	7538.806	7533.956
AD-1684752	sense	MSTN	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7383.514
	antis	MSTN	usUfsauuAfuUfUfguucUfuUfgccaususa	7461.807	7457.976
AD-1684750	sense	MSTN	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7383.514
	antis	MSTN	(Pmmds)usUfsauuAfuUfUfguucUfuUfgccaususa	7704.117	7699.942
AD-1684751	sense	MSTN	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7383.514
	antis	MSTN	(tPmmds)usUfsauuAfuUfUfguucUfuUfgccaususa	7704.113	7699.942
AD-68685	sense	MSTN	asusggcaAfaGfAfAfcaaauaauaaL10	7765.662	7761.684
	antis	MSTN	VPusUfsauuAfuUfUfguucUfuUfgccaususa	7538.806	7533.956

Example 11: *In vivo* Effect of Intravenously Administered Lipid Conjugated dsRNA Agents Targeting SOD1

The effect of dsRNA agents targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, were examined for knockdown in muscle tissues.

The design of this study is shown in Table 22.

Briefly, female C57BL/6 mice, 6-8 weeks of age (N=3 per group), were intravenously administered a single 2 mg/kg dose of AD-1427062, a dsRNA agent targeting SOD1. The modified nucleotide sequences of the sense and antisense strands of AD-1427062 are provided in Table 23.

On Day 14 post-dose, quadriceps, heart, lungs, liver, and other tissues were collected, immediately flash-frozen in liquid nitrogen and stored at -80°C. Tissues were ground with a tissue grinder (Geno/Grinder 2010 SPEX Sample Prep) at 1250 rpm for 1.5 minute. RNA extraction and purification were conducted per protocols as described in Qiagen's RNeasy 96 Universal Tissue Handbook. Resulting RNA (1500 ng) was used for cDNA synthesis with the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit. Levels of target mRNA were quantified by RT-qPCR using a Roche LightCycler® 480. Relative change in target mRNA expression was determined with ΔΔCt method by doubly normalizing to GAPDH and the average of controls.

The results, shown in Figure 10, demonstrate that administration of a single dose of a dsRNA agent targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, potently knocks down SOD1 expression in muscle tissues.

Table 22. Study Design

Group	siRNA	N	I.V. Dose	sac	Collection
1	PBS	3	NA	D14	Left quad, right quad, diaphragm, gastrocnemius
2	AD-1427062		2 mg/kg		

Table 23

Duplex ID	Strand	Target	Sequence 5' to 3'	Molecular Weight	Molecular Weight Found
AD-1427062	sense	SOD1	csasuuu(Uda)AfaUfCfCfucacucuasasa	7128.132	7124.348
	antis	SOD1	VPusUfsuagAfgUfGfaggaUfuAfaaaugsasg	7851.156	7847.154

Example 12: *In Vivo* Multidose Comparison of Intravenously or Subcutaneously Administered Lipid Conjugated dsRNA Agents Targeting SOD1

The effect of route of administration of lipid conjugated dsRNA agents targeting SOD1, *e.g.*, dsRNA agents targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand) on the mRNA levels of SOD1 in skeletal and cardiac muscle was examined. The design of this study is shown in Table 24.

Briefly, female C57BL/6 mice, 6-8 weeks of age (N=3 per group), were intravenously or subcutaneously administered a single 2 mg/kg dose or a 1 mg/kg dose on Day 0 followed by a second

1 mg/kg dose 1 week later (1 mg/kg dose X2) of AD-1812376, a dsRNA agent targeting SOD1. The modified nucleotide sequences of the sense and antisense strands of AD-1812376 are provided in Table 25.

On Day 28 post-dose, quadriceps, heart, lungs, liver, and other tissues were collected, immediately flash-frozen in liquid nitrogen and stored at -80°C. Tissues were ground with a tissue grinder (Geno/Grinder 2010 SPEX Sample Prep) at 1250 rpm for 1.5 minute. RNA extraction and purification were conducted per protocols as described in Qiagen's RNeasy 96 Universal Tissue Handbook. Resulting RNA (1500 ng) was used for cDNA synthesis with the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit. Levels of target mRNA were quantified by RT-qPCR using a Roche LightCycler® 480. Relative change in target mRNA expression was determined with ΔΔCt method by doubly normalizing to GAPDH and the average of controls.

The result, shown in Figures 11 and 12, demonstrate that dsRNA agents targeting SOD1 and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, knocks down SOD1 expression in muscle tissues irrespective of intravenous or subcutaneous administration.

Table 24: Study design

Group	siRNA	Route of Admin.	Dose	sac	N	Collection
1	PBS	IV	NA	D21	3	Quad, heart, adipose
2	AD-1812376	IV	2 mg/kg	D28		
3	AD-1812376	Subcutaneous	2 mg/kg			
4	AD-1812376	IV	1+1 mg/kg			
5	AD-1812376	Subcutaneous	1+1 mg/kg			

Table 25

Duplex ID	**Strand**	**Target**	**Sequence 5' to 3'**	**Molecular Weight**	**Molecular Weight Found**
AD-1812376	sense	SOD1	ususggg(Cda)AfaAfGfGfuggaaaugsasa	7468.423	7464.473
	antis	SOD1	VPusUfscauUfuCfCfaccuUfuGfcccaasgsu	7532.881	7529.035

Example 13: *In vivo* Dose Response of Intravenously Administered lipid conjugated siRNA agent targeting Myostatin in Monkeys

The effect of dsRNA agents targeting Myostatin (MTSN) and comprising one or more C_{22} hydrocarbon chains conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand, were examined for knockdown in muscle tissues.

The modified nucleotide sequences of the duplexes used in this study are provided in Table 26.

The effect of the agents in Table 26 was examined *in vivo* in non-human primates as summarized in Table 27. Briefly, animals were intravenously administered a single 2 mg/kg or 5 mg/kg dose of the agent on Day 0. On Day 56, animals were sacrificed and heart, gastrocnemius and quadriceps tissues were collected.

Table 26.

Duplex ID	Strand	Target	Sequence 5' to 3'	Molecular Weight	Molecular Weight Found
AD-1640773	sense	MSTN	asusggc(Ada)AfaGfAfAfcaaauaausasa	7387.441	7383.514
	antis	MSTN	VPusUfsauuAfuUfUfguucUfuUfgccaususa	7538.806	7533.956

Table 27. Study Design

Group	siRNA	N	I.V. Dose	sac	Collection
1	PBS	2	NA	D56	Quad, gastrocnemius
2	AD-1640773	3	2 mg/kg		
3	AD-1640773	3	5 mg/kg		

For quantification of MTSN protein concentration, tissues were pulverized into powder and samples lysates were prepared in mass to volume ratio of 1:20, and clarified lysate was used for Myostatin mRNA and total protein analysis. Tissue lysates were analyzed for MTSN protein using a commercially available sandwich ELISA per the manufacturer instructions (R&D Systems, DGDF80). In brief, standards and samples were added to microplates pre-coated with a monoclonal antibody specific for mature MTSN. Mature MTSN present in samples and standards were immobilized onto microplates. Plates were washed to remove unbound material, followed by addition of horseradish peroxidase-conjugated monoclonal antibody specific for mature MTSN. Plates were washed to remove any unbound antibody-enzyme reagent, and a substrate solution was added to each well. The substrate reacts with immobilized antibody-enzyme complex to generate a color product proportional to the amount of MTSN present in the unknown samples or standards. The color development is stopped and the intensity of the color is measured. For tissue, the mean concentration of GDF-8 protein (pg/mL) was adjusted to total protein concentration (mg/mL), and values were reported in pg/mg. The mean tissue MTSN concentration for each animal was normalized to the mean GDF-8 concentration of control animals to determine the fraction of myostatin protein relative to control.

The results of administration of a single 5 mg/kg dose of AD-1640773, a dsRNA agents targeting Myostatin (MTSN) and comprising one or more C_{22} hydrocarbon chains conjugated to

position 6 on the sense strand, counting from the 5'-end of the sense strand, on MTSN protein concentrations is provided Fig. 13C and demonstrates that intravenous administraton of the dsRNA agent knocksdown MTSN protein in muscle tissue.

For quantification of MTSN mRNA knockdown, heart, quadriceps, and gastrocnemius tissues were analyzed for myostatin (MSTN) mRNA using an exploratory reverse transcription quantitative polymerase chain reaction (RT-qPCR) method as outlined below.

Frozen heart, quadriceps, and gastrocnemius tissue samples were pulverized using a Genogrinder, further homogenized with a TissueLyser LT for 2 minutes with 50 cycles/sec before being lysed in 350 µL Buffer MR1 plus 6 µL reducing agent TCEP from the MACHEREY-NAGEL NucleoMag® RNA kit. Lysates were cleared by centrifugation at 5600×g for 5 minutes in a tabletop centrifuge. RNA was isolated from cleared lysates using the NucleoMag® RNA kit according to the manufacturer's protocol. RNA concentration and integrity were measured using the RNA ScreenTape Analysis on an Agilent TapeStation instrument. All RNA samples were diluted to 7.14 ng/µL in nuclease-free water, from which 14 µL, or 100 ng, was used for complementary DNA (cDNA) synthesis in a reverse transcription (RT) reaction using the Invitrogen™ SuperScript™ IV VILO™ Master Mix in a total reaction volume of 20 µL. For quantitative polymerase chain reaction (qPCR) reactions, 2 µL, containing 10 ng of cDNA, was used in a total reaction volume of 20 µL. qPCR was performed using the Applied Biosystems™ TaqMan® Fast Advanced Master Mix in the Applied Biosystems™ ViiA7 Real-Time PCR System. Commercially available, Macaca fascicularis-specific MSTN, peptidyl-prolyl cis-trans isomerase B (PPIB), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) TaqMan® primer/probes were used for qPCR analysis. Each sample was analyzed in duplicate by qPCR, with each reaction a duplex of fluorescein amidite (FAM)-labeled MSTN plus VIC (Aequorea Victoria Green Fluorescent Protein)-labeled GAPDH or PPIB. Data were acquired using ThermoFisher QuantStudio v1.3 software with 40 cycles and data acquisition at the end of each cycle.

Cycle threshold (Ct) values were determined using the ThermoFisher QuantStudio v1.3 software. The cycle threshold was set to 0.2 for the 40-cycle qPCR assay. The Delta-Delta Threshold Cycle (Relative Quantification) [ΔΔCt (RQ)] method was used to calculate fold-change in MSTN mRNA between control and treated samples using Microsoft® Office Excel 2016 (Microsoft Corporation [Redmond, WA]). First, the difference between MSTN and each housekeeping gene was calculated by subtracting the average housekeeping gene Ct from the average MSTN Ct for each duplex qPCR reaction. The difference between the delta Ct of each sample and the delta Ct of the average delta Ct of the control group (vehicle treated) was used to calculate the fold-change in MSTN mRNA. The fold-change in MSTN mRNA from all housekeeping genes was then averaged using a geometric mean and was reported as the "%mRNA remaining", where the control group "%mRNA" remaining was set to 100% remaining.

The results of administration of single 2 mg/kg or 5 mg/kg doses of AD-1640773, a dsRNA agents targeting Myostatin (MTSN) and comprising one or more C_{22} hydrocarbon chains conjugated

to position 6 on the sense strand, counting from the 5'-end of the sense strand, on MTSN mRNA knockdown are provided Figs. 13A and B and demonstrates that intravenous administraton of the dsRNA agent knocks down MTSN mRNA in muscle tissue.

Example 14: dsRNA Synthesis

siRNAs targeting the human leptin (LEP) gene (human: GenBank NM_000230.3, NCBI GeneID: 3952) were designed using custom R and Python scripts. The human LEP REFSEQ NM_000230.3 mRNA has a length of 3427 bases. siRNAs targeting the mouse leptin (Lep) gene (mouse: GenBank NM_008493.3, NCBI GeneID: 16846 were designed using custom R and Python scripts. The mouse Lep REFSEQ NM_008493.3 mRNA has a length of 3257bases.

siRNAs comprising a GalNAc conjugate targeting ligand were designed and synthesized as described above. siRNAs comprising an unsaturated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, were designed and synthesized as described above.

Detailed lists of the modified LEP sense and antisense strand nucleotide sequences comprising an unsarurated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, are shown in Tables 29 and 35, and the corresponding unmodified LEP sense and antisense nucleotide sequences are shown in Tables 28 and 34.

Detailed lists of the modified LEP sense and antisense strand nucleotide sequences comprising a GalNAc conjugate targeting ligand are shown in Tables 31 and 33, and the corresponding unmodified LEP sense and antisense nucleotide sequences are shown in Table 30 and 32.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

Table 28. Unmodified Sense and Antisense Strand Sequences of LEP dsRNA Agents

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949134	AAUGUGUUUUCUGAAUAACAA		1436	1456	UUGUUAUUCAGAAAACACAUUCU		1434	1456
AD-1949135	UGAAUCAAAGCAGUUAACUUA		2158	2178	UAAGUUAACUGCUUUGAUUCAAG		2156	2178
AD-1949136	GUGAAACUUCCUAAGUAUAAA		3024	3044	UUUAUACUUAGGAAGUUUCACAC		3022	3044
AD-1949137	UCUCGUAACUGGUUUCAUUUA		1255	1275	UAAAUGAAACCAGUUACGAGAGA		1253	1275
AD-1949138	UGCACUUUGUAACAUGUUUAA		2433	2453	UUAAACAUGUUACAAAGUGCAAG		2431	2453
AD-1949139	CUGUCCAAGGAAACUUGAAUA		2143	2163	UAUUCAAGUUUCCUUGGACAGAU		2141	2163
AD-1949140	UGAAGAAAACUCCUUUAGCAA		1951	1971	UUGCUAAAGGAGUUUUCUUCACC		1949	1971
AD-1949141	GGAGUGAGAUCAUUUUCUUAA		1479	1499	UUAAGAAAAUGAUCUCACUCCUU		1477	1499
AD-1949142	UUGUCUGUUUUUGUAACUUAA		3048	3068	UUAAGUUACAAAAACAGACAACC		3046	3068
AD-1949143	GCAUGCCAAAUUGUAGUUCUA		1864	1884	UAGAACUACAAUUUGGCAUGCAA		1862	1884
AD-1949144	AAGGGUAAAGAAGUUUGAUAA		2839	2859	UUAUCAAACUUCUUUACCCUUCA		2837	2859
AD-1949145	UUAGGAUGUUUUUCAUGAAAA		1513	1533	UUUUCAUGAAAAACAUCCUAAGC		1511	1533
AD-1949146	CCUCAUCAAGACAAUUGUCAA		156	176	UUGACAAUUGUCUUGAUGAGGGU		154	176
AD-1949147	UCACAUUCAGGAAGAUGAAAA		2057	2077	UUUUCAUCUUCCUGAAUGUGACC		2055	2077
AD-1949148	UUUGUCAAGUGUCAUAUGUAA		1363	1383	UUACAUAUGACACUUGACAAAAC		1361	1383
AD-1949149	CCUUUGAAACAAGAUAACUGA		2490	2510	UCAGUUAUCUUGUUUCAAAGGGA		2488	2510
AD-1949150	CUCCUUUUGCUUGAAACCAAA		727	747	UUUGGUUUCAAGCAAAAGGAGGC		725	747
AD-1949151	CACCAGGAUCAAUGACAUUUA		174	194	UAAAUGUCAUUGAUCCUGGUGAC		172	194
AD-1949152	CUUCAAAUCCAUCCAGAAUAA		1634	1654	UUAUUCUGGAUGGAUUUGAAGCA		1632	1654
AD-1949153	GGGCUGAAAGCCAUUUGUUGA		1663	1683	UCAACAAAUGGCUUUCAGCCCUU		1661	1683
AD-1949154	GAGCUGAACCCAUUUUGAGUA		1176	1196	UACUCAAAAUGGGUUCAGCUCAG		1174	1196
AD-1949155	GCUGUGUUUUUGCUAUCACAA		2108	2128	UUGUGAUAGCAAAAACACAGCAG		2106	2128
AD-1949156	AUCCAGGACUCUGUCAAUUUA		661	681	UAAAUUGACAGAGUCCUGGAUAA		659	681
AD-1949157	UUCCAGAAACGUGAUCCAAAA		327	347	UUUUGGAUCACGUUUCUGGAAGG		325	347

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949158	CAUGGCAGUGUUCCUAUUUGA		1838	1858	UCAAAUAGGAACACUGCCAUGUA		1836	1858
AD-1949159	AAGCCUUUUGCUCACAAAACA		2978	2998	UGUUUUGUGAGCAAAAGGCUUAA		2976	2998
AD-1949160	CUACUGUGACUGAUGUUACAA		1275	1295	UUGUAACAUCAGUCACAGUAGAA		1273	1295
AD-1949161	UGGAAGCACAUGUUUAUUUAA		1035	1055	UUAAAUAAACACAUGUGCUUCCAGC		1033	1055
AD-1949162	GCAUCUUAGCUUCUAUUAUAA		2462	2482	UUAUAAUAGAAGCUAAGAUGCCC		2460	2482
AD-1949163	UUCAGCCAUCAACAAGAGUUA		832	852	UAACUCUUGUUGAUGGCUGAAGA		830	852
AD-1949164	AGCUCACCCAAUAAACAUUAA		2232	2252	UUAAUGUUUAUUGGGUGAGCUUC		2230	2252
AD-1949165	UGGCCCUAUCUUUUCUAUGUA		94	114	UACAUAGAAAAGAUAGGGCCAAA		92	114
AD-1949166	GAUCACACUCUGGGUUUAUUA		1817	1837	UAAUAAACCCAGAGUGUGAUCAA		1815	1837
AD-1949167	UCACCGGUUUGGACUUCAUUA		227	247	UAAUGAAGUCCAAACCGGUGACU		225	247
AD-1949168	GAGCGAGAUUCCGUCUUAAAA		3349	3369	UUUUAAGACGGAAUCUCGCUCUG		3347	3369
AD-1949169	UUAUCUUCUGCAAUUGCUUAA		1496	1516	UUAAGCAAUUGCAGAAGAUAAGA		1494	1516
AD-1949170	GAGUAGCAAGUUCUAGAGAAA		2872	2892	UUUCUCUAGAACUUGCUACUCUU		2870	2892
AD-1949171	CCUGUUUUGUUGGAAGGUUUA		2719	2739	UAAACCUUCCAACAAAAACAGGCU		2717	2739
AD-1949172	ACAGGAGAAAUCGCUUAAACCA		3271	3291	UGGUUUAAGCGAUUCUCCUGUCU		3269	3291
AD-1949173	ACUCUUCCAAAGGCAUAAGAA		699	719	UUCUUAUGCCUUUGGAAGAGUGG		697	719
AD-1949174	GAUGGAUUUGAAGCAAAGCAA		1072	1092	UUGCUUUGCUUCAAAUCCAUCCA		1070	1092
AD-1949175	CUCCUCCAAACAGAAAGUCAA		210	230	UUGACUUUCUGUUUGGAGGAGAC		208	230
AD-1949176	CUGGAAAAGAGGAGUUUCGAA		2651	2671	UUCGAAACUCCUCUUUUCCAGAA		2649	2671
AD-1949177	CAGUUUCCAAUCCCAUAGAUA		1146	1166	UAUCUAUGGGAUUGGAAACUGCA		1144	1166
AD-1949178	ACCUGGCACAAUGGCUAAUUA		2996	3016	UAAUUAGCCAUUGUGCCAGGUUU		2994	3016
AD-1949179	UCCAAGGACCAGGUUAUUUUA		1331	1351	UAAAAUAACCUGGUCCUUGGAGA		1329	1351
AD-1949180	CCUGGGAAGGAAAAUGCAUUA		45	65	UAAUGCAUUUUCCUUCCCAGGAU		43	65
AD-1949181	AGUCCAAGAUGACACCAAAAA		135	155	UUUUUGGUGUCAUCUUGGACUUU		133	155
AD-1949182	UCCUCACAACCACCUAAUCAA		2944	2964	UUGAUUAGGUGGUUGUGAGGAUC		2942	2964
AD-1949183	AGCAAGGCCAAAAUUACCAAA		1903	1923	UUUGGUAAUUUUGGCCUUGCUUG		1901	1923

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949184	CACUGAAUGCCUCAAUGUGAA		882	902	UUCACAUUGAGGCAUUCAGUGAG		880	902
AD-1949185	UUACAUCACAGUGUUUGCAAA		1290	1310	UUUGCAAACACUGUGAUGUAACA		1288	1310
AD-1949186	UCCAAGGAGUUUCCAUGAAGAA		977	997	UUCUUCAUGGAACUCCUUGGAAU		975	997
AD-1949187	AGGAUUGAAGAGCAUUGCAUA		630	650	UAUGCAAUGCUCUUCAAUCCUGG		628	650
AD-1949188	AAGGGAGAAAGGAUCUAGAAUA		1419	1439	UAUUCUAGAUCCUUCUCCCUUCU		1417	1439
AD-1949189	UAUUAAAGGAGUUAAGAGUAA		2857	2877	UUACUCUUAACUCCUUUAAUAUC		2855	2877
AD-1949190	GUGGUCUUUCCUAUCAUGGAA		1722	1742	UUCCAUGAUAGGAAAGACCACUC		1720	1742
AD-1949191	UCUGCAUUUUAUUCUGGAUGA		1056	1076	UCAUCCAGAAUAAAAUGCAGAAU		1054	1076
AD-1949192	UCCUGCAAGGACUACGUUAAA		580	600	UUUAACGUAGUCCUUGCAGGAAG		578	600
AD-1949193	GUUCCCUCUGAGAAUUCCAAA		962	982	UUUGGAAUUCUCAGAGGGAACCU		960	982
AD-1949194	GAAGCUGAUGCUUUGCUUCAA		1619	1639	UUGAAGCAAAGCAUCAGCUUCUC		1617	1639
AD-1949195	AAAUACAUAAGACCAUAACAA		2527	2547	UUGUUAUGGUCUUAUGUAUUUUC		2525	2547
AD-1949196	CCUUUGGAUGACCAGAACAAA		941	961	UUUGUUCUGGUCAUCCAAAGGCU		939	961
AD-1949197	GGAGAUGCAGAGGUAAAAGUA		2746	2766	UACUUUUACCUCUGCAUCUCCAC		2744	2766
AD-1949198	UUGGGUUCAUCUGAGCAAGAA		1206	1226	UUCUUGCUCAGAUGAACCCAACC		1204	1226
AD-1949199	GCUCUCCAGUUAGUUCUCUCA		1239	1259	UGAGAGAACUAACUGGAGAGCCA		1237	1259
AD-1949200	AGUGUGAGCAGUGAGUUACAA		2763	2783	UUGUAACUCACUGCUCACACUUU		2761	2783
AD-1949201	UACACAGGAUCCUAUUCUCAA		754	774	UUGAGAAUAGGAUCCUGUGUAUA		752	774
AD-1949202	GUGCGAUUCUUGUGGCUUUA		75	95	UAAAGCCACACAAGAAUCCGCACAG		73	95
AD-1949203	AUCAGGCUGAGGUGUCUUAAA		2960	2980	UUUAAGACACCUCAGCCUGAUUA		2958	2980
AD-1949204	UUCGAGGUAGAGUUUGAAGGA		2666	2686	UCCUUCAAACUCUACCUCGAAAC		2664	2686
AD-1949205	UCCUGACCUUAUCCAAGAUGA		263	283	UCAUCUUGGAUAAGGUCAGGAUG		261	283
AD-1949206	CCUUGAAGGUCACUCUUCCUA		564	584	UAGGAAGAGUGACCUUCAAGGCC		562	584
AD-1949207	CUGGCUUUCUCCGACUGCUAA		1698	1718	UUAGCAGUCCGGAGAAAGCCAGAG		1696	1718
AD-1949209	UUGCAUUCCCAGUGGUCAAAA		2277	2297	UUUUGACCACUGGGAAUGCAAGA		2275	2297
AD-1949210	CAGCUGAACAGCCAAAUGCAA		2337	2357	UUGCAUUUGGCUGUUCAGCUGCU		2335	2357

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949211	AUACCCAGAGCAUUACGUGAA		2589	2609	UUCACGUAAUGCUCUGGGUAUCA		2587	2609
AD-1949212	CUGGCAGUCUACCAACAGAUA		292	312	UAUCUGUUGGUAGACUGCCAGUG		290	312
AD-1949213	AGAGCUGCUCUGGAAAAUGUA		2916	2936	UACAUUUUCCAGAGCAGCUCUCA		2914	2936
AD-1949214	GAGCACCUGCUUCAUGCUCAA		2185	2205	UUGAGCAUGAAGCAGGUGCUCAG		2183	2205
AD-1949215	GGUGGGAAAUGGUAUGAGCUA		2374	2394	UAGCUCAUACCAUUUCCCACCUG		2372	2394
AD-1949216	GUGUGGUGGGUUCUUUGGAAA		1459	1479	UUUCCAAAGAACCCACCACACAA		1457	1479
AD-1949217	GUUCUUGUCUGAUUGGCUCAA		1879	1899	UUGAGCCAAUCAGACAAGAACUA		1877	1899
AD-1949218	GAUCACAAGGUCACUAGAUGA		3145	3165	UCAUCUAGUGACCUUGUGAUCCC		3143	3165
AD-1949219	CUCCGGGAUCUUCUUCACGUA		367	387	UACGUGAAGAAGAUCCCGGAGGU		365	387
AD-1949220	GUCCUGGAAGCUUCAGGCUAA		457	477	UUAGCCUGAAGCUUCCAGGACAC		455	477
AD-1949221	AUUUCCCUGACUCCUCUAAGA		677	697	UCUUAGAGGAGUCAGGGAAAUUG		675	697
AD-1949222	UGACUGCGAUCUUCAGAGCAA		1760	1780	UUGCUCUGAAGAUCGCAGUCACC		1758	1780
AD-1949223	CCAGCUUCUCCCAGGCUCUUUA		1092	1112	UAAAGAGCCUGGAGAAGCUGGUG		1090	1112
AD-1949224	UCUGGCUUCCCAGGUAUCUCCA		610	630	UGGAGAUACCUGGAAGCCAGAGU		608	630
AD-1949225	CCUGGCCAACAUGGUGAAACA		3173	3193	UGUUUCACCAUGUUGGCCAGGAU		3171	3193
AD-1949226	GUUUUUACUCCCAGUGGUGAAA		1935	1955	UUUCACCACUGGAGUAAAAACCC		1933	1955
AD-1949227	AUGCAUGGUGCAGUUGACAGA		2352	2372	UCUGUCAACUGCACCAUGCAUUU		2350	2372
AD-1949228	AGAGUACAGUGAGCCAAGAUA		3301	3321	UAUCUUGGCUCACUGUACUCUCC		3299	3321
AD-1949229	AACACAAAAGUUAGCUGAGCA		3208	3228	UGCUCAGCUAACUUUUGUGUUUU		3206	3228
AD-1949230	UCCCAGCAACACAAGCUGGAA		1019	1039	UUCCAGCUUGUGUUGCUGGGAGU		1017	1039
AD-1949231	AGACCUGACAAGCACUGCUAA		1981	2001	UUAGCAGUGCUUGUCAGGUCUCA		1979	2001
AD-1949232	AUUUCACACACGCAGUCAGUA		190	210	UACUGACUGCGUGUGUGAAAUGU		188	210
AD-1949233	CAGGUAAUGAGGGACUGGAAA		2611	2631	UUUCCAGUCCCUCAUUACCUGGC		2609	2631
AD-1949234	UGAGGGAUGUGAAUUGCCUGA		2689	2709	UCAGGCAAUUCACAUCCCUCACC		2687	2709
AD-1949235	GAUCCUCACCAGUAUGCCUUA		309	329	UAAGGCAUACUGGUGAGGAUCUG		307	329
AD-1949236	CUGGCCUUCUCUAAGAGCUGA		388	408	UCAGCUCUUAGAGAAGGCCAGCA		386	408

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949237	GAUUCCCACCAAGGUCUUCAA		816	836	UUGAAGACCUUGGUGGGAAUCCC		814	836
AD-1949238	AUAUCCAACGACCUGGAGAAA		346	366	UUUCUCCAGGUCGUUGGAUAUUU		344	366
AD-1949239	CAUGCUGAAGGGACCUUGAAA		2821	2841	UUUCAAGGUCCCUUCAGCAUGGC		2819	2841
AD-1949240	UGGUGCUAUAGGCUGGAGAAA		2213	2233	UUUCUCCAGCCUAUAGCACCAGU		2211	2233
AD-1949241	CAUCCACACACGCAGGAACUA		1000	1020	UAGUUCCUGCGUGUGUGGAUGUG		998	1020
AD-1949242	UUGCCCUGAGUGGAUCUCCAA		1315	1335	UUGGAGAUCCACUCAGGGCAACA		1313	1335
AD-1949243	CGCACUCACACCCAUGUGCCAAA		2298	2318	UUUGGCACAUGGGUGAGUGCGGU		2296	2318
AD-1949244	GCGGUUGCAAGGCCCAAGAAA		19	39	UUUCUUGGGCCUUGCAACCGCUG		17	39
AD-1949245	CUGGAGAGAAGUUUCUGGCCA		1575	1595	UGGCCAGAAACUUCUCUCCAGGG		1573	1595
AD-1949246	GCCUGCAGAGAGAAGCCUGUA		2704	2724	UACAGGCUUCUCUCUGCAGGCAA		2702	2724
AD-1949247	CCUCUGAAUGGUCCAGGGUUA		1797	1817	UAACCCUGGACCAUUCAGAGGGU		1795	1817
AD-1949248	CAUUAAGAUUGAGGCCUGCCA		2247	2267	UGGCAGGCCUCAAUCUUAAUGUU		2245	2267
AD-1949249	GAGACCGAGCGCUUUCUGGAA		2636	2656	UUCCAGAAAGCGCUCCGGUCUCCC		2634	2656
AD-1949250	AGCGAGAGGCAGAGAAAGAAA		2782	2802	UUUCUUUCUCUGCCUCUCGCUGU		2780	2802
AD-1949251	UGCCACCAUCCUGCUGCUGUA		2093	2113	UACAGCAGCAGGAUGGUGGCAGA		2091	2113
AD-1949252	CUGCCCUCAGGGAUCUUGCAA		2262	2282	UUGCAAGAUCCCUGAGGGCAGGC		2260	2282
AD-1949253	CCAAGCUGUGCCCAUCCAAAA		114	134	UUUUGGAUGGGCACAGCUUGGAC		112	134
AD-1949254	CACAGGGAACCCUGCUUGCAA		2417	2437	UUGCAAGCAGGGUUCCCUGUGGG		2415	2437
AD-1949255	AUAGCCCAGGUCCUCUGAUAA		2572	2592	UUAUCAGAGGACCUGGGCUAUAG		2570	2592
AD-1949256	GUCUCUGCAGGACAUGCUGUA		513	533	UACAGCAUGUCCUGCAGAGACCC		511	533
AD-1949257	ACGGUCCCACACUGGUGACUA		1745	1765	UAGUCACCAGUGUGGGACCGUCA		1743	1765
AD-1949258	AGUGGGCUGCAUCUGGGAUUA		800	820	UAAUCCCAGAUGCAGCCCACUCU		798	820
AD-1949259	CCUUCCCACUGGAGGUCACAA		2042	2062	UUGUGACCUCCAGUGGGAAGGGC		2040	2062
AD-1949260	ACUGCACUCCCGCCUGAUGAA		3327	3347	UUCAUCAGGCCGGAGUGCAGUGG		3325	3347
AD-1949261	AGUGGCCUGGAGACCUUGGAA		424	444	UUCCAAGGUCUCCAGGCCACUGG		422	444
AD-1949262	GUCCACCCAGCAAAGAGUGGA		785	805	UCCACUCUUUGCUGGGUGGACCC		783	805

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1949263	GGCUACUCCACAGAGGUGGUA		472	492	UACCACCUCUGUGGAGUAGCCUG		470	492
AD-1949264	UGGCAGGACCAGGACUAUAGA		2556	2576	UCUAUAGUCCUGGUCCUGCCACC		2554	2576
AD-1949265	GUUAAGGGAAGGAACUCUGGA		595	615	UCCAGAGUUCCUUCCCUUAACGU		593	615
AD-1949266	ACAGUGGGUGGUGGAUCUGUA		2127	2147	UACAGAUCCACCACCCACUGUGU		2125	2147
AD-1949267	GAGCUGGCAAAGGUGGCUCUA		1224	1244	UAGAGCCACCUUUGCCAGCUCUU		1222	1244
AD-1949268	CCACUCGGGAGGCUGAGACAA		3254	3274	UUGUCUCAGCCUCCCGAGUGGCU		3252	3274

Table 29. Modified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949134	asasugu(Gda)UfuUfUfCfugaauaacsasa		VPusUfsguuAfuUfCfagaaAfaCfacauuscsu		AGAAUGUGUUUUCUGAAUAACAU	
AD-1949135	usgsaau(Cda)AfaAfGfCfaguuaacususa		VPusAfsaguUfaAfCfugcuUfuGfauucasasg		CUUGAAUCAAAGCAGUUAACUUU	
AD-1949136	gsusgaa(Ada)CfuUfCfCfuaaguauasasa		VPusUfsuauAfcUfUfaggaAfgUfuucacsasc		GUGUGAAACUUCCUAAGUAUAAA	
AD-1949137	uscsucg(Uda)AfaCfUfGfguuucauususa		VPusAfsaauGfaAfAfccagUfuAfcgagasgsa		UCUCUCGUAACUGGUUUCAUUUC	
AD-1949138	usgscac(Uda)UfuGfUfAfacauguuusasa		VPusUfsaaaCfaUfGfuuacAfaAfgugcasasg		CUUGCACUUUGUAACAUGUUUAC	
AD-1949139	csusguc(Cda)AfaGfGfAfaacuugaasusa		VPusAfsuucAfaGfUfuuccUfuGfgacagsasu		AUCUGUCCAAGGAAACUUGAAUC	
AD-1949140	usgsaag(Ada)AfaAfCfUfccuuuagcsasa		VPusUfsgcuAfaAfGfgaguUfuUfcuucascsc		GGUGAAGAAAACUCCUUUAGCAG	
AD-1949141	gsgsagu(Gda)AfgAfUfCfauuuucuusasa		VPusUfsaagAfaAfAfugauCfuCfacuccsusu		AAGGAGUGAGAUCAUUUUCUUAU	
AD-1949142	ususguc(Uda)GfuUfUfUfuguaacuusasa		VPusUfsaagUfuAfCfaaaaAfcAfgacaascsc		GGUUGUCUGUUUUUGUAACUUAA	
AD-1949143	gscsaug(Cda)CfaAfAfUfuguaguucsusa		VPusAfsgaaCfuAfCfaauuUfgGfcaugcsasa		UUGCAUGCCAAAUUGUAGUUCUU	
AD-1949144	asasggg(Uda)AfaAfGfAfaguuugausasa		VPusUfsaucAfaAfCfuucuUfuAfcccuuscsa		UGAAGGGUAAAGAAGUUUGAUAU	
AD-1949145	ususagg(Ada)UfgUfUfUfuucaugaasasa		VPusUfsuucAfuGfAfaaaaCfaUfccuaasgsc		GCUUAGGAUGUUUUUCAUGAAAA	
AD-1949146	cscsuca(Uda)CfaAfGfAfcaauugucsasa		VPusUfsgacAfaUfUfgucuUfgAfugaggsgsu		ACCCUCAUCAAGACAAUUGUCAC	
AD-1949147	uscsaca(Uda)UfcAfGfGfaagaugaasasa		VPusUfsuucAfuCfUfuccuGfaAfugugascsc		GGUCACAUUCAGGAAGAUGAAAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949148	usususgu(Cda)AfaGfUfGfucauaugusasa		VPusUfsacaUfaUfGfacacUfuGfacaaasasc		GUUUUGUCAAGUGUCAUAUGUAG	
AD-1949149	cscsuuu(Gda)AfaAfCfAfagauaacusgsa		VPusCfsaguUfaUfCfuuguUfuCfaaaggsgsa		UCCCUUUGAAACAAGAUAACUGA	
AD-1949150	csusccu(Uda)UfuGfCfUfugaaccasasa		VPusUfsuggUfuUfCfaagcAfaAfaggagsgsc		GCCUCCUUUUGCUUGAAACCAAA	
AD-1949151	csascca(Gda)GfaUfCfAfaugacauususa		VPusAfsaauGfuCfAfuugaUfcCfuggugsasc		GUCACCAGGAUCAAUGACAUUUC	
AD-1949152	csusuca(Ada)AfuCfCfAfuccagaauasa		VPusUfsauuCfuGfGfauggAfuUfugaagscsa		UGCUUCAAAUCCAUCCAGAAUAA	
AD-1949153	gsgsgcu(Gda)AfaAfGfCfcauuguusgsa		VPusCfsaacAfaAfUfggcuUfuCfagcccsusu		AAGGGCUGAAAGCCAUUUGUUGG	
AD-1949154	gsasgcu(Gda)AfaCfCfCfauuugagsusa		VPusAfscucAfaAfAfugggUfuCfagcucsasg		CUGAGCUGAACCCAUUUUGAGUG	
AD-1949155	gscsugu(Gda)UfuUfUfUfgcuaucacsasa		VPusUfsgugAfuAfGfcaaaAfaCfacagcsasg		CUGCUGUGUUUUUGCUAUCACAC	
AD-1949156	asuscca(Gda)GfaCfUfCfugucaauususa		VPusAfsaauUfgAfCfagagUfcCfuggausasa		UUAUCCAGGACUCUGUCAAUUUC	
AD-1949157	ususcca(Gda)AfaAfCfGfugauccaasasa		VPusUfsuugGfaUfCfacguUfuCfuggaasgsg		CCUUCCAGAAACGUGAUCCAAAU	
AD-1949158	csasugg(Cda)AfgUfGfUfucccuauuusgsa		VPusCfsaaaUfaGfGfaaacaCfuGfccaugsusa		UACAUGGCAGUGUUCCUAUUUGG	
AD-1949159	asasgcc(Uda)UfuUfGfCfucacaaaascsa		VPusGfsuuuUfgUfGfagcaAfaAfggcuusasa		UUAAGCCUUUUGCUCACAAAACC	
AD-1949160	csusacu(Gda)UfgAfCfUfgauguuacsasa		VPusUfsguaAfcAfUfcaguCfaCfaguagsasa		UUCUACUGUGACUGAUGUUACAU	
AD-1949161	usggsgaa(Gda)CfaCfAfUfguuauuusasa		VPusUfsaaaUfaAfAfcaugUfgCfuuccasgsc		GCUGGAAGCACAUGUUUAUUUAU	
AD-1949162	gscsauc(Uda)UfaGfCfUfucuauuausasa		VPusUfsauaAfuAfGfaagcUfaAfgaugcscsc		GGGCAUCUUAGCUUCUAUUAUAG	
AD-1949163	ususcag(Cda)CfaUfCfAfacaagagususa		VPusAfsacuCfuUfGfuugaUfgGfcugaasgsa		UCUUCAGCCAUCAACAAGAGUUG	
AD-1949164	asgscuc(Ada)CfcCfAfAfuaaacauusasa		VPusUfsaauGfuUfUfauugGfgUfgagcususc		GAAGCUCACCCAAUAAACAUUAA	
AD-1949165	usgsgcc(Cda)UfaUfCfUfuucuagususa		VPusAfscauAfgAfAfaagaUfaGfggccasasa		UUUGGCCCUAUCUUUUCUAUGUC	
AD-1949166	gsasuca(Cda)AfcUfCfUfgggauuaususa		VPusAfsauaAfaCfCfcagaGfuGfugaucsasa		UUGAUCACACUCUGGGUUUAUUA	
AD-1949167	uscsacc(Gda)GfuUfUfGfgacuucaususa		VPusAfsaugAfaGfUfccaaAfcCfggugascsu		AGUCACCGGUUUGGACUUCAUUC	
AD-1949168	gsasgcg(Ada)GfaUfUfCfcgucuaaasasa		VPusUfsuuaAfgAfCfggaaUfcUfcgcucusug		CAGAGCGAGAUUCCGUCUUAAAA	
AD-1949169	ususauc(Uda)UfcUfGfCfaauugcuusasa		VPusUfsaagCfaAfUfugcaGfaAfgauaasgsa		UCUUAUCUUCUGCAAUUGCUUAG	
AD-1949170	gsasgua(Gda)CfaAfGfUfucuagagasasa		VPusUfsucuCfuAfGfaacuUfgCfuacucsusu		AAGAGUAGCAAGUUCUAGAGAAG	
AD-1949171	cscsugu(Uda)UfuGfUfUfggaaggususa		VPusAfsaacCfuUfCfcaacAfaAfacaggscsu		AGCCUGUUUUGUUGGAAGGUUUG	
AD-1949172	ascsagg(Ada)GfaAfUfCfgcuaaacscsa		VPusGfsguUfaAfGfcgauUfcUfccuguscsu		AGACAGGAGAAUCGCUUAAACCU	
AD-1949173	ascsucu(Uda)CfcAfAfAfggcauaagsasa		VPusUfscuuAfuGfCfcuuuGfgAfagagusgsg		CCACUCUUCCAAAGGCAUAAGAC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949174	gsasugg(Ada)UfuUfGfAfagcaaagcsasa		VPusUfsgcuUfuGfCfuucaAfaUfccaucscsa		UGGAUGGAUUUGAAGCAAAGCAC	
AD-1949175	csusccu(Cda)CfaAfAfCfagaaagucsasa		VPusUfsgacUfuUfCfuguuUfgGfaggagsasc		GUCUCCUCCAAACAGAAAGUCAC	
AD-1949176	csusgga(Ada)AfaGfAfGfgaguucgsasa		VPusUfscgaAfaCfUfccucUfuUfuccagsasa		UUCUGGAAAAGAGGAGUUUCGAG	
AD-1949177	csasguu(Uda)CfcAfAfUfcccauagasusa		VPusAfsucuAfuGfGfgauuGfgAfaacugscsa		UGCAGUUUCCAAUCCCAUAGAUG	
AD-1949178	ascscug(Gda)CfaCfAfAfuggcuaaususa		VPusAfsauuAfgCfCfauugUfgCfcaggususu		AAACCUGGCACAAUGGCUAAUUC	
AD-1949179	uscscaa(Gda)GfaCfCfAfgguauuususa		VPusAfsaaaUfaAfCfcuggUfcCfuuggasgsa		UCUCCAAGGACCAGGUUAUUUUA	
AD-1949180	cscsugg(Gda)AfaGfGfAfaaaugcaususa		VPusAfsaugCfaUfUfuuccUfuCfccaggsasu		AUCCUGGGAAGGAAAAUGCAUUG	
AD-1949181	asgsucc(Ada)AfgAfUfGfacaccaaasasa		VPusUfsuuuGfgUfGfucauCfuUfggacususu		AAAGUCCAAGAUGACACCAAAAC	
AD-1949182	uscscuc(Ada)CfaAfCfCfaccuaaucsasa		VPusUfsgauUfaGfGfuggaUfgUfgaggasusc		GAUCCUCACAACCACCUAAUCAG	
AD-1949183	asgscaa(Gda)GfcCfAfAfaauuaccasasa		VPusUfsuggUfaAfUfuuugGfcCfuugcususg		CAAGCAAGGCCAAAAUUACCAAA	
AD-1949184	csascug(Ada)AfuGfCfCfucaaugugsasa		VPusUfscacAfuUfGfaggcAfuUfcagugsasg		CUCACUGAAUGCCUCAAUGUGAC	
AD-1949185	ususaca(Uda)CfaCfAfGfuguuugcasasa		VPusUfsugcAfaAfCfacugUfgAfuguaascsa		UGUUACAUCACAGUGUUUGCAAU	
AD-1949186	uscscaa(Gda)GfaGfUfUfccaugaagsasa		VPusUfscuuCfaUfGfgaacUfcCfuuggasasu		AUUCCAAGGAGUUCCAUGAAGAC	
AD-1949187	asgsgau(Uda)GfaAfGfAfgcauugcasusa		VPusAfsugcAfaUfGfcucuUfcAfauccusgsg		CCAGGAUUGAAGAGCAUUGCAUG	
AD-1949188	asasggg(Ada)GfaAfGfGfaucuagaasusa		VPusAfsuucUfaGfAfuccuUfcUfcccuuscsu		AGAAGGGAGAAGGAUCUAGAAUG	
AD-1949189	usasuua(Ada)AfgGfAfGfuuaagagusasa		VPusUfsacuCfuUfAfacucCfuUfuaauasusc		GAUAUUAAAGGAGUUAAGAGUAG	
AD-1949190	gsusggu(Cda)UfuUfCfCfuaucauggsasa		VPusUfsccaUfgAfUfaggaAfaGfaccacsusc		GAGUGGUCUUUCCUAUCAUGGAG	
AD-1949191	uscsugc(Ada)UfuUfUfAfuuucggausgsa		VPusCfsaucCfaGfAfauaaAfaUfgcagasasu		AUUCUGCAUUUUAUUCUGGAUGG	
AD-1949192	uscscug(Cda)AfaGfGfAfcuacguuasasa		VPusUfsuaaCfgUfAfguccUfuGfcaggasasg		CUUCCUGCAAGGACUACGUUAAG	
AD-1949193	gsusucc(Cda)UfcUfGfAfgaauuccasasa		VPusUfsuggAfaUfUfcucaGfaGfggaacscsu		AGGUUCCCUCUGAGAAUUCCAAG	
AD-1949194	gsasagc(Uda)GfaUfGfCfuuugcuucsasa		VPusUfsgaaGfcAfAfagcaUfcAfgcuucsusc		GAGAAGCUGAUGCUUUGCUUCAA	
AD-1949195	asasaua(Cda)AfuAfAfGfaccauaacsasa		VPusUfsguuAfuGfGfucuuAfuGfuauuususc		GAAAAUACAUAAGACCAUAACAG	
AD-1949196	cscsuuu(Gda)GfaUfGfAfccagaacasasa		VPusUfsuguUfcUfGfgucaUfcCfaaaggscsu		AGCCUUUGGAUGACCAGAACAAG	
AD-1949197	gsgsaga(Uda)GfcAfGfAfgguaaaagsusa		VPusAfscuuUfuAfCfcucuGfcAfucuccsasc		GUGGAGAUGCAGAGGUAAAAGUG	
AD-1949198	ususggg(Uda)UfcAfUfCfugagcaagsasa		VPusUfscuuGfcUfCfagauGfaAfcccaascsc		GGUUGGGUUCAUCUGAGCAAGAG	
AD-1949199	gscsucu(Cda)CfaGfUfUfaguucucuscsa		VPusGfsagaGfaAfCfuaacUfgGfagagcscsa		UGGCUCUCCAGUUAGUUCUCUCG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949200	asgsugu(Gda)AfgCfAfGfugaguuacsasa		VPusUfsguaAfcUfCfacugCfuCfacacususu		AAAGUGUGAGCAGUGAGUUACAG	
AD-1949201	usascac(Ada)GfgAfUfCfcuauucucsasa		VPusUfsgagAfaUfAfggauCfcUfguguasusa		UAUACACAGGAUCCUAUUCUCAC	
AD-1949202	gsusgcg(Gda)AfuUfCfUfuguggcuusasa		VPusAfsaagCfcAfCfaagaAfuCfcgcacsasg		CUGUGCGGAUUCUUGUGGCUUUG	
AD-1949203	asuscag(Gda)CfuGfAfGfugucuuasasa		VPusUfsuaaGfaCfAfccucAfgCfcugaususa		UAAUCAGGCUGAGGUGUCUUAAG	
AD-1949204	ususcga(Gda)GfuAfGfAfguuugaagsgsa		VPusCfscuuCfaAfAfcucuAfcCfucgaasasc		GUUUCGAGGUAGAGUUUGAAGGA	
AD-1949205	uscscug(Ada)CfcUfUfUfAfuccaagausgsa		VPusCfsaucUfuGfGfauaaGfgUfcaggasusg		CAUCCUGACCUUAUCCAAGAUGG	
AD-1949206	cscsuug(Ada)AfgGfUfCfacucuuccsusa		VPusAfsggaAfgAfGfugacCfuUfcaaggscsc		GGCCUUGAAGGUCACUCUUCCUG	
AD-1949207	csusggc(Uda)UfuCfUfUfCfgacugcusasa		VPusUfsagcAfgUfCfggagAfaAfgccagsasg		CUCUGGCUUUCUCCGACUGCUAG	
AD-1949209	ususgca(Uda)UfcCfCfAfguggucaasasa		VPusUfsuugAfcCfAfcuggGfaAfugcaasgsa		UCUUGCAUUCCCAGUGGUCAAAC	
AD-1949210	csasgcu(Gda)AfaCfAfGfccaaaugcsasa		VPusUfsgcaUfuUfGfgcugUfuCfagcugscsu		AGCAGCUGAACAGCCAAAUGCAU	
AD-1949211	asusacc(Cda)AfgAfGfCfauuacgugsasa		VPusUfscacGfuAfAfugcuCfuGfgguauscsa		UGAUACCCAGAGCAUUACGUGAG	
AD-1949212	csusggc(Ada)GfuCfUfAfccaacagasusa		VPusAfsucuGfuUfGfguagAfcUfgccagsusg		CACUGGCAGUCUACCAACAGAUC	
AD-1949213	asgsagc(Uda)GfcUfCfUfggaaaaugsusa		VPusAfscauUfuUfCfcagaGfcAfgcucuscsa		UGAGAGCUGCUCUGGAAAAUGUG	
AD-1949214	gsasgca(Cda)CfuGfCfUfucaugcucsasa		VPusUfsgagCfaUfGfaagcAfgGfugcucsasg		CUGAGCACCUGCUUCAUGCUCAG	
AD-1949215	gsgsugg(Gda)AfaAfUfGfguaugagcsusa		VPusAfsgcuCfaUfAfccauUfuCfccaccsusg		CAGGUGGGAAAUGGUAUGAGCUG	
AD-1949216	gsusgug(Gda)UfgGfGfUfucuuuggasasa		VPusUfsuccAfaAfGfaaccCfaCfcacacsasa		UUGUGUGGUGGGUUCUUUGGAAG	
AD-1949217	gsusucu(Uda)GfuCfUfGfauuggcucsasa		VPusUfsgagCfcAfAfucagAfcAfagaacsusa		UAGUUCUUGUCUGAUUGGCUCAC	
AD-1949218	gsasuca(Cda)AfaGfGfUfcacuagausgsa		VPusCfsaucUfaGfUfgaccUfuGfugaucscsc		GGGAUCACAAGGUCACUAGAUGG	
AD-1949219	csusccg(Gda)GfaUfCfUfucuucacgsusa		VPusAfscguGfaAfGfaagaUfcCfcggagsgsu		ACCUCCGGGAUCUUCUUCACGUG	
AD-1949220	gsusccu(Gda)GfaAfGfCfuucaggcusasa		VPusUfsagcCfuGfAfagcuUfcCfaggacsasc		GUGUCCUGGAAGCUUCAGGCUAC	
AD-1949221	asusuuc(Cda)CfuGfAfCfuccucuaasgsa		VPusCfsuuaGfaGfGfagucAfgGfgaaaususg		CAAUUUCCCUGACUCCUCUAAGC	
AD-1949222	usgsacu(Gda)CfgAfUfCfuucagagcsasa		VPusUfsgcuCfuGfAfagauCfgCfagucascsc		GGUGACUGCGAUCUUCAGAGCAG	
AD-1949223	cscsagc(Uda)UfcUfCfCfaggcucuususa		VPusAfsaagAfgCfCfuggaGfaAfgcuggsusg		CACCAGCUUCUCCAGGCUCUUUG	
AD-1949224	uscsugg(Cda)UfuCfCfAfgguaucucscsa		VPusGfsgagAfuAfCfcuggAfaGfccagasgsu		ACUCUGGCUUCCAGGUAUCUCCA	
AD-1949225	cscsugg(Cda)CfaAfCfAfuggugaaascsa		VPusGfsuuuCfaCfCfauguUfgGfccaggsasu		AUCCUGGCCAACAUGGUGAAACC	
AD-1949226	gsusuuu(Uda)AfcUfCfCfaguggugasasa		VPusUfsucaCfcAfCfuggaGfuAfaaaacscsc		GGGUUUUUACUCCAGUGGUGAAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949227	asusgca(Uda)GfgUfGfCfaguugacasgsa		VPusCfsuguCfaAfCfugcaCfcAfugcaususu		AAAUGCAUGGUGCAGUUGACAGC	
AD-1949228	asgsagu(Ada)CfaGfUfGfagccaagasusa		VPusAfsucuUfgGfCfucacUfgUfacucuscsc		GGAGAGUACAGUGAGCCAAGAUC	
AD-1949229	asascac(Ada)AfaAfGfUfuagcugagscsa		VPusGfscucAfgCfUfaacuUfuUfguguususu		AAAACACAAAAGUUAGCUGAGCG	
AD-1949230	uscscca(Gda)CfaAfCfAfcaagcuggsasa		VPusUfsccaGfcUfUfguguUfgCfugggasgsu		ACUCCCAGCAACACAAGCUGGAA	
AD-1949231	asgsacc(Uda)GfaCfAfAfgcacugcusasa		VPusUfsagcAfgUfGfcuugUfcAfggucuscsa		UGAGACCUGACAAGCACUGCUAG	
AD-1949232	asusuuc(Ada)CfaCfAfCfgcagucagsusa		VPusAfscugAfcUfGfcgugUfgUfgaaausgsu		ACAUUUCACACACGCAGUCAGUC	
AD-1949233	csasggu(Ada)AfuGfAfGfggacuggasasa		VPusUfsuccAfgUfCfccucAfuUfaccugsgsc		GCCAGGUAAUGAGGGACUGGAAC	
AD-1949234	usgsagg(Gda)AfuGfUfGfaauugccusgsa		VPusCfsaggCfaAfUfucacAfuCfccucascsc		GGUGAGGGAUGUGAAUUGCCUGC	
AD-1949235	gsasucc(Uda)CfaCfCfAfguaugccususa		VPusAfsaggCfaUfAfcuggUfgAfggaucsusg		CAGAUCCUCACCAGUAUGCCUUC	
AD-1949236	csusggc(Cda)UfuCfUfCfuaagagcusgsa		VPusCfsagcUfcUfUfUfagagAfaGfgccagscsa		UGCUGGCCUUCUCUAAGAGCUGC	
AD-1949237	gsasuuc(Cda)CfaCfCfAfaggucuucsasa		VPusUfsgaaGfaCfCfuuggUfgGfgaaucscsc		GGGAUUCCCACCAAGGUCUUCAG	
AD-1949238	asusauc(Cda)AfaCfGfAfccuggagasasa		VPusUfsucuCfcAfGfgucgUfuGfgauaususu		AAAUAUCCAACGACCUGGAGAAC	
AD-1949239	csasugc(Uda)GfaAfGfGfgaccuugasasa		VPusUfsucaAfgGfUfcccuUfcAfgcaugsgsc		GCCAUGCUGAAGGGACCUUGAAG	
AD-1949240	usgsgug(Cda)UfaUfAfGfgcuggagasasa		VPusUfsucuCfcAfGfccuaUfaGfcaccasgsu		ACUGGUGCUAUAGGCUGGAGAAG	
AD-1949241	csasucc(Ada)CfaCfAfCfgcaggaacsusa		VPusAfsguuCfcUfGfcgugUfgUfggaugsusg		CACAUCCACACACGCAGGAACUC	
AD-1949242	ususgcc(Cda)UfgAfGfUfggaucuccsasa		VPusUfsggaGfaUfCfcacuCfaGfggcaascsa		UGUUGCCCUGAGUGGAUCUCCAA	
AD-1949243	csgscac(Uda)CfaCfCfCfauggccasasa		VPusUfsuggCfaCfAfugggUfgAfgugcgsgsu		ACCGCACUCACCCAUGUGCCAAG	
AD-1949244	gscsggu(Uda)GfcAfAfGfgcccaagasasa		VPusUfsucuUfgGfGfccuuGfcAfaccgcsusg		CAGCGGUUGCAAGGCCCAAGAAG	
AD-1949245	csusgga(Gda)AfgAfAfGfuuucuggcscsa		VPusGfsgccAfgAfAfAfacuuCfuCfuccagsgsg		CCCUGGAGAAGUUUCUUGGCCC	
AD-1949246	gscscug(Cda)AfgAfGfAfgaagccugsusa		VPusAfscagGfcUfUfUfcucuCfuGfcaggcsasa		UUGCCUGCAGAGAAGCCUGUU	
AD-1949247	cscsucu(Gda)AfaUfGfGfuccaggususa		VPusAfsaccCfuGfGfaccaUfuCfagaggsgsu		ACCCUCUGAAUGGUCCAGGGUUG	
AD-1949248	csasuua(Ada)GfaUfUfGfaggccugcscsa		VPusGfsgcaGfgCfCfucaaUfcUfuaaugsusu		AACAUUAAGAUUGAGGCCUGCCC	
AD-1949249	gsasgac(Cda)GfaGfCfGfcuuucuggsasa		VPusUfsccaGfaAfAfgcgcUfcGfgucucscsc		GGGAGACCGAGCGCUUUCUGGAA	
AD-1949250	asgscga(Gda)AfgGfCfAfgagaaagasasa		VPusUfsucuUfuCfUfcugcCfuCfucgcusgsu		ACAGCGAGAGGCAGAGAAAGAAG	
AD-1949251	usgscca(Cda)CfaUfCfCfugcugcugsusa		VPusAfscagCfaGfCfaggaUfgGfuggcasgsa		UCUGCCACCAUCCUGCUGCUGUG	
AD-1949252	csusgcc(Cda)UfcAfGfGfgaucuugcsasa		VPusUfsgcaAfgAfUfcccuGfaGfggcagsgsc		GCCUGCCCUCAGGGAUCUUGCAU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1949253	cscsaag(Cda)UfgUfGfCfccauccaasasa		VPusUfsuugGfaUfGfggcaCfaGfcuuggsasc		GUCCAAGCUGUGCCCAUCCAAAA	
AD-1949254	csascag(Gda)GfaAfCfCfcugcuugcsasa		VPusUfsgcaAfgCfAfggguUfcCfcugugsgsg		CCCACAGGGAACCCUGCUUGCAC	
AD-1949255	asusagc(Cda)CfaGfGfUfccucugausasa		VPusUfsaucAfgAfGfgaccUfgGfgcuausasg		CUAUAGCCCAGGUCCUCUGAUAC	
AD-1949256	gsuscuc(Uda)GfcAfGfGfacaugcugsusa		VPusAfscagCfaUfGfuccuGfcAfgagacscsc		GGGUCUCUGCAGGACAUGCUGUG	
AD-1949257	ascsggu(Cda)CfcAfCfAfcuggugacsusa		VPusAfsgucAfcCfAfguguGfgGfaccguscsa		UGACGGUCCCACACUGGUGACUG	
AD-1949258	asgsugg(Gda)CfuGfCfAfucugggaususa		VPusAfsaucCfcAfGfaugcAfgCfccacuscsu		AGAGUGGGCUGCAUCUGGGAUUC	
AD-1949259	cscsuuc(Cda)CfaCfUfGfgaggucacsasa		VPusUfsgugAfcCfUfccagUfgGfgaaggsgsc		GCCCUUCCCACUGGAGGUCACAU	
AD-1949260	ascsugc(Ada)CfuCfCfGfgccugaugsasa		VPusUfscauCfaGfGfccggAfgUfgcagusgsg		CCACUGCACUCCGGCCUGAUGAC	
AD-1949261	asgsugg(Cda)CfuGfGfAfgaccuuggsasa		VPusUfsccaAfgGfUfcuccAfgGfccacusgsg		CCAGUGGCCUGGAGACCUUGGAC	
AD-1949262	gsuscca(Cda)CfcAfGfCfaaagagugsgsa		VPusCfscacUfcUfUfugcuGfgGfuggacscsc		GGGUCCACCCAGCAAAGAGUGGG	
AD-1949263	gsgscua(Cda)UfcCfAfCfagagguggsusa		VPusAfsccaCfcUfCfugugGfaGfuagccsusg		CAGGCUACUCCACAGAGGUGGUG	
AD-1949264	usgsgca(Gda)GfaCfCfAfggacuauasgsa		VPusCfsuauAfgUfCfcuggUfcCfugccascsc		GGUGGCAGGACCAGGACUAUAGC	
AD-1949265	gsusuaa(Gda)GfgAfAfGfgaacucugsgsa		VPusCfscagAfgUfUfccuuCfcCfuuaacsgsu		ACGUUAAGGGAAGGAACUCUGGC	
AD-1949266	ascsagu(Gda)GfgUfGfGfuggaucugsusa		VPusAfscagAfuCfCfaccaCfcCfacugusgsu		ACACAGUGGGUGGUGGAUCUGUC	
AD-1949267	gsasgcu(Gda)GfcAfAfAfgguggcucsusa		VPusAfsgagCfcAfCfcuuuGfcCfagcucsusu		AAGAGCUGGCAAAGGUGGCUCUC	
AD-1949268	cscsacu(Cda)GfgGfAfGfgcugagacsasa		VPusUfsgucUfcAfGfccucCfcGfaguggscsu		AGCCACUCGGGAGGCUGAGACAG	

Table 30. Unmodified Sense and Antisense Strand Sequences of LEP dsRNA Agents

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3		Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3	
			Start Position	End Position			Start Position	End Position
AD-1945272	GCGGUUGCAAGGCCCAAGAAA		19	39	UUUCUUGGGCCUUGCAACCGCUG		17	39
AD-1945298	CCUGGGAAGGAAAAUGCAUUA		45	65	UAAUGCAUUUUCCUUCCCAGGAU		43	65
AD-1945308	GUGCGGAUUCUUGUGGCUUUA		75	95	UAAAGCCACAAGAAUCCGCACAG		73	95
AD-1945327	UGGCCCUAUCUUUUCUAUGUA		94	114	UACAUAGAAAAGAUAGGGCCAAA		92	114
AD-1945347	CCAAGCUGUGCCCAUCCAAAA		114	134	UUUUGGAUGGGCACAGCUUGGAC		112	134

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3		Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3	
			Start Position	End Position			Start Position	End Position
AD-1945372	AGUCCAAGAUGACACCAAAAA		135	155	UUUUUGGUGUCAUCUUGGACUUU		133	155
AD-1945386	CCUCAUCAAGACAAUUGUCAA		156	176	UUGACAAUUGUCUUGAUGAGGGU		154	176
AD-1945401	AUUUCACACACGCAGUCAGUA		190	210	UACUGACUGCGUGUGUGAAAUGU		188	210
AD-1945422	CUCCUCCAAACAGAAAGUCAA		210	230	UUGACUUUCUGUUUGGAGGAGAC		208	230
AD-1945439	UCACCGGUUUGGACUUCAUUA		227	247	UAAUGAAGUCCAAACCGGUGACU		225	247
AD-1945454	UCCUGACCUUAUCCCAAGAUGA		263	283	UCAUCUUGGAUAAGGUCAGGAUG		261	283
AD-1945483	CUGGCAGUCUACCAACAGAUA		292	312	UAUCUGUUGGUAGACUGCCAGUG		290	312
AD-1945500	GAUCCUCACCAGUAUGCCUUA		309	329	UAAGGCAUACUGGUGAGGAUCUG		307	329
AD-1945518	UUCCAGAAACGUGAUCCAAAA		327	347	UUUUGGAUCACGUUUCUGGAAGG		325	347
AD-1945537	AUAUCCAACGACCUGGAGAAA		346	366	UUUCUCCAGGUCGUUGGAUAUUU		344	366
AD-1945558	CUCCGGGAUCUUCUUCACGUA		367	387	UACGUGAAGAAGAUCCCGGAGGU		365	387
AD-1945579	CUGGCCUUCUCUAAGAGCUGA		388	408	UCAGCUCUUAGAGAAGGCCAGCA		386	408
AD-1945611	AGUGGCCUGGAGACCUUGGAA		424	444	UUCCAAGGUCUCCAGGCCACUGG		422	444
AD-1945624	GUCCUGGAAGCUUCAGGCUAA		457	477	UUAGCCUGAAGCUUCCAGGACAC		455	477
AD-1945639	GGCUACUCCCACAGAGGUGGUA		472	492	UACCACCUCUGUGGAGUAGCCUG		470	492
AD-1945658	GUCUCUGCAGGACAUGCUGUA		513	533	UACAGCAUGUCCUGCAGAGACCC		511	533
AD-1945708	CCUUGAAGGUCACUCUUCCUA		564	584	UAGGAAGAGUGACCUUCAAGGCC		562	584
AD-1945724	UCCUGCAAGGACUACGUUAAA		580	600	UUUAACGUAGUCCUUGCAGGAAG		578	600
AD-1945739	GUUAAGGGAAGGAACUCUGGA		595	615	UCCAGAGUUCCUUCCCUUAACGU		593	615
AD-1945754	UCUGGCUUCCAGGUAUCUCCA		610	630	UGGAGAUACCUGGAAGCCAGAGU		608	630
AD-1945774	AGGAUUGAAGAGCAUUGCAUA		630	650	UAUGCAAUGCUCUUCAAUCCUGG		628	650
AD-1945785	AUCCAGGACUCUGUCAAUUUA		661	681	UAAAUUGACAGAGUCCUGGAUAA		659	681
AD-1945801	AUUUCCCUGACUCCCUCUAAGA		677	697	UCUUAGAGGGAGUCAGGGAAAUUG		675	697
AD-1945823	ACUCUUCCAAAGGCAUAAGAA		699	719	UUCUUAUGCCUUUGGAAGAGUGG		697	719
AD-1945851	CUCCUUUUGCUUGAAACCAAA		727	747	UUUGGUUUCAAGCAAAAGGAGGC		725	747
AD-1945878	UACACAGGAUCCUAUUCUCAA		754	774	UUGAGAAUAGGAUCCUGUGUAUA		752	774

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3		Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3	
			Start Position	End Position			Start Position	End Position
AD-1945888	GUCCACCCAGCAAAGAGUGGA		785	805	UCCACUCUUUGCUGGGUGGACCC		783	805
AD-1945903	AGUGGGCUGCAUCUGGGAUUA		800	820	UAAUCCCAGAUGCAGCCCACUCU		798	820
AD-1945919	GAUUCCCACCAAGGUCUUUCAA		816	836	UUGAAGACCUUGGUGGGAAUCCC		814	836
AD-1945935	UUCAGCCAUCAACAAGAGUUA		832	852	UAACUCUUGUUGAUGGCUGAAGA		830	852
AD-1945947	CACUGAAUGCCUCAAUGUGAA		882	902	UUCACAUUGAGGCAUUCAGUGAG		880	902
AD-1945966	CCUUUGGAUGACCAGAACAAA		941	961	UUUGUUCUGGUCAUCCAAAGGCU		939	961
AD-1945987	GUUCCCUCUGAGAAUUCCAAA		962	982	UUUGGAAUUCUCAGAGGGAACCU		960	982
AD-1946002	UCCAAGGAGUUCCCAUGAAGAA		977	997	UUCUUCAUGGAACUCCUUGGAAU		975	997
AD-1946025	CAUCCACACACGCAGGAACUA		1000	1020	UAGUUCCUGCGUGUGUGGAUGUG		998	1020
AD-1946044	UCCCAGCAACACAAGCUGGAA		1019	1039	UUCCAGCUUGUGUUGCUGGGAGU		1017	1039
AD-1946060	UGGAAGCACAUGUUUAUUUAA		1035	1055	UUAAAUAAACAUGUGCUUCCAGC		1033	1055
AD-1946076	UCUGCAUUUUAUUCUGGAUGA		1056	1076	UCAUCCAGAAUAAAAUGCAGAAU		1054	1076
AD-1946092	GAUGGAUUUGAAGCAAAGCAA		1072	1092	UUGCUUUGCUUCAAAUCCAUCCA		1070	1092
AD-1946112	CCAGCUUCUCCAGGCUCUUUA		1092	1112	UAAAGAGCCUGGAGAAGCUGGUG		1090	1112
AD-1946129	CAGUUUCCAAUCCCAUAGAUA		1146	1166	UAUCUAUGGGAUUGGAAACUGCA		1144	1166
AD-1946159	GAGCUGAACCCAUUUUGAGUA		1176	1196	UACUCAAAAUGGGUUCAGCUCAG		1174	1196
AD-1946189	UUGGGUUCAUCUGAGCAAGAA		1206	1226	UUCUUGCUCAGAUGAACCCAACC		1204	1226
AD-1946207	GAGCUGGCAAAGGUGGCUCUA		1224	1244	UAGAGCCACCUUUGCCAGCUCUU		1222	1244
AD-1946222	GCUCUCCAGUUAGUUCUCUCA		1239	1259	UGAGAGAACUAACUGGAGAGCCA		1237	1259
AD-1946238	UCUCGUAACUGGUUUCAUUUA		1255	1275	UAAAUGAAACCAGUUACGAGAGA		1253	1275
AD-1946258	CUACUGUGACUGAUGUUACAA		1275	1295	UUGUAACAUCAGUCACAGUAGAA		1273	1295
AD-1946273	UUACAUCACAGUGUUUGCAAA		1290	1310	UUUGCAAACACUGUGAUGUAACA		1288	1310
AD-1946298	UUGCCCUGAGUGGAUCUCCAA		1315	1335	UUGGAGAUCCACUCAGGGCAACA		1313	1335
AD-1946314	UCCAAGGACCAGGUUAUUUUA		1331	1351	UAAAAUAACCUGGUCCUUGGAGA		1329	1351
AD-1946336	UUUGUCAAGUGUCAUAUGUAA		1363	1383	UUACAUAUGACACUUGACAAAAC		1361	1383
AD-1946366	AAGGGAGAAGGAUCUAGAAUA		1419	1439	UAUUCUAGAUCCUUCUCCCUUCU		1417	1439

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1946383	AAUGUGUUUUCUGAAUAACAA		1436	1456	UUGUUAUUCAGAAAACACAUUCU		1434	1456
AD-1946402	GUGUGGUGGGUUCUUUGGAAA		1459	1479	UUUCCAAAGAACCCACCACACAA		1457	1479
AD-1946422	GGAGUGAGAUCAUUUUCUUAA		1479	1499	UUAAGAAAAUGAUCUCACUCCUU		1477	1499
AD-1946438	UUAUCUUCUGCAAUUGCUUAA		1496	1516	UUAAGCAAUUGCAGAAGAUAAGA		1494	1516
AD-1946455	UUAGGAUGUUUUUCAUGAAAA		1513	1533	UUUUCAUGAAAAACAUCCUAAGC		1511	1533
AD-1946472	CUGGAGAGAAGUUUCUGGCCA		1575	1595	UGGCCAGAAACUUCUCUCCAGGG		1573	1595
AD-1946496	GAAGCUGAUGCUUUGCUUCAA		1619	1639	UUGAAGCAAAGCAUCAGCUUCUC		1617	1639
AD-1946511	CUUCAAAUCCAUCCAGAAUAA		1634	1654	UUAUUCUGGAUGGAUUUGAAGCA		1632	1654
AD-1946540	GGGCUGAAAGCCAUUUGUUGA		1663	1683	UCAACAAAUGGCUUUCAGCCCUU		1661	1683
AD-1946555	CUGGCUUUCUCCGACUGCUAA		1698	1718	UUAGCAGUCGGAGAAAGCCAGAG		1696	1718
AD-1946579	GUGGUCUUUCCUAUCAUGGAA		1722	1742	UUCCAUGAUAGGAAAGACCACUC		1720	1742
AD-1946602	ACGGUCCCACACUGGUGACUA		1745	1765	UAGUCACCAGUGUGGGACCGUCA		1743	1765
AD-1946617	UGACUGCGAUCUUCAGAGCAA		1760	1780	UUGCUCUGAAGAUCGCAGUCACC		1758	1780
AD-1946634	CCUCUGAAUGGUCCAGGGUUA		1797	1817	UAACCCUGGACCAUUCAGAGGGU		1795	1817
AD-1946654	GAUCACACUCUGGGUUUAUUA		1817	1837	UAAUAAACCCAGAGUGUGAUCAA		1815	1837
AD-1946675	CAUGGCAGUGUUCCUAUUUGA		1838	1858	UCAAAUAGGAACACUGCCAUGUA		1836	1858
AD-1946681	GCAUGCCAAAUUGUAGUUCUA		1864	1884	UAGAACUACAAUUUGGCAUGCAA		1862	1884
AD-1946696	GUUCUUGUCUGAUUGGCUCAA		1879	1899	UUGAGCCAAUCAGACAAGAACUA		1877	1899
AD-1946720	AGCAAGGCCAAAAUUACCAAA		1903	1923	UUUGGUAAUUUUGGCCUUGCUUG		1901	1923
AD-1946729	GUUUUUACUCCAGUGGUGAAA		1935	1955	UUUCACCACUGGAGUAAAAACCC		1933	1955
AD-1946745	UGAAGAAAACUCCUUUAGCAA		1951	1971	UUGCUAAAGGAGUUUUCUUCACC		1949	1971
AD-1946775	AGACCUGACAAGCACUGCUAA		1981	2001	UUAGCAGUGCUUGUCAGGUCUCA		1979	2001
AD-1946816	CCUUCCCACUGGAGGUCACAA		2042	2062	UUGUGACCUCCAGUGGGAAGGGC		2040	2062
AD-1946831	UCACAUUCAGGAAGAUGAAAA		2057	2077	UUUUCAUCUUCCUGAAUGUGACC		2055	2077
AD-1946845	UGCCACCAUCCUGCUGCUGUA		2093	2113	UACAGCAGCAGGAUGGUGGCAGA		2091	2113
AD-1946860	GCUGUGUUUUUGCUAUCACAA		2108	2128	UUGUGAUAGCAAAAACACAGCAG		2106	2128

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1946879	ACAGUGGGUGGUGGAUCUGUA		2127	2147	UACAGAUCCACCACCCACUGUGU		2125	2147
AD-1946895	CUGUCCAAGGAAACUUGAAUA		2143	2163	UAUUCAAGUUUCCUUGGACAGAU		2141	2163
AD-1946910	UGAAUCAAAGCAGUUAACUUA		2158	2178	UAAGUUAACUGCUUUGAUUCAAG		2156	2178
AD-1946937	GAGCACCUGCUUCAUGCUCAA		2185	2205	UUGAGCAUGAAGCAGGUGCUCAG		2183	2205
AD-1946965	UGGUGCUAUAGGCUGGAGAAA		2213	2233	UUUCUCCAGCCUAUAGCACCAGU		2211	2233
AD-1946984	AGCUCACCCAAUAAACAUUAA		2232	2252	UUAAUGUUUAUUGGGUGAGCUUC		2230	2252
AD-1946998	CAUUAAGAUUGAGGCCUGCCA		2247	2267	UGGCAGGCCUCAAUCUUAAUGUU		2245	2267
AD-1947013	CUGCCCUCAGGGAUCUUGCAA		2262	2282	UUGCAAGAUCCCUGAGGGCAGGC		2260	2282
AD-1947028	UUGCAUUCCCAGUGGUCAAAA		2277	2297	UUUUGACCACUGGGAAUGCAAGA		2275	2297
AD-1947049	CGCACUCACCCAUGUGCCAAA		2298	2318	UUUGGCACAUGGGUGAGUGCGGU		2296	2318
AD-1947068	CAGCUGAACAGCCAAAUGCAA		2337	2357	UUGCAUUUGGCUGUUCAGCUGCU		2335	2357
AD-1947083	AUGCAUGGUGCAGUUGACAGA		2352	2372	UCUGUCAACUGCACCAUGCAUUU		2350	2372
AD-1947105	GGUGGGAAAUGGUAUGAGCUA		2374	2394	UAGCUCAUACCAUUUCCCACCUG		2372	2394
AD-1947113	CACAGGGAACCCUGCUUGCAA		2417	2437	UUGCAAGCAGGGUUCCCUGUGGG		2415	2437
AD-1947129	UGCACUUUGUAACAUGUUUAA		2433	2453	UUAAACAUGUUACAAAGUGCAAG		2431	2453
AD-1947158	GCAUCUUAGCUUCUAUUAUAA		2462	2482	UUAUAAUAGAAGCUAAGAUGCCC		2460	2482
AD-1947186	CCUUUGAAACAAGAUAACUGA		2490	2510	UCAGUUAUCUUGUUUCAAAGGGA		2488	2510
AD-1947204	AAAUACAUAAGACCAUAACAA		2527	2547	UUGUUAUGGUCUUAUGUAUUUUC		2525	2547
AD-1947233	UGGCAGGACCAGGACUAUAGA		2556	2576	UCUAUAGUCCUGGUCCUGCCACC		2554	2576
AD-1947249	AUAGCCCAGGUCCUCUGAUAA		2572	2592	UUAUCAGAGGACCUGGGCUAUAG		2570	2592
AD-1947266	AUACCCAGAGCAUUACGUGAA		2589	2609	UUCACGUAAUGCUCUGGGUAUCA		2587	2609
AD-1947288	CAGGUAAUGAGGGACUGGAAA		2611	2631	UUUCCAGUCCCUCAUUACCUGGC		2609	2631
AD-1947313	GAGACCGAGCGCUUUCUGGAA		2636	2656	UUCCAGAAAGCGCUCGGUCUCCC		2634	2656
AD-1947328	CUGGAAAAGAGGAGUUUCGAA		2651	2671	UUCGAAACUCCUCUUUUCCAGAA		2649	2671
AD-1947343	UUCGAGGUAGAGUUUGAAGGA		2666	2686	UCCUUCAAACUCUACCUCGAAAC		2664	2686
AD-1947366	UGAGGGAUGUGAAUUGCCUGA		2689	2709	UCAGGCAAUUCACAUCCCUCACC		2687	2709

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_000230.3 Start Position	NM_000230.3 End Position
AD-1947381	GCCUGCAGAGAGAAGCCUGUA		2704	2724	UACAGGCUUCUCUCUGCAGGCAA		2702	2724
AD-1947396	CCUGUUUUGUUGGAAGGUUUA		2719	2739	UAAACCUUCCAACAAAACAGGCU		2717	2739
AD-1947423	GGAGAUGCAGAGGUAAAAGUA		2746	2766	UACUUUUACCUCUGCAUCUCCAC		2744	2766
AD-1947440	AGUGUGAGCAGUGAGUUACAA		2763	2783	UUGUAACUCACUGCUCACACUUU		2761	2783
AD-1947459	AGCGAGAGGCAGAGAAAGAAA		2782	2802	UUUCUUUCUCUGCCUCUCGCUGU		2780	2802
AD-1947489	CAUGCUGAAGGGACCUUGAAA		2821	2841	UUUCAAGGUCCCUUCAGCAUGGC		2819	2841
AD-1947507	AAGGGUAAAGAAGUUUGAUAA		2839	2859	UUAUCAAACUUCUUUACCCUUCA		2837	2859
AD-1947522	UAUUAAAGGAGUUAAGAGUAA		2857	2877	UUACUCUUAACUCCUUUAAUAUC		2855	2877
AD-1947537	GAGUAGCAAGUUCUAGAGAAA		2872	2892	UUUCUCUAGAACUUGCUACUCUU		2870	2892
AD-1947581	AGAGCUGCUCUGGAAAAUGUA		2916	2936	UACAUUUUCCAGAGCAGCUCUCA		2914	2936
AD-1947609	UCCUCACAACCACCUAAUCAA		2944	2964	UUGAUUAGGUGGUUGUGAGGAUC		2942	2964
AD-1947625	AUCAGGCUGAGGUGUCUUAAA		2960	2980	UUUAAGACACCUCAGCCUGAUUA		2958	2980
AD-1947643	AAGCCUUUUGCUCACAAAACA		2978	2998	UGUUUUGUGAGCAAAAGGCUUAA		2976	2998
AD-1947661	ACCUGGCACAAUGGCUAAUUA		2996	3016	UAAUUAGCCAUUGUGCCAGGUUU		2994	3016
AD-1947689	GUGAAACUUCCUAAGUAUAAA		3024	3044	UUUAUACUUAGGAAGUUUCACAC		3022	3044
AD-1947713	UUGUCUGUUUUUGUAACUUAA		3048	3068	UUAAGUUACAAAAACAGACAACC		3046	3068
AD-1947724	CCUGGCCAACAUGGUGAAACA		15292	15312	UGUUUCACCAUGUUGGCCAGGAU		15290	15312
AD-1947747	GAUCACAAGGUCACUAGAUGA		3145	3165	UCAUCUAGUGACCUUGUGAUCCC		3143	3165
AD-1947787	AACACAAAAGUUAGCUGAGCA		3208	3228	UGCUCAGCUAACUUUUGUGUUUU		3206	3228
AD-1947821	CCACUCGGGAGGCUGAGACAA		3254	3274	UUGUCUCAGCCUCCCGAGUGGCU		3252	3274
AD-1947838	ACAGGAGAAUCGCUUAAACCA		3271	3291	UGGUUUAAGCGAUUCUCCUGUCU		3269	3291
AD-1947868	AGAGUACAGUGAGCCAAGAUA		3301	3321	UAUCUUGGCUCACUGUACUCUCC		3299	3321
AD-1947891	ACUGCACUCCGGCCUGAUGAA		3327	3347	UUCAUCAGGCCGGAGUGCAGUGG		3325	3347
AD-1947913	GAGCGAGAUUCCGUCUUAAAA		3349	3369	UUUUAAGACGGAAUCUCGCUCUG		3347	3369

Table 31. Modified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1945272	gscsgguuGfcAfAfGfgcccaagaaaL96		VPusUfsucuUfgGfGfccuuGfcAfaccgcsusg		CAGCGGUUGCAAGGCCCAAGAAG	
AD-1945298	cscsugggAfaGfGfAfaaaugcauuaL96		VPusAfsaugCfaUfUfuuccUfuCfccaggsasu		AUCCUGGGAAGGAAAAUGCAUUG	
AD-1945308	gsusgcggAfuUfCfUfuguggcuuuaL96		VPusAfsaagCfcAfCfaagaAfuCfcgcacsasg		CUGUGCGGAUUCUUGUGGCUUUG	
AD-1945327	usgsgcccUfaUfCfUfuuucuauguaL96		VPusAfscauAfgAfAfaagaUfaGfggccasasa		UUUGGCCCUAUCUUUUCUAUGUC	
AD-1945347	cscsaagcUfgUfGfCfccauccaaaaL96		VPusUfsuugGfaUfGfggcaCfaGfcuuggsasc		GUCCAAGCUGUGCCCAUCCAAAA	
AD-1945372	asgsuccaAfgAfUfGfacaccaaaaaL96		VPusUfsuuuGfgUfGfucauCfuUfggacususu		AAAGUCCAAGAUGACACCAAAAC	
AD-1945386	cscsucauCfaAfGfAfcaauugucaaL96		VPusUfsgacAfaUfUfgucuUfgAfugaggsgsu		ACCCUCAUCAAGACAAUUGUCAC	
AD-1945401	asusuucaCfaCfAfCfgcagucaguaL96		VPusAfscugAfcUfGfcgugUfgUfgaaausgsu		ACAUUUCACACACGCAGUCAGUC	
AD-1945422	csusccucCfaAfAfCfagaaagucaaL96		VPusUfsgacUfuUfCfuguuUfgGfaggagsasc		GUCUCCUCCAAACAGAAAGUCAC	
AD-1945439	uscsaccgGfuUfUfGfgacuucauuaL96		VPusAfsaugAfaGfUfccaaAfcCfggugascsu		AGUCACCGGUUUGGACUUCAUUC	
AD-1945454	uscscugaCfcUfUfAfuccaagaugaL96		VPusCfsaucUfuGfGfauaaGfgUfcaggasusg		CAUCCUGACCUUAUCCAAGAUGG	
AD-1945483	csusggcaGfuCfUfAfccaacagauaL96		VPusAfsucuGfuUfGfguagAfcUfgccagsusg		CACUGGCAGUCUACCAACAGAUC	
AD-1945500	gsasuccuCfaCfCfAfguaugccuuaL96		VPusAfsaggCfaUfAfcuggUfgAfggaucsusg		CAGAUCCUCACCAGUAUGCCUUC	
AD-1945518	ususccagAfaAfCfGfugauccaaaaL96		VPusUfsuugGfaUfCfacguUfuCfuggaasgsg		CCUUCCAGAAACGUGAUCCAAAU	
AD-1945537	asusauccAfaCfGfAfccuggagaaaL96		VPusUfsucuCfcAfGfgucgUfuGfgauaususu		AAAUAUCCAACGACCUGGAGAAC	
AD-1945558	csusccggGfaUfCfUfucuucacguaL96		VPusAfscguGfaAfGfaagaUfcCfcggagsgsu		ACCUCCGGGAUCUUCUUCACGUG	
AD-1945579	csusggccUfuCfUfCfuaagagcugaL96		VPusCfsagcUfcUfUfagagAfaGfgccagscsa		UGCUGGCCUUCUCUAAGAGCUGC	
AD-1945611	asgsuggcCfuGfGfAfgaccuuggaaL96		VPusUfsccaAfgGfUfcuccAfgGfccacusgsg		CCAGUGGCCUGGAGACCUUGGAC	
AD-1945624	gsusccugGfaAfGfCfuucaggcuaaL96		VPusUfsagcCfuGfAfagcuUfcCfaggacsasc		GUGUCCUGGAAGCUUCAGGCUAC	
AD-1945639	gsgscuacUfcCfAfCfagaggugguaL96		VPusAfsccaCfcUfCfugugGfaGfuagccsusg		CAGGCUACUCCACAGAGGUGGUG	
AD-1945658	gsuscucuGfcAfGfGfacaugcuguaL96		VPusAfscagCfaUfGfuccuGfcAfgagacscsc		GGGUCUCUGCAGGACAUGCUGUG	
AD-1945708	cscsuugaAfgGfUfCfacucuuccuaL96		VPusAfsggaAfgAfGfugacCfuUfcaaggscsc		GGCCUUGAAGGUCACUCUUCCUG	
AD-1945724	uscscugcAfaGfGfAfcuacguuaaaL96		VPusUfsuaaCfgUfAfguccUfuGfcaggasasg		CUUCCUGCAAGGACUACGUUAAG	
AD-1945739	gsusuaagGfgAfAfGfgaacucuggaL96		VPusCfscagAfgUfUfccuuCfcCfuuaacsgsu		ACGUUAAGGGAAGGAACUCUGGC	
AD-1945754	uscsuggcUfuCfCfAfgguaucuccaL96		VPusGfsgagAfuAfCfcuggAfaGfccagasgsu		ACUCUGGCUUCCAGGUAUCUCCA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1945774	asgsgauuGfaAfGfAfgcauugcauaL96		VPusAfsugcAfaUfGfcucuUfcAfauccusgsg		CCAGGAUUGAAGAGCAUUGCAUG	
AD-1945785	asusccagGfaCfUfCfugucaauuuaL96		VPusAfsaauUfgAfCfagagUfcCfuggausasa		UUAUCCAGGACUCUGUCAAUUUC	
AD-1945801	asusuuccCfuGfAfCfuccucuaagaL96		VPusCfsuuaGfaGfGfagucAfgGfgaaaususg		CAAUUUCCCUGACUCCCUCUAAGC	
AD-1945823	ascsucuuCfcAfAfAfggcauaagaaL96		VPusUfscuuAfuGfCfcuuuGfgAfagagusgsg		CCACUCUUCCAAAGGCAUAAGAC	
AD-1945851	csusccuuUfuGfCfUfugaaaccaaaL96		VPusUfsuggUfuUfUfCfaagcAfaAfaggagsgsc		GCCUCCUUUUGCUUGAAACCAAA	
AD-1945878	usascacaGfgAfUfCfcuauucucaaL96		VPusUfsggaGfaUfAfggauCfcUfguguasusa		UAUACACAGGAUCCUAUUCUCAC	
AD-1945888	gsusccacCfcAfGfCfaaagaguggaL96		VPusCfscacUfcUfUfugcuGfgGfuggacscsc		GGGUCCACCCAGCAAAGAGUGGG	
AD-1945903	asgsugggCfuGfCfAfucugggauuaL96		VPusAfsaucCfcAfGfaugcAfgCfccacuscsu		AGAGUGGGCUGCAUCUGGGAUUC	
AD-1945919	gsasuuccCfaCfCfAfaggucuucaaL96		VPusUfsgaaGfaCfCfuuggUfgGfgaaucscsc		GGGAUUCCCACCAAGGUCUUCAG	
AD-1945935	ususcagcCfaUfCfCfAfacaagaguuaL96		VPusAfsacuCfuUfGfuugaUfgGfcugaasgsa		UCUUCAGCCAUCAACAAGAGUUG	
AD-1945947	csascugaAfuGfCfCfCfucaaugugaaL96		VPusUfscacAfuUfGfaggcAfuUfcagugsasg		CUCACUGAAUGCCUCAAUGUGAC	
AD-1945966	cscsuuugGfaUfGfAfccagaacaaaL96		VPusUfsuguUfcUfGfgucaUfcCfaaaggscsu		AGCCUUUGGAUGACCAGAACAAG	
AD-1945987	gsusucccUfcUfGfAfgaauuccaaaL96		VPusUfsuggAfaUfUfcucaGfaGfggaacscsu		AGGUUCCCUCUGAGAAUUCCAAG	
AD-1946002	uscscaagGfaGfUfUfccaugaagaaL96		VPusUfscuuCfaUfGfgaacUfcCfuuggasasu		AUUCCAAGGAGUUCCAUGAAGAC	
AD-1946025	csasuccaCfaCfAfCfgcaggaacuaL96		VPusAfsguuCfcUfGfcgugUfgUfggaugsusg		CACAUCCACACACGCAGGAACUC	
AD-1946044	uscsccagCfaAfCfAfcaagcuggaaL96		VPusUfsccaGfcUfUfguguUfgCfugggasgsu		ACUCCCAGCAACACAAGCUGGAA	
AD-1946060	usgsgaagCfaCfAfUfguuauuuaaaL96		VPusUfsaaaUfaAfAfcaugUfgCfuuccasgsc		GCUGGAAGCACAUGUUUAUUUAU	
AD-1946076	uscsugcaUfuUfUfAfuucuggaugaL96		VPusCfsaucCfaGfAfauaaAfaUfgcagasasu		AUUCUGCAUUUUAUUCUGGAUGG	
AD-1946092	gsasuggaUfuUfGfAfagcaaagcaaL96		VPusUfsgcuUfuGfCfuucaAfaUfccaucscsa		UGGAUGGAUUUGAAGCAAAGCAC	
AD-1946112	cscsagcuUfcUfCfCfCfaggcucuuuaL96		VPusAfsaagAfgCfCfuggaGfaAfgcuggsusg		CACCAGCUUCUCCCAGGCUCUUUG	
AD-1946129	csasguuuCfcAfAfUfCfccauagaauaL96		VPusAfsucuAfuGfGfgauuGfgAfaacugscsa		UGCAGUUUCCAAUCCCAUAGAUG	
AD-1946159	gsasgcugAfaCfCfCfCfauuuugaguaL96		VPusAfscucAfaAfAfuggguUfuCfagcucsasg		CUGAGCUGAACCCAUUUUUGAGUG	
AD-1946189	ususgggUfcAfUfCfUfugagcaagaaL96		VPusUfscuuGfcUfUfCfagauGfaAfcccaascsc		GGUUGGGUUCAUCUGAGCAAGAG	
AD-1946207	gsasgcugGfcAfAfAfgguggcucuaL96		VPusAfsgagCfcAfCfcuuuGfcCfagcucsusu		AAGAGCUGGCAAAGGUGGCUCUC	
AD-1946222	gscsucucCfaGfUfUfaguucucucaL96		VPusGfsagaGfaAfCfuaacUfgGfagagcscsa		UGGCUCUCUCCAGUUAGUUCUCUCG	
AD-1946238	uscsucguAfaCfUfCfguuucauuuaL96		VPusAfsaauGfaAfAfccagUfuAfcgagasgsa		UCUCUCGUAACUGGUUUCAUUUC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1946258	csusacugUfgAfCfUfgauguuacaaL96		VPusUfsguaAfcAfUfcaguCfaCfaguagsasa		UUCUACUGUGACUGAUGUUACAU	
AD-1946273	ususacauCfaCfAfGfuguuugcaaaL96		VPusUfsugcAfaAfCfacugUfgAfuguaascsa		UGUUACAUCACAGUGUUUGCAAU	
AD-1946298	ususgcccUfgAfGfUfggaucuccaaL96		VPusUfsggaGfaUfCfcacuCfaGfggcaascsa		UGUUGCCCUGAGUGGAUCUCCAA	
AD-1946314	uscscaagGfaCfCfAfgguuauuuuaL96		VPusAfsaaaUfaAfCfcuggUfcCfuuggasgsa		UCUCCAAGGACCAGGUUAUUUUA	
AD-1946336	ususugucAfaGfUfGfucauauguaaL96		VPusUfsacaUfaUfGfcacaCfuUfGfacaaasasc		GUUUUGUCAAGUGUCAUAUGUAG	
AD-1946366	asasggaGfaAfGfGfaucuagaauaL96		VPusAfsuucUfaGfAfuccuUfcUfcccuuscsu		AGAAGGGAGAAGGAUCUAGAAUG	
AD-1946383	asasugugUfuUfUfCfugaauaacaaL96		VPusUfsguuAfuUfCfagaaAfaCfacauuscsu		AGAAUGUGUUUUCUGAAUAACAU	
AD-1946402	gsusguggUfgGfGfUfucuuuggaaaL96		VPusUfsuccAfaAfGfGfaaccCfaCfcacacsasa		UUGUGUGGUGGGUUCUUUGGAAG	
AD-1946422	gsgsagugAfgAfUfCfauuuucuuaaL96		VPusUfsaagAfaAfAfGfuguuCfuCfacuccsusu		AAGGAGUGAGAUCAUUUUCUUAU	
AD-1946438	ususaucuUfcUfGfCfaauugcuuaaL96		VPusUfsaagCfaAfUfugcaGfaAfgauaasgsa		UCUUAUCUUCUGCAAUUGCUUAG	
AD-1946455	ususaggaUfgUfUfUfuucaugaaaaL96		VPusUfsuucAfuGfGfAfaaaaCfaUfccuaasgsc		GCUUAGGAUGUUUUUCAUGAAAA	
AD-1946472	csusggagAfgAfAfGfuuucuggccaL96		VPusGfsgccAfgAfAfacuuCfuCfuccagsgsg		CCCUGGAGAGAAGUUUCUGGCCC	
AD-1946496	gsasagcuGfaUfGfCfCfuuugcuucaaL96		VPusUfsgaaGfcAfAfagcaUfcAfgcuucsusc		GAGAAGCUGAUGCUUUGCUUCAA	
AD-1946511	csusucaaAfuCfCfAfuccagaauaaL96		VPusUfsauuCfuGfGfauggAfuUfugaagscsa		UGCUUCAAAUCCAUCCAGAAUAA	
AD-1946540	gsgsgcugAfaAfGfGfCfcauuuguugaL96		VPusCfsaacAfaAfUfUfggcuUfuCfagcccsusu		AAGGGCUGAAAGCCAUUUGUUGG	
AD-1946555	csusggcuUfuCfUfCfCfgacugcuaaL96		VPusUfsagcAfgUfUfCfggagAfaAfgccagsasg		CUCUGGCUUUCUCCGACUGCUAG	
AD-1946579	gsusggucUfuUfUfCfCfuaucauggaaL96		VPusUfsccaUfgAfUfaggaAfaGfaccacsusc		GAGUGGUCUUUCCUAUCAUGGAG	
AD-1946602	ascsggucCfcAfCfAfcuggugacuaL96		VPusAfsgucAfcCfAfguguGfgGfaccguscsa		UGACGGUCCCACACUGGUGACUG	
AD-1946617	usgsacugCfgAfUfCfCfuucagagcaaL96		VPusUfsgcuCfuGfAfagauCfgCfagucascsc		GGUGACUGCGAUCUUCAGAGCAG	
AD-1946634	cscsucugAfaUfUfGfGfuccaggguuaL96		VPusAfsaccCfuGfGfaccaUfuCfagagggsgsu		ACCCUCUGAAUGGUCCAGGGUUG	
AD-1946654	gsasucacAfcUfCfUfggguuauuaaL96		VPusAfsauaAfaCfCfCfcagaGfuGfgugaucsasa		UUGAUCACACUCUGGGUUUAUUA	
AD-1946675	csasuggcAfgUfGfUfuccuauuugaL96		VPusCfsaaaUfaGfGfGfaacaCfuGfccaugsusa		UACAUGGCAGUGUUCCUAUUUGG	
AD-1946681	gscsaugcCfaAfAfUfuguaguucuaL96		VPusAfsgaaCfuAfAfCfaauuUfgGfcaugcsasa		UUGCAUGCCAAAUUGUAGUUCUU	
AD-1946696	gsusucuuGfuCfUfGfauuggcucaaL96		VPusUfsggaGfcAfAfucagAfcAfagaacsusa		UAGUUCUUGUCUGAUUGGCUCAC	
AD-1946720	asgscaagGfcCfAfAfaauuaccaaaL96		VPusUfsuggUfaAfUfuuugGfcCfuugcususg		CAAGCAAGGCCAAAAUUACCAAA	
AD-1946729	gsusuuuuAfcUfCfCfaguggugaaaL96		VPusUfsucaCfcAfCfuggaGfuAfaaaacscsc		GGGUUUUUACUCCAGUGGUGAAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1946745	usgsaagaAfaAfCfUfccuuuagcaaL96		VPusUfsgcuAfaAfGfgaguUfuUfcuucascsc		GGUGAAGAAAACUCCUUUAGCAG	
AD-1946775	asgsaccuGfaCfAfAfgcacugcuaaL96		VPusUfsagcAfgUfGfcuugUfcAfggucuscsa		UGAGACCUGACAAGCACUGCUAG	
AD-1946816	cscsuuccCfaCfUfUfGfgaggucacaaL96		VPusUfsgugAfcCfUfccagUfgGfgaaggsgsc		GCCCUUCCCACUGGAGGUCACAU	
AD-1946831	uscsacauUfcAfGfGfGfaagaugaaaaL96		VPusUfsuucAfuCfUfuccuGfaAfugugascsc		GGUCACAUUCAGGAAGAUGAAAG	
AD-1946845	usgsccacCfaUfCfCfUfugcugcuguaL96		VPusAfscagCfaGfCfaggaUfgGfuggcasgsa		UCUGCCACCAUCCUGCUGCUGUG	
AD-1946860	gscsugugUfuUfUfUfGfcauucacaaL96		VPusUfsgugAfuAfGfcaaaAfaCfacagcsasg		CUGCUGUGUUUUUGCUAUCACAC	
AD-1946879	ascsaguGfgUfGfGfGfuggaucuguaL96		VPusAfscagAfuCfCfaccaCfcCfacugusgsu		ACACAGUGGGUGGGUGGAUCUGUC	
AD-1946895	csusguccAfaGfGfGfAfaacuugaauaL96		VPusAfsuucAfaGfUfUfuuccUfuGfgacagsasu		AUCUGUCCAAGGAAACUUGAAUC	
AD-1946910	usgsaaucAfaAfGfCfCfaguuaacuuaL96		VPusAfsaguUfaAfCfUfgcuUfuGfauucasasg		CUUGAAUCAAAGCAGUUAACUUU	
AD-1946937	gsasgcacCfuGfCfUfUfucaugcucaaL96		VPusUfsgagCfaUfGfaagcAfgGfugcucsasg		CUGAGCACCUGCUUCAUGCUCAG	
AD-1946965	usgsggugCfuAfUfAfGfgcuggagaaaL96		VPusUfsucuCfcAfGfccuaUfaGfcaccasgsu		ACUGGUGCUAUAGGCUGGAGAAG	
AD-1946984	asgscucaCfcCfAfAfUfuaaacauuaaL96		VPusUfsaauGfuUfUfauugGfgUfgagcususc		GAAGCUCACCCAAUAAACAUUAA	
AD-1946998	csasuuaaGfaUfUfUfGfaggccugccaL96		VPusGfsgcaGfgCfCfucaaUfcUfuaaugsusu		AACAUUAAGAUUGAGGCCUGCCC	
AD-1947013	csusgcccUfcAfGfGfGfgaucuugcaaL96		VPusUfsgcaAfgAfUfcccuGfaGfggcagsgsc		GCCUGCCCUCAGGGAUCUUGCAU	
AD-1947028	ususgcauUfcCfCfCfAfguggucaaaaL96		VPusUfsuugAfcCfAfcuggGfaAfugcaasgsa		UCUUGCAUUCCCAGUGGUCAAAC	
AD-1947049	csgscacuCfaCfCfCfUfaugugccaaaL96		VPusUfsuggCfaCfAfugggUfgAfgugcgsgsu		ACCGCACUCACCCAUGUGCCAAG	
AD-1947068	csasgcugAfaCfAfGfGfccaaaugcaaL96		VPusUfsgcaUfuUfUfGfgcugUfuCfagcugscsu		AGCAGCUGAACAGCCAAAUGCAU	
AD-1947083	asusgcauGfgUfGfCfCfaguugacagaL96		VPusCfsuguCfaAfCfUfgcaCfcAfugcaususu		AAAUGCAUGGUGCAGUUGACAGC	
AD-1947105	gsgsugggAfaAfUfUfGfguaugagcuaL96		VPusAfsgcuCfaUfAfccauUfuCfccaccsusg		CAGGUGGGAAAUGGUAUUGAGCUG	
AD-1947113	csascaggGfaAfCfCfCfcugcuugcaaL96		VPusUfsgcaAfgCfAfggguUfcCfcugugsgsg		CCCACAGGGAACCCUGCUUGCAC	
AD-1947129	usgscacuUfuGfUfUfAfacauguuuaaL96		VPusUfsaaaCfaUfGfuuacAfaAfgugcasasg		CUUGCACUUUGUAACAUGUUUAC	
AD-1947158	gscsaucuUfaGfCfUfUfucuauuauaaL96		VPusUfsauaAfuAfGfaagcUfaAfgaugcscsc		GGGCAUCUUAGCUUCUAUUAUAG	
AD-1947186	cscsuuugAfaAfCfAfAfagauaacugaL96		VPusCfsaguUfaUfUfCfuuguUfuCfaaaggsgsa		UCCCUUUGAAACAAGAUAAACUGA	
AD-1947204	asasauacAfuAfAfGfGfaccauaacaaL96		VPusUfsguuAfuGfGfucuuAfuGfuauuususc		GAAAAUACAUAAGACCAUAAACAG	
AD-1947233	usgsgcagGfaCfCfAfAfggacuauaagaL96		VPusCfsuauAfgUfUfCfcuggUfcCfugccascsc		GGUGGCAGGACCAGGACCAUAUAGC	
AD-1947249	asusagccCfaGfGfUfUfccucugauuaaL96		VPusUfsaucAfgAfGfGfgaccUfgGfgcuausasg		CUAUAGCCCAGGUCCUCUGAUAC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1947266	asusacccAfgAfGfCfauuacgugaaL96		VPusUfscacGfuAfAfugcuCfuGfgguauscsa		UGAUACCCAGAGCAUUACGUGAG	
AD-1947288	csasgguaAfuGfAfGfggacuggaaaL96		VPusUfsuccAfgUfCfccucAfuUfaccuggsgsc		GCCAGGUAAUGAGGGACUGGAAC	
AD-1947313	gsasgaccGfaGfCfGfcuuucuggaaL96		VPusUfsccaGfaAfAfgcgcUfcGfgucucscsc		GGGAGACCGAGCGCUUUCUGGAA	
AD-1947328	csusggaaAfaGfAfGfgaguuucgaaL96		VPusUfscgaAfaCfUfccucUfuUfuccagsasa		UUCUGGAAAAGAGGAGUUUCGAG	
AD-1947343	ususcgagGfuAfGfAfguugaaggaL96		VPusCfscuuCfaAfAfcucuAfcCfucgaasasc		GUUUCGAGGUAGAGUUUGAAGGA	
AD-1947366	usgsagggAfuGfUfGfaauugccugaL96		VPusCfsaggCfaAfUfucacAfuCfccucascsc		GGUGAGGGAUGUGAAUUGCCUGC	
AD-1947381	gscscugcAfgAfGfAfgaaccuguaL96		VPusAfscagGfcUfUfucucCfuGfcaggcsasa		UUGCCUGCAGAGAAGAAGCCUGUU	
AD-1947396	cscsuguuUfuGfUfUfUfggaagguuuaL96		VPusAfsaacCfuUfCfcaacAfaAfacaggscsu		AGCCUGUUUUGUUGGAAGGUUUG	
AD-1947423	gsgsagauGfcAfGfAfGfaguaaaaguaL96		VPusAfscuuUfuAfCfcucuGfcAfucuccsasc		GUGGAGAUGCAGAGGUAAAAGUG	
AD-1947440	asgsugugAfgCfAfGfugaguuacaaL96		VPusUfsguaAfcUfCfacugCfuCfacacususu		AAAGUGUGAGCAGUGAGUUACAG	
AD-1947459	asgscgagAfgGfCfAfgagaaagaaaL96		VPusUfsucuUfuCfUfcugcCfuCfucgcusgsu		ACAGCGAGAGGCAGAGAAAGAAG	
AD-1947489	csasugcuGfaAfGfGfgaccuugaaaL96		VPusUfsucaAfgGfUfcccuUfcAfgcaugsgsc		GCCAUGCUGAAGGGACCUUGAAG	
AD-1947507	asasgguAfaAfGfAfaguuugauaaL96		VPusUfsaucAfaAfCfuucuUfuAfcccuuscsa		UGAAGGGUAAAGAAGUUUGAUAU	
AD-1947522	usasuuaaAfgGfAfGfuuaagaguaaL96		VPusUfsacuCfuUfAfacucCfuUfuaauasusc		GAUAUUAAAGGAGUUAAGAGUAG	
AD-1947537	gsasguagCfaAfGfUfucuagagaaaL96		VPusUfsucuCfuAfGfaacuUfgCfuacucsusu		AAGAGUAGCAAGUUCUAGAGAAG	
AD-1947581	asgsagcuGfcUfCfUfggaaaauguaL96		VPusAfscauUfuUfCfcagaGfcAfgcucuscsa		UGAGAGCUGCUCUGGAAAAUGUG	
AD-1947609	uscscucaCfaAfCfCfcaccuaaucaaL96		VPusUfsgauUfaGfGfugguUfgUfgaggasusc		GAUCCUCACACCACCCUAAUCAG	
AD-1947625	asusgaggCfuGfAfGfgugcuuaaaL96		VPusUfsuaaGfaCfAfccucAfgCfcugaususa		UAAUCAGGCUGAGGUGUCUUAAG	
AD-1947643	asasgccuUfuUfGfCfcucacaaaacaL96		VPusGfsuuuUfgUfGfagcaAfaAfggcuusasa		UUAAGCCUUUUGCUCACAAAACC	
AD-1947661	ascscuggCfaCfAfAfuggcuaauuaL96		VPusAfsauuAfgCfCfauugUfgCfcaggususu		AAACCUGGCACAAUGGCUAAUUC	
AD-1947689	gsusgaaaCfuUfCfCfuaaguauaaaL96		VPusUfsuauAfcUfUfUfaggaAfgUfuucacsasc		GUGUGAAACUUCCUAAGUAUAAA	
AD-1947713	ususgucuGfuUfUfUfuguaacuuaaL96		VPusUfsaagUfuAfCfaaaaAfcAfgacaascsc		GGUUGUCUGUUUUUGUAACUUAA	
AD-1947724	cscsuggcCfaAfCfAfuggugaaacaL96		VPusGfsuuuCfaCfCfauguUfgGfccaggsasu		AGCCUGGCCAACAUGGUGAAACC	
AD-1947747	gsasucacAfaGfGfUfcacuagaugaL96		VPusCfsaucUfaGfUfgaccUfuGfugaucscsc		GGGAUCACAAGGUCACUAGAUGG	
AD-1947787	asascacaAfaAfGfUfuagcugagcaL96		VPusGfscucAfgCfUfaacuUfuUfgugususu		AAAACACAAAAGUUAGCUGAGCG	
AD-1947821	cscsacucGfgGfAfGfgcugagacaaL96		VPusUfsgucUfcAfGfccucCfcGfaguggscsu		AGCCACUCGGGAGGCUGAGACAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1947838	ascsaggaGfaAfUfCfgcuuaaaccaL96		VPusGfsguuUfaAfGfcgauUfcUfccuguscsu		AGACAGGAGAAUCGCUUAAACCU	
AD-1947868	asgsaguaCfaGfUfGfagccaagauaL96		VPusAfsucuUfgGfCfucacUfgUfacucuscsc		GGAGAGUACAGUGAGCCAAGAUC	
AD-1947891	ascsugcaCfuCfCfGfgccugaugaaL96		VPusUfscauCfaGfGfccggAfgUfgcagusgsg		CCACUGCACUCCGGCCUGAUGAC	
AD-1947913	gsasgcgaGfaUfUfCfcgucuuaaaaL96		VPusUfsuuaAfgAfCfggaaUfcUfcgcucsusg		CAGAGCGAGAUUCCGUCUUAAAA	

Table 32. Unmodified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_008493.3 Start Position	NM_008493.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_008493.3 Start Position	NM_008493.3 End Position
AD-1646889	CACCAGGAUCAAUGACAUUUA		176	196	UAAAUGUCAUUGAUCCUGGUGAC		174	196

Table 33. Modified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence 5' to 3'	SEQ ID NO.
AD-1646889	csasccagGfaUfCfAfaugacauuuaL96		VPusAfsaauGfuCfAfuugaUfcCfuggugsasc		GUCACCAGGAUCAAUGACAUUUC	

Table 34. Unmodified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO:	NM_008493.3 Start Position	NM_008493.3 End Position	Antisense Strand Sequence 5' to 3'	SEQ ID NO:	NM_008493.3 Start Position	NM_008493.3 End Position
AD-1888031	UUGAAGUGUAGUUUUAUACAA				UUGUAUAAAACUACACUUCAAGC			
AD-1888032	GUGACUGGUUUUGUUUCUAUA				UAUAGAAACAAAACCAGUCACCA			

Table 35. Modified Sense and Antisense Strand Sequences of LEP dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.
AD-1888031	ususgaa(Gda)UfgUfAfGfuuuuauacsasa		VPusUfsguaUfaAfAfacuaCfaCfuucaasgsc	
AD-1888032	gsusgac(Uda)GfgUfUfUfuguuucuasusa		VPusAfuagAfaAfCfaaaaCfcAfgucacscsa	

Example 15: *In vitro* Screening of dsRNA agents targeting Leptin

In vitro Dual-Luciferase and Endogenous Screening Assays

Hepa1-6 cells cells were transfected by adding 50 µL of siRNA duplexes and 75 ng of a plasmid, comprising partial sequences of human LEP (NM_000230.3), per well along with 100 µL of Opti-MEM plus 0.5 µL of Lipofectamine 2000 per well (Invitrogen, Carlsbad CA. cat # 13778-150) and then incubated at room temperature for 15 minutes. The mixture was then added to the cells which are re-suspended in 35 µL of fresh complete media. The transfected cells were incubated at 37°C in an atmosphere of 5% CO2. Single-dose experiments were performed at 10 nM.

Twenty-four hours after the siRNAs and pV205 plasmid are transfected; Firefly (transfection control) and Renilla (fused to LEP target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 75 µL of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 75 µL of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenches the firefly luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (LEP) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done with n=4.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human AGT, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the **ΔΔ**Ct method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: 5'-cuuAcGcuGAGuAcuucGAdTsdT-3' and antisense 5'-UCGAAGuACUcAGCGuAAGdTsdT-3'. The results of the dual-luciferase assays of the agents listed in Tables 30 and 31 are provided in Table 36.

Table 36. Single Dose Reporter Screen for Human LEP in Hepa1-6 Cells

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1947913.1	43.603	2.447
AD-1947891.1	58.640	1.210
AD-1947868.1	31.474	0.969
AD-1947838.1	46.521	2.454
AD-1947821.1	83.789	4.438
AD-1947787.1	36.331	1.427
AD-941419.1	47.050	2.614
AD-1947724.1	30.677	0.743
AD-1947747.1	34.652	1.368
AD-1947713.1	40.581	2.760
AD-1947689.1	43.628	1.151
AD-1947661.1	37.525	1.138
AD-1947643.1	38.039	3.247
AD-1947625.1	38.507	1.343
AD-1947609.1	72.801	1.141
AD-1947581.1	79.702	1.776
AD-1947537.1	37.364	2.772
AD-1947522.1	35.942	0.882
AD-1947507.1	36.951	0.626
AD-1947489.1	46.426	1.774
AD-1947459.1	81.536	2.751
AD-1947440.1	61.008	1.210

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1947423.1	53.159	1.375
AD-1947396.1	56.099	1.389
AD-1947381.1	61.241	3.458
AD-1947366.1	50.593	2.097
AD-1947343.1	42.641	1.768
AD-1947328.1	43.813	1.044
AD-1947313.1	40.401	2.279
AD-1947288.1	46.571	2.579
AD-1947266.1	46.348	0.904
AD-1947249.1	53.461	2.807
AD-1947233.1	81.878	3.064
AD-1947204.1	41.342	0.563
AD-1947186.1	35.394	1.249
AD-1947158.1	41.903	1.667
AD-1947129.1	45.153	1.855
AD-1947113.1	42.953	2.207
AD-1947105.1	52.166	2.202
AD-1947083.1	42.067	2.123
AD-1947068.1	41.200	0.885
AD-1947049.1	50.618	2.650
AD-1947028.1	51.813	2.991
AD-1947013.1	61.578	2.017
AD-1946998.1	60.723	0.755
AD-1946984.1	49.100	0.725
AD-1946965.1	47.300	1.205
AD-1946937.1	58.866	1.661
AD-1946910.1	31.610	1.426
AD-1946895.1	23.692	0.569
AD-1946879.1	65.108	2.337
AD-1946860.1	65.307	2.102
AD-1946845.1	79.606	0.818
AD-1946831.1	33.578	0.286
AD-1946816.1	72.449	3.972
AD-1946775.1	50.696	1.851
AD-1946745.1	54.226	2.705
AD-1946729.1	48.246	1.728
AD-1946720.1	60.826	1.540
AD-1946696.1	29.266	1.250
AD-1946681.1	38.420	1.814

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1946675.1	47.619	1.832
AD-1946654.1	39.292	1.833
AD-1946634.1	45.094	2.684
AD-1946617.1	38.242	1.563
AD-1946602.1	51.676	1.117
AD-1946579.1	41.965	3.569
AD-1946555.1	44.655	3.361
AD-1946540.1	50.290	2.364
AD-1946511.1	36.491	4.415
AD-1946496.1	35.628	0.542
AD-1946472.1	40.298	0.781
AD-1946455.1	15.510	0.230
AD-1946438.1	28.956	0.710
AD-1946422.1	19.287	0.467
AD-1946402.1	29.636	1.066
AD-1946383.1	8.690	0.152
AD-1946366.1	23.871	1.745
AD-1946336.1	6.380	0.297
AD-1946314.1	38.183	1.490
AD-1946298.1	35.136	0.953
AD-1946273.1	7.719	0.650
AD-1946258.1	6.801	0.428
AD-1946238.1	10.955	0.470
AD-1946222.1	13.814	1.065
AD-1946207.1	29.775	1.684
AD-1946189.1	21.264	1.258
AD-1946159.1	28.747	2.396
AD-1946129.1	32.971	1.969
AD-1946112.1	48.966	2.897
AD-1946092.1	23.863	1.513
AD-1946076.1	40.021	4.141
AD-1946060.1	14.895	0.784
AD-1946044.1	48.890	1.195
AD-1946025.1	37.091	0.805
AD-1946002.1	10.544	0.417
AD-1945987.1	16.883	0.930
AD-1945966.1	15.010	0.264
AD-1945947.1	7.818	0.374
AD-1945935.1	11.128	0.309

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message Remaining	SD
AD-1945919.1	16.097	0.745
AD-1945903.1	41.216	1.166
AD-1945888.1	80.942	4.615
AD-1945878.1	8.663	0.727
AD-1945851.1	12.559	1.161
AD-1945823.1	57.799	1.818
AD-1945801.1	9.824	0.799
AD-1945785.1	11.348	0.382
AD-1945774.1	9.080	0.648
AD-1945754.1	19.588	1.001
AD-1945739.1	19.742	1.110
AD-1945724.1	25.956	0.638
AD-1945708.1	76.824	4.360
AD-1945658.1	59.073	2.903
AD-1945639.1	33.564	1.280
AD-1945624.1	38.002	1.267
AD-1945611.1	82.959	2.823
AD-1945579.1	12.399	0.311
AD-1945558.1	40.552	2.467
AD-1945537.1	77.963	1.551
AD-1945518.1	9.156	0.630
AD-1945500.1	20.272	1.212
AD-1945483.1	15.547	0.946
AD-1945454.1	13.943	0.452
AD-1945439.1	10.560	0.408
AD-1945422.1	13.636	1.277
AD-1945401.1	11.282	0.322
AD-1646889.1	10.741	0.392
AD-1945386.1	9.855	0.659
AD-1945372.1	21.061	0.495
AD-1945347.1	15.416	0.808
AD-1945327.1	6.105	0.196
AD-1945308.1	17.009	0.767
AD-1945298.1	7.306	0.188
AD-1945272.1	89.310	1.201
Positive control (Rluc directed siRNA)	0.522	0.077

Example 16: dsRNA Synthesis

siRNAs targeting the human phospholamban (PLN) gene (human: GenBank NM_002667.5, NCBI GeneID: 5350) and the human calcium/calmodulin dependent protein kinase II delta (CAMK2D) gene (human: GenBank NM_001321571.2, NCBI GeneID: 817) were designed using custom R and Python scripts.

The human PLN REFSEQ NM_002667.5 mRNA has a length of 2989 bases.

The human CAMK2D REFSEQ NM_001321571.2 mRNA has a length of 5785 bases.

siRNAs comprising a GalNAc conjugate targeting ligand were designed and synthesized as described above. siRNAs comprising an unsaturated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, were designed and synthesized as described above.

Detailed lists of the modified PLN sense and antisense strand nucleotide sequences comprising an unsarurated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, are shown in Table 38, and the corresponding unmodified PLN sense and antisense nucleotide sequences are shown in Table 37.

Detailed lists of the modified PLN sense and antisense strand nucleotide sequences comprising a GalNAc conjugate targeting ligand are shown in Table 40, and the corresponding unmodified PLN sense and antisense nucleotide sequences are shown in Table 39.

Detailed lists of the modified CAMK2D sense and antisense strand nucleotide sequences comprising an unsarurated C_{22} hydrocarbon chain conjugated to the sense strand at position 6, counting from the 5'-end of the sense strand, are shown in Table 42, and the corresponding unmodified CAMK2D sense and antisense nucleotide sequences are shown in Table 41.

Detailed lists of the modified CAMK2D sense and antisense strand nucleotide sequences comprising a GalNAc conjugate targeting ligand are shown in Table 44, and the corresponding unmodified CAMK2D sense and antisense nucleotide sequences are shown in Table 43.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

Table 37. Unmodified Sense and Antisense Strand Sequences of PLN dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011169	CUAUUUUUCUUCCUCUAUCAU		1888	1908	AUGAUAGAGGAAGAAAAAUAGCU		1886	1908
AD-2011170	UAUCAAUUUCUGUCUCAUCUU		290	310	AAGAUGAGACAGAAAUUGAUAAA		288	310
AD-2011171	AACAUAAAAGUCUUCAUUCUU		1694	1714	AAGAAUGAAGACUUUUAUGUUGA		1692	1714
AD-2011172	UCUCAGUAAACAGAAAUAACU		2624	2644	AGUUAUUUCUGUUUACUGAGAAA		2622	2644
AD-2011173	AUCUUAAUAUGUCUCUUGCUU		306	326	AAGCAAGAGACAUAUUAAGAUGA		304	326
AD-2011174	CACAAAUUUCUAUCCCAAAUU		619	639	AAUUUGGGAUAGAAAUUUGUGAG		617	639
AD-2011175	CUUCCAAUAACUCAUAAAACU		2034	2054	AGUUUUAUGAGUUAUUGGAAGAU		2032	2054
AD-2011176	UAUCAGAAUCUACAUUCUAAU		890	910	AUUAGAAUGUAGAUUCUGAUAGU		888	910
AD-2011177	UAUGAAUUCUCUCUCCAAAUU		1368	1388	AAUUUGGAGAGAGAAUUCAUAUA		1366	1388
AD-2011178	UAACAACAGAAUCUAAUCUUU		1148	1168	AAAGAUUAGAUUCUGUUGUUAGU		1146	1168
AD-2011179	CCUCACAUCUGUUAUCUUAUU		847	867	AAUAAGAUAACAGAUGUGAGGAG		845	867
AD-2011180	CACUUAAAACUUCAGACUUCU		159	179	AGAAGUCUGAAGUUUUAAGUGGU		157	179
AD-2011181	CAUUCUCAUUGUCUUCACAUU		1708	1728	AAUGUGAAGACAAUGAGAAUGAA		1706	1728
AD-2011182	GACCAUUUCAGAACAUCUUCU		2018	2038	AGAAGAUGUUCUGAAAUGGUCAG		2016	2038
AD-2011183	CCUUACUGAUAACAUAAACAU		1538	1558	AUGUUUAUGUUAUCAGUAAGGUU		1536	1558
AD-2011184	UCUCUGAAGUUCUGCUACAAU		347	367	AUUGUAGCAGAACUUCAGAGAAG		345	367
AD-2011185	CUACAGAAUCUAUUUAUCAAU		276	296	AUUGAUAAAUAGAUUCUGUAGCU		274	296
AD-2011186	UAAGACUUCAGAAUGAUUUUU		1246	1266	AAAAAUCAUUCUGAAGUCUUAAG		1244	1266
AD-2011187	AAGUAUUUUUCAGGUCUUCAU		769	789	AUGAAGACCUGAAAAAUACUUAG		767	789
AD-2011188	ACUGUUGAUUUCCUCAACAUU		595	615	AAUGUUGAGGAAAUCAACAGUUG		593	615
AD-2011189	CCUAUACUGCAUAAUCCAACU		2148	2168	AGUUGGAUUAUGCAGUAUAGGUG		2146	2168
AD-2011190	CUGUUCUAAGACAUAUGAUCU		1069	1089	AGAUCAUAUGUCUUAGAACAGAU		1067	1089
AD-2011191	UCUACUAUAGAAUAAGUUCUU		2834	2854	AAGAACUUAUUCUAUAGUAGAAC		2832	2854
AD-2011192	AUGCUCAAAUAUGUUCUACUU		2820	2840	AAGUAGAACAUAUUUGAGCAUUU		2818	2840

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011193	UUCUAAAAACAUGGUUACUAAU		2245	2265	AUUAGUAACCAUGUUUUUAGAAGA		2243	2265
AD-2011194	GGAAAAUAUAUUCACCAAACU		2076	2096	AGUUUGGUGAAUAUAUUUUCCAG		2074	2096
AD-2011195	CAAAAUCUUAACUACCUAAUU		1498	1518	AAUUAGGUAGUUAAGAUUUUGCG		1496	1518
AD-2011196	UUUUCAAUUUCUCCUCUGACU		2001	2021	AGUCAGAGGAGAAAUUGAAAAUU		1999	2021
AD-2011197	UCUUUCUCUCGACCACUUAAU		146	166	AUUAAGUGGUCGAGAGAAAGAUA		144	166
AD-2011198	GCUUACCAUACUAUAUCUUUU		1211	1231	AAAAGAUAUAGUAUGGUAAGCUA		1209	1231
AD-2011199	UUCACCAAGUAUCAAAGUAAU		785	805	AUUACUUUGAUACUUGGUGAAGA		783	805
AD-2011200	CAUCAGCUUAAAAAUCUGUCAU		389	409	AUGACAGAUUUUAAGCUGAUGUG		387	409
AD-2011201	ACUAUUGACCAUAAACCUUAU		1523	1543	AUAAGGUUUAUGGUCAAUAGUAG		1521	1543
AD-2011202	CUAUAUUCCUACAAUAAAGUU		1598	1618	AACUUUAUUGUAGGAAUAUAGUG		1596	1618
AD-2011203	UACAAGCUGGAAAUUCCUAAU		2448	2468	AUUAGGAAUUUCCAGCUUGUAGA		2446	2468
AD-2011204	UACUGAGCUAAAUUAUAGAUU		1942	1962	AAUCUAUAAUUUAGCUCAGUAGA		1940	1962
AD-2011205	UGCCAACAAGUUCACUUCAUU		677	697	AAUGAAGUGAACUUGUUGGCAGU		675	697
AD-2011206	CUACCUAAAAGAAGACAGUUU		99	119	AAACUGUCUUCUUUUAGGUAGCC		97	119
AD-2011207	AGACAUGUUACUAAUAUAACU		2186	2206	AGUUAUAUUAGUAACAUGUCUUC		2184	2206
AD-2011208	UUCAUACAACACAAUACUCUU		52	72	AAGAGUAUUGUGUUGUAUGAAGU		50	72
AD-2011209	AUGCCACAUUAACAUCUUUUU		931	951	AAAAAGAUGUUAAUGUGGCAUAG		929	951
AD-2011210	CACUGGUUUUAGUAAAUUACU		2511	2531	AGUAAUUUACUAAAAACCAGUGAG		2509	2531
AD-2011211	CUCGCUCAGCUAUAAGAAGAU		217	237	AUCUUCUUAUAGCUGAGCGAGUG		215	237
AD-2011212	UCUUCAUUCUUUGAUAGAAAU		2656	2676	AUUUCUAUCAAAGAAUGAAGAAC		2654	2676
AD-2011213	CAAGAUUAAGACUAAAACUUU		482	502	AAAGUUUUAGUCUUAAUCUUGAC		480	502
AD-2011214	AAGCUAGAGAAAAUGUUAUUU		1618	1638	AAAUAACAUUUUUCUCUAGCUUAC		1616	1638
AD-2011215	AAACUUUGGUAAUUUUAAGUUU		2092	2112	AAACUUAAAAUUACCAAAGUUUUGG		2090	2112
AD-2011216	CAUAUCACUAAUAUACUAACU		1132	1152	AGUUAGUAUAUUAGUGAUAUGAC		1130	1152
AD-2011217	UUGCUGAUCUGUAUCAUCGUU		321	341	AACGAUGAUACAGAUCAGCAAGA		319	341
AD-2011218	AUCUGUUGGAUCUUGUAAACU		521	541	AGUUUACAAGAUCCAACAGAUGA		519	541

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011219	AUGUAGGUAAAAUCAUAAAAUCU		1050	1070	AGAUUUAUGAUUUACCUACAUGU		1048	1070
AD-2011220	CUGAUUAGUCAUAUUCCUUUUU		2578	2598	AAAAGGAAUAUGACUAAUCAGUU		2576	2598
AD-2011221	CAUCUAUUACAUCUACAGCUU		1432	1452	AAGCUGUAGAUGUAAUAGAUGGG		1430	1452
AD-2011222	AAAAUAGUUUACACCUAUACU		2135	2155	AGUAUAGGUGUAAACUAUUUUAG		2133	2155
AD-2011223	GGAGACACUAUUAAAUUUUCU		2329	2349	AGAAAAUUUAAUAGUGUCUCCUU		2327	2349
AD-2011224	AGUAGAAAGCUUAUAAACAAU		2473	2493	AUUGUUUAUAAGCUUUCUACUUG		2471	2493
AD-2011225	UCUUACACAAGUGUUGCUAAU		2297	2317	AUUAGCAACACUUGUGUAAGAAA		2295	2317
AD-2011226	AACUAUUUGUAGUAACUAUCU		874	894	AGAUAGUUACUACAAAUAGUUCU		872	894
AD-2011227	UUACUAAAAGAAUAUGUAACU		2258	2278	AGUUACAUAUUCUUUUAGUAACC		2256	2278
AD-2011228	UCAAAAUAGUCCACUGACUCU		828	848	AGAGUCAGUGGACUAUUUUGAAU		826	848
AD-2011229	ACAGGAAAACAAUAUUGUAUU		419	439	AAUACAAUAUUGUUUUCCUGUCU		417	439
AD-2011230	GUGUAUAAAAUGCAACUGUUU		581	601	AAACAGUUGCAUUUUAUACACUU		579	601
AD-2011231	CUAUACUGUGAUGAUCACAGU		70	90	ACUGUGAUCAUCACAGUAUAGAG		68	90
AD-2011232	GCCAUACUCUUACAUAAUAAU		984	1004	AUUAUUAUGUAAGAGUAUGGCCU		982	1004
AD-2011233	GAAUCACAGAAUUCUAGUACU		1030	1050	AGUACUAGAAUUCUGUGAUUCUU		1028	1050
AD-2011234	ACACAUAUUUUGCGUGUUAUU		1566	1586	AAUAACACGCAAAAUAUGUGUUA		1564	1586
AD-2011235	UUGAUUACACUGUUUGUUACU		2596	2616	AGUAACAAACAGUGUAAUCAAAG		2594	2616
AD-2011236	CCAGCUAUGCUAUUUAUAAUU		1962	1982	AAUUAUAAAUAGCAUAGCUGGAU		1960	1982
AD-2011237	CAACUGAAGUAAAAAUUGAGUU		2053	2073	AACUCAAUUUUACUUCAGUUGUU		2051	2073
AD-2011238	GUGUAAUUAACCAUAUCUUCU		2228	2248	AGAAGAUAUGGUUAAUUACACAU		2226	2248
AD-2011239	UCUAUCAACCAAAUGGUAAGU		1901	1921	ACUUACCAUUUGGUUGAUAGAGG		1899	1921
AD-2011240	UGAGGAUUACAGAAUACUAUU		2692	2712	AAUAGUAUUCUGUAAUCCUCACA		2690	2712
AD-2011241	GUGGAAAAUUUGAACUGAUUU		2564	2584	AAAUCAGUUCAAAUUUUCCACUU		2562	2584
AD-2011242	AUGGAGAAAGUCCAAUACCUU		195	215	AAGGUAUUGGACUUUCUCCCAUGA		193	215
AD-2011243	AAAUCAGAAUCACUAUAUUAU		2947	2967	AUAAUAUAGUGAUUCUGAUUUGC		2945	2967
AD-2011244	CUUCCAUUCCAGCCUAACAUU		1275	1295	AAUGUUAGGCUGGAAUGGAAGAC		1273	1295

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011245	AAUGAAGUGUCAUUAUUCAAU		812	832	AUUGAAUAAUGACACUUCAUUUG		810	832
AD-2011246	CUUCAUCAUAAAGUGUAAAGU		2754	2774	ACUUUACACUUUAUGAUGAAGAA		2752	2774
AD-2011247	CCAAAUCUUUUCUGAAGAUGU		633	653	ACAUCUUCAGAAAAGAUUUGGGA		631	653
AD-2011248	UUUGGAAUCAUGAAACCUUAU		1228	1248	AUAAGGUUUCAUGAUUCCAAAGA		1226	1248
AD-2011249	AAACACCCGUAAGACUUCAUU		37	57	AAUGAAGUCUUACGGGUGUUUAG		35	57
AD-2011250	AACUCAAUAGUGAAGGAGACU		2315	2335	AGUCUCCUUCACUAUUGAGUUAG		2313	2335
AD-2011251	UCUUCAUUUAAGGCACUGUAU		1164	1184	AUACAGUGCCUUAAAUGAAGAUU		1162	1184
AD-2011252	GGUCUUGCAGUCUUGUCUUAU		1773	1793	AUAAGACAAGACUGCAAGACCAA		1771	1793
AD-2011253	UAUCUGAGCUAGAGUUACCUU		1190	1210	AAGGUAACUCUAGCUCAGAUAAU		1188	1210
AD-2011254	AACUGUAAUAGGAUAUAGCUU		1870	1890	AAGCUAUAUCCUAUUACAGUUGA		1868	1890
AD-2011255	AUGAAAAGGGCUUUAUUUUCU		541	561	AGAAAAUAAAGCCCUUUUCAUGU		539	561
AD-2011256	CAGAAAGUAUCCCUAGUCUUU		2536	2556	AAAGACUAGGGAUACUUUCUGUA		2534	2556
AD-2011257	UGCCAGCUUUUUAUCUUUCUU		133	153	AAGAAAGAUAAAAAGCUGGCAGC		131	153
AD-2011258	CUUUUGAGGUGAAUAUAAUUU		718	738	AAAUUAUAUUCACCUCAAAAGAG		716	738
AD-2011259	UCAGGGAUAUCUGAAGAACAU		2395	2415	AUGUUCUUCAGAUAUCCCUGAAC		2393	2415
AD-2011260	CAACAAGCACGUCAAAAGCUU		258	278	AAGCUUUUGACGUGCUUGUUGAG		256	278
AD-2011261	AUUGUAUAACAGACCACUUCU		432	452	AGAAGUGGUCUGUUAUACAAUAU		430	452
AD-2011262	UUAAUGUUGCCUUUUAUAUUU		2894	2914	AAAUAUAAAAGGCAACAUUAAGC		2892	2914
AD-2011263	AUUGACCUUGGUUUCUUACAU		2284	2304	AUGUAAGAAACCAAGGUCAAUAU		2282	2304
AD-2011264	UAUGAUCAACAGAUGAGAACU		1082	1102	AGUUCUCAUCUGUUGAUCAUAUG		1080	1102
AD-2011265	GCAGAGAUUUCUUAAGUGACU		2420	2440	AGUCACUUAAGAAAUCUCUGCCC		2418	2440
AD-2011266	GAAGAGUUUCUUUGUGAAAAU		459	479	AUUUUCACAAAGAAACUCUUCUA		457	479
AD-2011267	CAACCAUUGAAAUGCCUCAAU		241	261	AUUGAGGCAUUUCAAUGGUUGAG		239	261
AD-2011268	AUCUAUUUUGCAGUCCACUCU		1922	1942	AGAGUGGACUGCAAAAUAGAUGC		1920	1942
AD-2011269	GUUACCAUAUGUAUUCAUCUU		505	525	AAGAUGAAUACAUAUGGUAACAA		503	525
AD-2011270	UUCUUAUCUUAAUUUACAGGU		2850	2870	ACCUGUAAAUUAAGAUAAGAACU		2848	2870

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011271	ACAGUUAUCUCAUAUUUGGCU		113	133	AGCCAAAUAUGAGAUAACUGUCU		111	133
AD-2011272	AACAAUUUUAAUUUCAGUUGU		2165	2185	ACAACUGAAAUUAAAAUUGUUGG		2163	2185
AD-2011273	GGCAAGGAAAAUAAAAGAUUU		1303	1323	AAAUCUUUUAUUUUCCUUGCCUG		1301	1323
AD-2011274	UCAUCGUGAUGCUUCUCUGAU		334	354	AUCAGAGAAGCAUCACGAUGAUA		332	354
AD-2011275	AAGAUGAAGAGAGUUUAGUUUUU		647	667	AAAAACUAAACUCUUCAUCUUCA		645	667
AD-2011276	CACUGUAGUGAAUUAUCUGAU		1177	1197	AUCAGAUAAUUCACUACAGUGCC		1175	1197
AD-2011277	CUACAACCUCUAGAUCUGCAU		361	381	AUGCAGAUCUAGAGGUUGUAGCA		359	381
AD-2011278	AUGAGAAUCAAGUAUGGAAAU		958	978	AUUUCCAUACUUGAUUCUCAUCA		956	978
AD-2011279	UUGUUCAAGGGUCAACUGUAU		1857	1877	AUACAGUUGACCCUUGAACAACA		1855	1877
AD-2011280	GAACCCAUGAGAGAUACUAGU		2350	2370	ACUAGUAUCUCUCAUGGGUUCAG		2348	2370
AD-2011281	AUGUGACAGUGAGAUUAGUCU		1113	1133	AGACUAAUCUCACUGUCACAUAU		1111	1133
AD-2011282	CUAUUCAUUAAAUGGAAGUGU		1670	1690	ACACUUCCAUUUAAUGAAUAGUA		1668	1690
AD-2011283	UCUUUUAAAGUUGAUGAGAAU		945	965	AUUCUCAUCAACUUUAAAAGAUG		943	965
AD-2011284	GGUGACAGAGUCAGAAAAACUU		9	29	AAGUUUUCUGACUCUGUCACCCA		7	29
AD-2011285	AAACAACAGGUGAUACACUCU		2487	2507	AGAGUGUAUCACCUGUUGUUUAU		2485	2507
AD-2011286	CCACUUCCUGAGUAGAAGAGU		445	465	ACUCUUCUACUCAGGAAGUGGUC		443	465
AD-2011287	AAAUCUUAUUCUGUGAGGAUU		2679	2699	AAUCCUCACAGAAUAAGAUUUUA		2677	2699
AD-2011288	AGUGGAAAGUGUUUGGUUCAU		2378	2398	AUGAACCAAACACUUUCCACUCC		2376	2398
AD-2011289	UUGUAUUUUUUCUAUGCCACU		918	938	AGUGGCAUAGAAAAAAUACAAUU		916	938
AD-2011290	GUGUUAUAUGUAUUAUACACU		1579	1599	AGUGUAUAAUACAUAUAACACGC		1577	1599
AD-2011291	AAAGAGUAGAGGAUGUGUAAU		2214	2234	AUUACACAUCCUCUACUCUUUUA		2212	2234
AD-2011292	AGUUUUAAAACUGCACUGCCU		661	681	AGGCAGUGCAGUUUUAAAACUAA		659	681
AD-2011293	AAGAUUUCCAGUGACAGAAAU		1317	1337	AUUUCUGUCACUGGAAAUCUUUU		1315	1337
AD-2011294	ACUGGUGGUUAAUAUGUGACU		1100	1120	AGUCACAUAUUAACCACCAGUUC		1098	1120
AD-2011295	UUGCAAUUCAAGCCCUUGUUU		1839	1859	AAACAAGGGCUUGAAUUGCAAGG		1837	1859
AD-2011296	AAGUGACGCCUCAUCUACAAU		2433	2453	AUUGUAGAUGAGGCGUCACUUAA		2431	2453

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2011297	AUUUUGCAGGUUGUCUUCCAU		1261	1281	AUGGAAGACAACCUGCAAAAUCA		1259	1281
AD-2011298	AUGUAACAUCAAUAUUGACCU		2271	2291	AGGUCAAUAUUGAUGUUACAUAU		2269	2291
AD-2011299	AAAGAAUAUUCAUGUAUAAGU		1812	1832	ACUUAUACAUGAAUAUUCUUUCC		1810	1832
AD-2011300	AAGUAAUAACACAAAUGAAGU		799	819	ACUUCAUUUGUGUUAUUACUUUG		797	819
AD-2011301	UACAGCUGACCCUUGAACAUU		1445	1465	AAUGUUCAAGGGUCAGCUGUAGA		1443	1465
AD-2011302	AUGGAAAAGUAAGGCCAUACU		971	991	AGUAUGGCCUUACUUUUCCAUAC		969	991
AD-2011303	GAACUUGUUGGCCCAUCUAUU		1419	1439	AAUAGAUGGGCCAACAAGUUCAU		1417	1439

Table 38. Modified Sense and Antisense Strand Sequences of PLN dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SE(ID NO
AD-2011169	csusauu(Uda)UfuCfUfUfccucuaucauL96		asUfsgauAfgAfGfgaagAfaAfaauagscsu		AGCUAUUUUUCUUCCUCUAUCAA	
AD-2011170	usasuca(Ada)UfuUfCfUfgucucaucuuL96		asAfsgauGfaGfAfcagaAfaUfugauasasa		UUUAUCAAUUUCUGUCUCAUCUU	
AD-2011171	asascau(Ada)AfaAfGfUfcuucauucuuL96		asAfsgaaUfgAfAfgacuUfuUfauguusgsa		UCAACAUAAAAGUCUUCAUUCUC	
AD-2011172	uscsuca(Gda)UfaAfAfCfagaaauaacuL96		asGfsuuaUfuUfCfuguuUfaCfugagasasa		UUUCUCAGUAAACAGAAAUAACU	
AD-2011173	asuscuu(Ada)AfuAfUfGfucucuugcuuL96		asAfsgcaAfgAfGfacauAfuUfaagausgsa		UCAUCUUAAUAUGUCUCUUGCUG	
AD-2011174	csascaa(Ada)UfuUfCfUfaucccaaauuL96		asAfsuuuGfgGfAfuagaAfaUfuugugsasg		CUCACAAAUUUCUAUCCCAAAUC	
AD-2011175	csususcc(Ada)AfuAfAfCfucauaaaacuL96		asGfsuuuUfaUfGfaguuAfuUfggaagsasu		AUCUUCCAAUAACUCAUAAAACA	
AD-2011176	usasuca(Gda)AfaUfCfUfacauucuaauL96		asUfsuagAfaUfGfuagaUfuCfugauasgsu		ACUAUCAGAAUCUACAUUCUAAA	
AD-2011177	usasuga(Ada)UfuCfUfCfucuccaaauuL96		asAfsuuuGfgAfGfagagAfaUfucauasusa		UAUAUGAAUUCUCUCUCCAAAUA	
AD-2011178	usasaca(Ada)CfaGfAfAfucuaaucuuuL96		asAfsagaUfuAfGfauucUfgUfuguuasgsu		ACUAACAACAGAAUCUAAUCUUC	
AD-2011179	cscsuca(Cda)AfuCfUfGfuuaucuuauuL96		asAfsuaaGfaUfAfacagAfuGfugaggsasg		CUCCUCACAUCUGUUAUCUUAUU	
AD-2011180	csascuu(Ada)AfaAfCfUfucagacuucuL96		asGfsaagUfcUfGfaaguUfuUfaagugsgsu		ACCACUUAAAACUUCAGACUUCC	
AD-2011181	csasuuc(Uda)CfaUfUfGfucuucacauuL96		asAfsuguGfaAfGfacaaUfgAfgaaugsasa		UUCAUUCUCAUUGUCUUCACAUU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO
AD-2011182	gsascca(Uda)UfuCfAfGfaacaucuucuL96		asGfsaagAfuGfUfucugAfaAfuggucsasg		CUGACCAUUUCAGAACAUCUUCC	
AD-2011183	cscsuua(Cda)UfgAfUfAfacauaaacauL96		asUfsguuUfaUfGfuuauCfaGfuaaggsusu		AACCUUACUGAUAACAUUAAACAG	
AD-2011184	uscsucu(Gda)AfaGfUfUfcugcuacaauL96		asUfsuguAfgCfAfgaacUfuCfagagasasg		CUUCUCUGAAGUUCUGCUACAAC	
AD-2011185	csusaca(Gda)AfaUfCfUfauuauucaauL96		asUfsugaUfaAfAfuagaUfuCfuguagscsu		AGCUACAGAAUCUAUUUAUCAAU	
AD-2011186	usasaga(Cda)UfuCfAfGfaaugauuuuuL96		asAfsaaaUfcAfUfucugAfaGfucuuasasg		CUUAAGACUUCAGAAUGAUUUUG	
AD-2011187	asasgua(Uda)UfuUfUfCfaggucuucauL96		asUfsgaaGfaCfCfugaaAfaAfuacuusasg		CUAAGUAUUUUUCAGGUCUUCAC	
AD-2011188	ascsugu(Uda)GfaUfUfUfccucaacauuL96		asAfsuguUfgAfGfgaaaUfcAfacagususg		CAACUGUUGAUUUCCUCAACAUG	
AD-2011189	cscsuau(Ada)CfuGfCfAfuaauccaacuL96		asGfsuugGfaUfUfaugcAfgUfauaggsusg		CACCUAUACUGCAUAAUCCAACA	
AD-2011190	csusguu(Cda)UfaAfGfAfcauaugaucuL96		asGfsaucAfuAfUfgucuUfaGfaacagsasu		AUCUGUUCUAAGACAUAUGAUCA	
AD-2011191	uscsuac(Uda)AfuAfGfAfauaaguucuuL96		asAfsgaaCfuUfAfuucuAfuAfguagasasc		GUUCUACUAUAGAAUAAGUUCUU	
AD-2011192	asusgcu(Cda)AfaAfUfAfuguucuacuuL96		asAfsguaGfaAfCfauauUfuGfagcaususu		AAAUGCUCAAAUAUGUUCUACUA	
AD-2011193	ususcua(Ada)AfaCfAfUfgguuacuaauL96		asUfsuagUfaAfCfcaugUfuUfuagaasgsa		UCUUCUAAAACAUGGUUACUAAA	
AD-2011194	gsgsaaa(Ada)UfaUfAfUfucaccaaacuL96		asGfsuuuGfgUfGfaauaUfaUfuuuccsasg		CUGGAAAAUAUAUUCACCAAACU	
AD-2011195	csasaaa(Uda)CfuUfAfAfcuaccuaauuL96		asAfsuuaGfgUfAfguuaAfgAfuuuugsgscsg		CGCAAAAUCUUAACUACCUAAUA	
AD-2011196	ususuuc(Ada)AfuUfUfCfuccucugacuL96		asGfsucaGfaGfGfagaaAfuUfgaaaasusu		AAUUUUCAAUUUCUCCUCUGACC	
AD-2011197	uscsuuu(Cda)UfcUfCfCfgfaccacuuaauL96		asUfsuaaGfuGfGfucgaGfaGfaaagasusa		UAUCUUUCUCCUCGACCACUUAAA	
AD-2011198	gscsuua(Cda)CfaUfAfCfuauaucuuuuL96		asAfsaagAfuAfUfaguaUfgGfuaagcsusa		UAGCUUACCAUACUAUAUCUUUG	
AD-2011199	ususcac(Cda)AfaGfUfUfAfucaaaguaauL96		asUfsuacUfuUfGfauacUfuGfgugaasgsa		UCUUCACCAAGUAUCAAAGUAAU	
AD-2011200	csasuca(Gda)CfuUfAfAfaaucugucauL96		asUfsgacAfgAfUfuuuaAfgCfugaugsusg		CACAUCAGCUUAAAAAUCUGUCAU	
AD-2011201	ascsuau(Uda)GfaCfCfCfAfuaaaccuuauL96		asUfsaagGfuUfUfauggUfcAfauagusasg		CUACUAUUGACCAUAAACCUUAC	
AD-2011202	csusaua(Uda)UfcCfUfAfcaauaaaguuL96		asAfscuuUfaUfUfguagGfaAfuauagsusg		CACUAUAUUCCUACAAUAAAGUA	
AD-2011203	usascaa(Gda)CfuGfGfAfaauuccuaauL96		asUfsuagGfaAfUfuuccAfgCfuuguasgsa		UCUACAAGCUGGAAAUUCCUAAA	
AD-2011204	usascug(Ada)GfcUfAfAfauuauagauuL96		asAfsucuAfuAfAfuuuaGfcUfcaguasgsa		UCUACUGAGCUAAAUUAUAGAUC	
AD-2011205	usgscca(Ada)CfaAfGfUfucacuucauuL96		asAfsugaAfgUfGfaacuUfgUfuggcasgsu		ACUGCCAACAAGUUCACUUCAUA	
AD-2011206	csusacc(Uda)AfaAfAfGfaagacaguuuL96		asAfsacuGfuCfUfucuuUfuAfgguagscsc		GGCUACCUAAAAGAAGACAGUUA	
AD-2011207	asgsaca(Uda)GfuUfAfCfuaauauaacuL96		asGfsuuaUfaUfUfaguaAfcAfugucususc		GAAGACAUGUUACUAAUAUAACU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO
AD-2011208	ususcau(Ada)CfaAfCfAfcaauacucuuL96		asAfsgagUfaUfUfguguUfgUfaugaasgsu		ACUUCAUACAACACAAUACUCUA	
AD-2011209	asusgcc(Ada)CfaUfUfAfacaucuuuuuL96		asAfsaaaGfaUfGfuuaaUfgUfggcausasg		CUAUGCCACAUUAACAUCUUUUA	
AD-2011210	csascug(Gda)UfuUfUfAfguaaauuacuL96		asGfsuaaUfuUfAfcuaaAfaCfcagugsasg		CUCACUGGUUUUAGUAAAUUACC	
AD-2011211	csuscgc(Uda)CfaGfCfUfauaagaagauL96		asUfscuuCfuUfAfuagcUfgAfgcgagsusg		CACUCGCUCAGCUAUAAGAAGAG	
AD-2011212	uscsuuc(Ada)UfuCfUfUfugauagaaauL96		asUfsuucUfaUfCfaaagAfaUfgaagasasc		GUUCUUCAUUCUUUGAUAGAAAU	
AD-2011213	csasaga(Uda)UfaAfGfAfcuaaaacuuuL96		asAfsaguUfuUfAfgucuUfaAfucuugsasc		GUCAAGAUUAAGACUAAAACUUA	
AD-2011214	asasgcu(Ada)GfaGfAfAfaauguuauuuL96		asAfsauaAfcAfUfuuucUfcUfagcuusasc		GUAAGCUAGAGAAAAUGUUAUUU	
AD-2011215	asasacu(Uda)UfgGfUfAfauuuaaguuuL96		asAfsacuUfaAfAfuuacCfaAfaguuusgsg		CCAAACUUUGGUAAUUUAAGUUG	
AD-2011216	csasuau(Cda)AfcUfAfAfuauacuaacuL96		asGfsuuaGfuAfUfauuaGfuGfauaugsasc		GUCAUAUCACUAAUAUACUAACA	
AD-2011217	ususgcu(Gda)AfuCfUfGfuaucaucguuL96		asAfscgaUfgAfUfacagAfuCfagcaasgsa		UCUUGCUGAUCUGUAUCAUCGUG	
AD-2011218	asuscug(Uda)UfgGfAfUfcuuguaaacuL96		asGfsuuuAfcAfAfgaucCfaAfcagausgsa		UCAUCUGUUGGAUCUUGUAAACA	
AD-2011219	asusgua(Gda)GfuAfAfAfucauaaaucuL96		asGfsauuUfaUfGfauuuAfcCfuacausgsu		ACAUGUAGGUAAAUCAUAAAUCU	
AD-2011220	csusgau(Uda)AfgUfCfAfuauuccuuuuL96		asAfsaagGfaAfUfaugaCfuAfaucagsusu		AACUGAUUAGUCAUAUUCCUUUG	
AD-2011221	csasucu(Ada)UfuAfCfAfucuacagcuuL96		asAfsgcuGfuAfGfauguAfaUfagaugsgsg		CCCAUCUAUUACAUCUACAGCUG	
AD-2011222	asasaau(Ada)GfuUfUfAfcaccuauacuL96		asGfsuauAfgGfUfguaaAfcUfauuuusasg		CUAAAAUAGUUUACACCUAUACU	
AD-2011223	gsgsaga(Cda)AfcUfAfUfuaaauuuucuL96		asGfsaaaAfuUfUfaauaGfuGfucuccsusu		AAGGAGACACUAUUAAAUUUUCU	
AD-2011224	asgsuag(Ada)AfaGfCfUfuauaaacaauL96		asUfsuguUfuAfUfaagcUfuUfcuacususg		CAAGUAGAAAGCUUAUAAACAAC	
AD-2011225	uscsuua(Cda)AfcAfAfGfuguugcuaauL96		asUfsuagCfaAfCfacuuGfuGfuaagasasa		UUUCUUACACAAGUGUUGCUAAC	
AD-2011226	asascua(Uda)UfuGfUfAfguaacuaucuL96		asGfsauaGfuUfAfcuacAfaAfuaguuscsu		AGAACUAUUUGUAGUAACUAUCA	
AD-2011227	ususacu(Ada)AfaAfGfAfauauguaacuL96		asGfsuuaCfaUfAfuucuUfuUfaguaascsc		GGUUACUAAAAGAAUAUGUAACA	
AD-2011228	uscsaaa(Ada)UfaGfUfCfcacugacucuL96		asGfsaguCfaGfUfggacUfaUfuuugasasu		AUUCAAAAUAGUCCACUGACUCC	
AD-2011229	ascsagg(Ada)AfaAfCfAfauauuguauuL96		asAfsuacAfaUfAfuuguUfuUfccuguscsu		AGACAGGAAAACAAUAUUGUAUA	
AD-2011230	gsusgua(Uda)AfaAfAfUfgcaacuguuuL96		asAfsacaGfuUfGfcauuUfuAfuacacsusu		AAGUGUAUAAAAUGCAACUGUUG	
AD-2011231	csusaua(Cda)UfgUfGfAfugaucacaguL96		asCfsuguGfaUfCfaucaCfaGfuauagsasg		CUCUAUACUGUGAUGAUCACAGC	
AD-2011232	gscscau(Ada)CfuCfUfUfacauaauaauL96		asUfsuauUfaUfGfuaagAfgUfauggcscsu		AGGCCAUACUCUUACAUAAUAAA	
AD-2011233	gsasauc(Ada)CfaGfAfAfuucuaguacuL96		asGfsuacUfaGfAfauucUfgUfgauucsusu		AAGAAUCACAGAAUUCUAGUACA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO
AD-2011234	ascsaca(Uda)AfuUfUfUfgcguguuauuL96		asAfsuaaCfaCfGfcaaaAfuAfugugususa		UAACACAUAUUUUGCGUGUUAUA	
AD-2011235	ususgau(Uda)AfcAfCfUfguuuguuacuL96		asGfsuaaCfaAfAfcaguGfuAfaucaasasg		CUUUGAUUACACUGUUUGUUACA	
AD-2011236	cscsagc(Uda)AfuGfCfUfauuuauaauuL96		asAfsuuaUfaAfAfuagcAfuAfgcuggsasu		AUCCAGCUAUGCUAUUUAUAAUU	
AD-2011237	csasacu(Gda)AfaGfUfAfaaauugaguuL96		asAfscucAfaUfUfuuacUfuCfaguugsusu		AACAACUGAAGUAAAAUUGAGUG	
AD-2011238	gsusgua(Ada)UfuAfAfCfcauaucuucuL96		asGfsaagAfuAfUfgguuAfaUfuacacsasu		AUGUGUAAUUAACCAUAUCUUCU	
AD-2011239	uscsuau(Cda)AfaCfCfAfaaugguaaguL96		asCfsuuaCfcAfUfuuggUfuGfauagasgsg		CCUCUAUCAACCAAAUGGUAAGC	
AD-2011240	usgsagg(Ada)UfuAfCfAfgaauacuauuL96		asAfsuagUfaUfUfcuguAfaUfccucascsa		UGUGAGGAUUACAGAAUACUAUA	
AD-2011241	gsusgga(Ada)AfaUfUfUfgaacugauuuL96		asAfsaucAfgUfUfcaaaUfuUfuccacsusu		AAGUGGAAAAUUUGAACUGAUUA	
AD-2011242	asusgga(Gda)AfaAfGfUfccaauaccuuL96		asAfsgguAfuUfGfgacuUfuCfuccausgsa		UCAUGGAGAAAGUCCAAUACCUC	
AD-2011243	asasauc(Ada)GfaAfUfCfacuauauuauL96		asUfsaauAfuAfGfugauUfcUfgauuusgsc		GCAAAUCAGAAUCACUAUAUUAA	
AD-2011244	csusucc(Ada)UfuCfCfAfgccuaacauuL96		asAfsuguUfaGfGfcuggAfaUfggaagsasc		GUCUUCCAUUCCAGCCUAACAUC	
AD-2011245	asasuga(Ada)GfuGfUfCfauuauucaauL96		asUfsugaAfuAfAfugacAfcUfucauususg		CAAAUGAAGUGUCAUUAUUCAAA	
AD-2011246	csusuca(Uda)CfaUfAfAfaguguaaaguL96		asCfsuuuAfcAfCfuuuaUfgAfugaagsasa		UUCUUCAUCAUAAAGUGUAAAGA	
AD-2011247	cscsaaa(Uda)CfuUfUfUfcugaagauguL96		asCfsaucUfuCfAfgaaaAfgAfuuuggsgsa		UCCCAAAUCUUUUCUGAAGAUGA	
AD-2011248	ususugg(Ada)AfuCfAfUfgaaaccuuauL96		asUfsaagGfuUfUfcaugAfuUfccaaasgsa		UCUUUGGAAUCAUGAAACCUUAA	
AD-2011249	asasaca(Cda)CfcGfUfAfagacuucauuL96		asAfsugaAfgUfCfuuacGfgGfuguuusasg		CUAAACACCCGUAAGACUUCAUA	
AD-2011250	asascuc(Ada)AfuAfGfUfgaaggagacuL96		asGfsucuCfcUfUfcacuAfuUfgaguusasg		CUAACUCAAUAGUGAAGGAGACA	
AD-2011251	uscsuuc(Ada)UfuUfAfAfggcacuguauL96		asUfsacaGfuGfCfcuuaAfaUfgaagasusu		AAUCUUCAUUUAAGGCACUGUAG	
AD-2011252	gsgsucu(Uda)GfcAfGfUfcuugucuuauL96		asUfsaagAfcAfAfgacuGfcAfagaccsasa		UUGGUCUUGCAGUCUUGUCUUAG	
AD-2011253	usasucu(Gda)AfgCfUfAfgaguuaccuuL96		asAfsgguAfaCfUfcuagCfuCfagauasasu		AUUAUCUGAGCUAGAGUUACCUA	
AD-2011254	asascug(Uda)AfaUfAfGfgauauagcuuL96		asAfsgcuAfuAfUfccuaUfuAfcaguusgsa		UCAACUGUAAUAGGAUAUAGCUA	
AD-2011255	asusgaa(Ada)AfgGfGfCfuuuauuuucuL96		asGfsaaaAfuAfAfagccCfuUfuucausgsu		ACAUGAAAAGGGCUUUAUUUUCA	
AD-2011256	csasgaa(Ada)GfuAfUfCfccuagucuuuL96		asAfsagaCfuAfGfggauAfcUfuucugsusa		UACAGAAAGUAUCCCUAGUCUUA	
AD-2011257	usgscca(Gda)CfuUfUfUfuaucuuucuuL96		asAfsgaaAfgAfUfaaaaAfgCfuggcasgsc		GCUGCCAGCUUUUUAUCUUUCUC	
AD-2011258	csusuuu(Gda)AfgGfUfGfaauauaauuuL96		asAfsauuAfuAfUfucacCfuCfaaaagsasg		CUCUUUUGAGGUGAAUAUAAUUU	
AD-2011259	uscsagg(Gda)AfuAfUfCfugaagaacauL96		asUfsguuCfuUfCfagauAfuCfccugasasc		GUUCAGGGAUAUCUGAAGAACAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO
AD-2011260	csasaca(Ada)GfcAfCfGfucaaaagcuuL96		asAfsgcuUfuUfGfacguGfcUfuguugsasg		CUCAACAAGCACGUCAAAAGCUA	
AD-2011261	asusugu(Ada)UfaAfCfAfgaccacuucuL96		asGfsaagUfgGfUfcuguUfaUfacaausasu		AUAUUGUAUAACAGACCACUUCC	
AD-2011262	ususaau(Gda)UfuGfCfCfuuuuauauuuL96		asAfsauaUfaAfAfaggcAfaCfauuaasgsc		GCUUAAUGUUGCCUUUUAUAUUU	
AD-2011263	asusuga(Cda)CfuUfGfGfuuucuuacauL96		asUfsguaAfgAfAfaccaAfgGfucaausasu		AUAUUGACCUUGGUUUCUUACAC	
AD-2011264	usasuga(Uda)CfaAfCfAfgaugagaacuL96		asGfsuucUfcAfUfcuguUfgAfucauasusg		CAUAUGAUCAACAGAUGAGAACU	
AD-2011265	gscsaga(Gda)AfuUfUfCfuuaagugacuL96		asGfsucaCfuUfAfagaaAfuCfucugcscsc		GGGCAGAGAUUUCUUAAGUGACG	
AD-2011266	gsasaga(Gda)UfuUfCfUfuugugaaaauL96		asUfsuuuCfaCfAfaagaAfaCfucuucsusa		UAGAAGAGUUUCUUUGUGAAAAG	
AD-2011267	csasacc(Ada)UfuGfAfAfaugccucaauL96		asUfsugaGfgCfAfuuucAfaUfgguugsasg		CUCAACCAUUGAAAUGCCUCAAC	
AD-2011268	asuscua(Uda)UfuUfGfCfaguccacucuL96		asGfsaguGfgAfCfugcaAfaAfuagausgsc		GCAUCUAUUUUGCAGUCCACUCU	
AD-2011269	gsusuac(Cda)AfuAfUfGfuauucaucuuL96		asAfsgauGfaAfUfacauAfuGfguaacsasa		UUGUUACCAUAUGUAUUCAUCUG	
AD-2011270	ususcuu(Ada)UfcUfUfAfauuuacagguL96		asCfscugUfaAfAfuuaaGfaUfaagaascsu		AGUUCUUAUCUUAAUUUACAGGG	
AD-2011271	ascsagu(Uda)AfuCfUfCfauauuuggcuL96		asGfsccaAfaUfAfugagAfuAfacuguscsu		AGACAGUUAUCUCAUAUUUGGCU	
AD-2011272	asascaa(Uda)UfuUfAfAfuuucaguuguL96		asCfsaacUfgAfAfauuaAfaAfuuguusgsg		CCAACAAUUUUAAUUUCAGUUGA	
AD-2011273	gsgscaa(Gda)GfaAfAfAfuaaaagauuuL96		asAfsaucUfuUfUfauuuUfcCfuugccsusg		CAGGCAAGGAAAAUAAAAGAUUU	
AD-2011274	uscsauc(Gda)UfgAfUfGfcuucucugauL96		asUfscagAfgAfAfgcauCfaCfgaugasusa		UAUCAUCGUGAUGCUUCUCUGAA	
AD-2011275	asasgau(Gda)AfaGfAfGfuuuaguuuuuL96		asAfsaaaCfuAfAfacucUfuCfaucuuscsa		UGAAGAUGAAGAGUUUAGUUUUA	
AD-2011276	csascug(Uda)AfgUfGfAfauuaucugauL96		asUfscagAfuAfAfuucaCfuAfcagugscsc		GGCACUGUAGUGAAUUAUCUGAG	
AD-2011277	csusaca(Ada)CfcUfCfUfagaucugcauL96		asUfsgcaGfaUfCfuagaGfgUfuguagscsa		UGCUACAACCUCUAGAUCUGCAG	
AD-2011278	asusgag(Ada)AfuCfAfAfguauggaaauL96		asUfsuucCfaUfAfcuugAfuUfcucauscsa		UGAUGAGAAUCAAGUAUGGAAAA	
AD-2011279	ususguu(Cda)AfaGfGfGfucaacuguauL96		asUfsacaGfuUfGfacccUfuGfaacaascsa		UGUUGUUCAAGGGUCAACUGUAA	
AD-2011280	gsasacc(Cda)AfuGfAfGfagauacuaguL96		asCfsuagUfaUfCfucucAfuGfgguucsasg		CUGAACCCAUGAGAGAUACUAGA	
AD-2011281	asusgug(Ada)CfaGfUfGfagauuagucuL96		asGfsacuAfaUfCfucacUfgUfcacausasu		AUAUGUGACAGUGAGAUUAGUCA	
AD-2011282	csusauu(Cda)AfuUfAfAfauggaaguguL96		asCfsacuUfcCfAfuuuaAfuGfaauagsusa		UACUAUUCAUUAAAUGGAAGUGG	
AD-2011283	uscsuuu(Uda)AfaAfGfUfugaugagaauL96		asUfsucuCfaUfCfaacuUfuAfaaagasusg		CAUCUUUUAAAGUUGAUGAGAAU	
AD-2011284	gsgsuga(Cda)AfgAfGfUfcagaaaacuuL96		asAfsguuUfuCfUfgacuCfuGfucaccscsa		UGGGUGACAGAGUCAGAAAACUC	
AD-2011285	asasaca(Ada)CfaGfGfUfgauacacucuL96		asGfsaguGfuAfUfcaccUfgUfuguuusasu		AUAAACAACAGGUGAUACACUCA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO
AD-2011286	cscsacu(Uda)CfcUfGfAfguagaagaguL96		asCfsucuUfcUfAfcucaGfgAfaguggsusc		GACCACUUCCUGAGUAGAAGAGU	
AD-2011287	asasauc(Uda)UfaUfUfCfugugaggauuL96		asAfsuccUfcAfCfagaaUfaAfgauuususa		UAAAAUCUUAUUCUGUGAGGAUU	
AD-2011288	asgsugg(Ada)AfaGfUfGfuuugguucauL96		asUfsgaaCfcAfAfacacUfuUfccacuscsc		GGAGUGGAAAGUGUUUGGUUCAG	
AD-2011289	ususgua(Uda)UfuUfUfUfcuaugccacuL96		asGfsuggCfaUfAfgaaaAfaAfuacaasusu		AAUUGUAUUUUUUCUAUGCCACA	
AD-2011290	gsusguu(Ada)UfaUfGfUfauuauacacuL96		asGfsuguAfuAfAfuacaUfaUfaacacsgsc		GCGUGUUAUAUGUAUUAUACACU	
AD-2011291	asasaga(Gda)UfaGfAfGfgauguguaauL96		asUfsuacAfcAfUfccucUfaCfucuuususa		UAAAAGAGUAGAGGAUGUGUAAU	
AD-2011292	asgsuuu(Uda)AfaAfAfCfugcacugccuL96		asGfsgcaGfuGfCfaguuUfuAfaaacusasa		UUAGUUUUAAAACUGCACUGCCA	
AD-2011293	asasgau(Uda)UfcCfAfGfugacagaaauL96		asUfsuucUfgUfCfacugGfaAfaucuususu		AAAAGAUUUCCAGUGACAGAAAA	
AD-2011294	ascsugg(Uda)GfgUfUfAfauaugugacuL96		asGfsucaCfaUfAfuuaaCfcAfccagususc		GAACUGGUGGUUAAUAUGUGACA	
AD-2011295	ususgca(Ada)UfuCfAfAfgcccuuguuuL96		asAfsacaAfgGfGfcuugAfaUfugcaasgsg		CCUUGCAAUUCAAGCCCUUGUUG	
AD-2011296	asasgug(Ada)CfgCfCfUfcaucuacaauL96		asUfsuguAfgAfUfgaggCfgUfcacuusasa		UUAAGUGACGCCUCAUCUACAAG	
AD-2011297	asususu(Gda)CfaGfGfUfugucuuccauL96		asUfsggaAfgAfCfaaccUfgCfaaaauscsa		UGAUUUUGCAGGUUGUCUUCCAU	
AD-2011298	asusgua(Ada)CfaUfCfAfauauugaccuL96		asGfsgucAfaUfAfuugaUfgUfuacausasu		AUAUGUAACAUCAAUAUUGACCU	
AD-2011299	asasaga(Ada)UfaUfUfCfauguauaaguL96		asCfsuuaUfaCfAfugaaUfaUfucuuuscsc		GGAAAGAAUAUUCAUGUAUAAGU	
AD-2011300	asasgua(Ada)UfaAfCfAfcaaaugaaguL96		asCfsuucAfuUfUfguguUfaUfuacuususg		CAAAGUAAUAACACAAAUGAAGU	
AD-2011301	usascag(Cda)UfgAfCfCfcuugaacauuL96		asAfsuguUfcAfAfggguCfaGfcuguasgsa		UCUACAGCUGACCCUUGAACAUG	
AD-2011302	asusgga(Ada)AfaGfUfAfaggccauacuL96		asGfsuauGfgCfCfuuacUfuUfuccausasc		GUAUGGAAAAGUAAGGCCAUACU	
AD-2011303	gsasacu(Uda)GfuUfGfGfcccaucuauuL96		asAfsuagAfuGfGfgccaAfcAfaguucsasu		AUGAACUUGUUGGCCCAUCUAUU	

Table 39. Unmodified Sense and Antisense Strand Sequences of PLN dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2004072	GGUGACAGAGUCAGAAAACUU		9	29	AAGUUUUCUGACUCUGUCACCCA		7	29
AD-2004080	AAACACCCGUAAGACUUCAUU		37	57	AAUGAAGUCUUACGGGUGUUUAG		35	57
AD-2004095	UUCAUACAACACAAUACUCUU		52	72	AAGAGUAUUGUGUUGUAUGAAGU		50	72

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2004113	CUAUACUGUGAUGAUCACAGU		70	90	ACUGUGAUCAUCACAGUAUAGAG		68	90
AD-2004142	CUACCUAAAAGAAGACAGUUU		99	119	AAACUGUCUUCUUUUAGGUAGCC		97	119
AD-2004156	ACAGUUAUCUCAUAUUUGGCU		113	133	AGCCAAAUAUGAGAUAACUGUCU		111	133
AD-2004176	UGCCAGCUUUUUAUCUUUCUU		133	153	AAGAAAGAUAAAAAGCUGGCAGC		131	153
AD-2004189	UCUUUCUCUCGACCACUUAAU		146	166	AUUAAGUGGUCGAGAGAAAGAUA		144	166
AD-2004202	CACUUAAAACUUCAGACUUCU		159	179	AGAAGUCUGAAGUUUUAAGUGGU		157	179
AD-2004238	AUGGAGAAAGUCCAAUACCUU		195	215	AAGGUAUUGGACUUUCUCCAUGA		193	215
AD-2004260	CUCGCUCAGCUAUAAGAAGAU		217	237	AUCUUCUUAUAGCUGAGCGAGUG		215	237
AD-2004284	CAACCAUUGAAAUGCCUCAAU		241	261	AUUGAGGCAUUUCAAUGGUUGAG		239	261
AD-2004301	CAACAAGCACGUCAAAAGCUU		258	278	AAGCUUUUGACGUGCUUGUUGAG		256	278
AD-2004319	CUACAGAAUCUAUUUAUCAAU		276	296	AUUGAUAAAUAGAUUCUGUAGCU		274	296
AD-2004329	UAUCAAUUUCUGUCUCAUCUU		290	310	AAGAUGAGACAGAAAUUGAUAAA		288	310
AD-2004345	AUCUUAAUAUGUCUCUUGCUU		306	326	AAGCAAGAGACAUAUUAAGAUGA		304	326
AD-2004360	UUGCUGAUCUGUAUCAUCGUU		321	341	AACGAUGAUACAGAUCAGCAAGA		319	341
AD-2004373	UCAUCGUGAUGCUUCUCUGAU		334	354	AUCAGAGAAGCAUCACGAUGAUA		332	354
AD-2004386	UCUCUGAAGUUCUGCUACAAU		347	367	AUUGUAGCAGAACUUCAGAGAAG		345	367
AD-2004400	CUACAACCUCUAGAUCUGCAU		361	381	AUGCAGAUCUAGAGGUUGUAGCA		359	381
AD-2004428	CAUCAGCUUAAAAUCUGUCAU		389	409	AUGACAGAUUUUAAGCUGAUGUG		387	409
AD-2004458	ACAGGAAAACAAUAUUGUAUU		419	439	AAUACAAUAUUGUUUUCCUGUCU		417	439
AD-2004468	AUUGUAUAACAGACCACUUCU		432	452	AGAAGUGGUCUGUUAUACAAUAU		430	452
AD-2004481	CCACUUCCUGAGUAGAAGAGU		445	465	ACUCUUCUACUCAGGAAGUGGUC		443	465
AD-2004495	GAAGAGUUUCUUUGUGAAAAU		459	479	AUUUUCACAAAGAAACUCUUCUA		457	479
AD-2004518	CAAGAUUAAGACUAAAACUUU		482	502	AAAGUUUUAGUCUUAAUCUUGAC		480	502
AD-2004539	GUUACCAUAUGUAUUCAUCUU		505	525	AAGAUGAAUACAUAUGGUAACAA		503	525
AD-2004555	AUCUGUUGGAUCUUGUAAACU		521	541	AGUUUACAAGAUCCAACAGAUGA		519	541
AD-2004575	AUGAAAAGGGCUUUAUUUUCU		541	561	AGAAAAUAAAGCCCUUUUCAUGU		539	561

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2004594	GUGUAUAAAAAUGCAACUGUUU		581	601	AAACAGUUGCAUUUUUAUACACUU		579	601
AD-2004608	ACUGUUGAUUUCCUCAACAUU		595	615	AAUGUUGAGGAAAUCAACAGUUG		593	615
AD-2004632	CACAAAUUUCUAUCCCAAAUU		619	639	AAUUUGGGAUAGAAAAUUUGUGAG		617	639
AD-2004646	CCAAAUCUUUUUCUGAAGAUGU		633	653	ACAUCUUCAGAAAAGAUUUGGGA		631	653
AD-2004660	AAGAUGAAGAGUUUAGUUUUU		647	667	AAAAACUAAACUCUUCAUCUUCA		645	667
AD-2004673	AGUUUUAAAAACUGCACUGCCU		661	681	AGGCAGUGCAGUUUUAAAACUAA		659	681
AD-2004689	UGCCAACAAGUUCACUUCAUU		677	697	AAUGAAGUGAACUUGUUGGCAGU		675	697
AD-2004718	CUUUUGAGGUGAAUAUAAUUU		718	738	AAAUUAUAUUUCACCUCAAAAGAG		716	738
AD-2004747	AAGUAUUUUUUCAGGUCUUCAU		769	789	AUGAAGACCUGAAAAAAUACUUAG		767	789
AD-2004763	UUCACCAAGUAUCAAAGUAAU		785	805	AUUACUUUGAUACUUGGUGAAGA		783	805
AD-2004777	AAGUAAUAACACAAAUGAAGU		799	819	ACUUCAUUUGUGUUAUUACUUUG		797	819
AD-2004790	AAUGAAGUGUCAUUAUUCAAU		812	832	AUUGAAUAAUGACACUUCAUUUG		810	832
AD-2004806	UCAAAAUAGUCCACUGACUCU		828	848	AGAGUCAGUGGACUAUUUUGAAU		826	848
AD-2004825	CCUCACAUCUGUUAUCUUAUU		847	867	AAUAAGAUAACAGAUGUGAGGAG		845	867
AD-2004837	AACUAUUUGUAGUAACUAUCU		874	894	AGAUAGUUACUACAAAUAGUUCU		872	894
AD-2004853	UAUCAGAAUCUACAUUCUAAU		890	910	AUUAGAAUGUAGAUUCUGAUAGU		888	910
AD-2004872	UUGUAUUUUUUCUAUGCCACU		918	938	AGUGGCAUAGAAAAAAUACAAUU		916	938
AD-2004885	AUGCCACAUUAACAUCUUUUU		931	951	AAAAAGAUGUUAAUGUGGCAUAG		929	951
AD-2004896	UCUUUUAAAGUUGAUGAGAAU		945	965	AUUCUCAUCAACUUUAAAAGAUG		943	965
AD-2004909	AUGAGAAUCAAGUAUGGAAAU		958	978	AUUUCCAUACUUGAUUCUCAUCA		956	978
AD-2004922	AUGGAAAAGUAAGGCCAUACU		971	991	AGUAUGGCCUUACUUUUCCAUAC		969	991
AD-2004935	GCCAUACUCUUACAUAAUAAU		984	1004	AUUAUUAUGUAAGAGUAUGGCCU		982	1004
AD-2004950	GAAUCACAGAAUUCUAGUACU		1030	1050	AGUACUAGAAUUCUGUGAUUCUU		1028	1050
AD-2004970	AUGUAGGUAAAUCAUAAAUCU		1050	1070	AGAUUUAUGAUUUACCUACAUGU		1048	1070
AD-2004989	CUGUUCUAAGACAUAUGAUCU		1069	1089	AGAUCAUAUGUCUUAGAACAGAU		1067	1089
AD-2005002	UAUGAUCAACAGAUGAGAACU		1082	1102	AGUUCUCAUCUGUUGAUCAUAUG		1080	1102

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2005020	ACUGGUGGUUAAUAUGUGACU		1100	1120	AGUCACAUAUUAACCACCAGUUC		1098	1120
AD-2005033	AUGUGACAGUGAGAUUAGUCU		1113	1133	AGACUAAUCUCACUGUCACAUAU		1111	1133
AD-2005051	CAUAUCACUAAUAUACUAACU		1132	1152	AGUUAGUAUAUUAGUGAUAUGAC		1130	1152
AD-2005067	UAACAACAGAAUCUAAUCUUU		1148	1168	AAAGAUUAGAUUCUGUUGUUAGU		1146	1168
AD-2005082	UCUUCAUUUAAGGCACUGUAU		1164	1184	AUACAGUGCCUUAAAUGAAGAUU		1162	1184
AD-2005095	CACUGUAGUGAAUUAUCUGAU		1177	1197	AUCAGAUAAAUUCACUACAGUGCC		1175	1197
AD-2005108	UAUCUGAGCUAGAGUUACCUU		1190	1210	AAGGUAACUCUAGCUCAGAUAAU		1188	1210
AD-2005129	GCUUACCAUACUAUAUCUUUU		1211	1231	AAAAGAUAUAGUAUGGUAAGCUA		1209	1231
AD-2005146	UUUGGAAUCAUGAAACCUUAU		1228	1248	AUAAGGUUUCAUGAUUCCAAAGA		1226	1248
AD-2005164	UAAGACUUCAGAAUGAUUUUU		1246	1266	AAAAAUCAUUCUGAAGUCUUAAG		1244	1266
AD-2005179	AUUUUGCAGGUUGUCUUCCAU		1261	1281	AUGGAAGACAACCUGCAAAAUCA		1259	1281
AD-2005193	CUUCCAUUCCAGCCUAACAUU		1275	1295	AAUGUUAGGCUGGAAUGGAAGAC		1273	1295
AD-2005221	GGCAAGGAAAAUAAAAGAUUU		1303	1323	AAAUCUUUUAUUUUCCUUGCCUG		1301	1323
AD-2005234	AAGAUUUCCAGUGACAGAAAU		1317	1337	AUUUCUGUCACUGGAAAUCUUUU		1315	1337
AD-2005250	UAUGAAUUCUCUCUCCAAAUU		1368	1388	AAUUUGGAGAGAGAAAUUCAUAUA		1366	1388
AD-2005274	GAACUUGUUGGCCCAUCUAUU		1419	1439	AAUAGAUGGGCCAACAAGUUCAU		1417	1439
AD-2005287	CAUCUAUUACAUCUACAGCUU		1432	1452	AAGCUGUAGAUGUAAUAGAUGGG		1430	1452
AD-2005300	UACAGCUGACCCUUGAACAUU		1445	1465	AAUGUUCAAGGGUCAGCUGUAGA		1443	1465
AD-2005325	CAAAAUCUUAACUACCUAAUU		1498	1518	AAUUAGGUAGUUAAGAUUUUGCG		1496	1518
AD-2005350	ACUAUUGACCAUAAACCUUAU		1523	1543	AUAAGGUUUAUGGUCAAUAGUAG		1521	1543
AD-2005365	CCUUACUGAUAACAUAAACAU		1538	1558	AUGUUUAUGUUAUCAGUAAGGUU		1536	1558
AD-2005384	ACACAUAUUUUGCGUGUUAUU		1566	1586	AAUAACACGCAAAAUAUGUGUUA		1564	1586
AD-2005396	GUGUUAUAUGUAUUAUACACU		1579	1599	AGUGUAUAAAUACAUAUAACACGC		1577	1599
AD-2005409	CUAUAUUCCUACAAUAAAGUU		1598	1618	AACUUUAUUGUAGGAAUAUAGUG		1596	1618
AD-2005429	AAGCUAGAGAAAAUGUUAUUU		1618	1638	AAAUAACAUUUUCUCUAGCUUAC		1616	1638
AD-2005447	CUAUUCAUUAAAUGGAAGUGU		1670	1690	ACACUUCCAUUUAAUGAAUAGUA		1668	1690

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2005471	AACAUAAAAGUCUUCAUUCUU		1694	1714	AAGAAUGAAGACUUUUAUGUUGA		1692	1714
AD-2005485	CAUUCUCAUUGUCUUCACAUU		1708	1728	AAUGUGAAGACAAUGAGAAUGAA		1706	1728
AD-2005527	GGUCUUGCAGUCUUGUCUUAU		1773	1793	AUAAGACAAGACUGCAAGACCAA		1771	1793
AD-2005530	AAAGAAUAUUCAUGUAUAAAGU		1812	1832	ACUUAUACAUGAAUAUUCUUUCC		1810	1832
AD-2005557	UUGCAAUUCAAGCCCUUGUUU		1839	1859	AAACAAGGGCUUGAAUUGCAAGG		1837	1859
AD-2005575	UUGUUCAAGGGUCAACUGUAU		1857	1877	AUACAGUUGACCCUUGAACAACA		1855	1877
AD-2005588	AACUGUAAAUAGGAUAUAGCUU		1870	1890	AAGCUAUAUCCUAUUACAGUUGA		1868	1890
AD-2005604	CUAUUUUUCUUCCUCUAUCAU		1888	1908	AUGAUAGAGGAAGAAAAAAUAGCU		1886	1908
AD-2005617	UCUAUCAACCAAAUGGUAAGU		1901	1921	ACUUACCAUUUGGUUGAUAGAGG		1899	1921
AD-2005638	AUCUAUUUUGCAGUCCACUCU		1922	1942	AGAGUGGACUGCAAAAUAGAUGC		1920	1942
AD-2005658	UACUGAGCUAAAUUAUAGAUU		1942	1962	AAUCUAUAAUUUAGCUCAGUAGA		1940	1962
AD-2005678	CCAGCUAUGCUAUUUAUAAUU		1962	1982	AAUUAUAAAUAGCAUAGCUGGAU		1960	1982
AD-2005687	UUUUCAAUUUCUCCUCUGACU		2001	2021	AGUCAGAGGAGAAAUUGAAAAUU		1999	2021
AD-2005704	GACCAUUUCAGAACAUCUUCU		2018	2038	AGAAGAUGUUCUGAAAUGGUCAG		2016	2038
AD-2005720	CUUCCAAUAACUCAUAAAACU		2034	2054	AGUUUUAUGAGUUAUUGGAAGAU		2032	2054
AD-2005737	CAACUGAAGUAAAAAUUGAGUU		2053	2073	AACUCAAUUUUACUUCAGUUGUU		2051	2073
AD-2005760	GGAAAAUAUAUUCACCAAACU		2076	2096	AGUUUGGUGAAUAUAUUUUCCAG		2074	2096
AD-2005826	AAACUUUGGUAAUUUAAGUUU		2092	2112	AAACUUAAAUUACCAAAGUUUGG		2090	2112
AD-2005849	AAAAUAGUUUACACCUAUACU		2135	2155	AGUAUAGGUGUAAACUAUUUUAG		2133	2155
AD-2005862	CCUAUACUGCAUAAUCCAACU		2148	2168	AGUUGGAUUAUGCAGUAUAGGUG		2146	2168
AD-2005923	AACAAUUUUAAUUUCAGUUGU		2165	2185	ACAACUGAAAUUAAAAUUGUUGG		2163	2185
AD-2005942	AGACAUGUUACUAAUAUAACU		2186	2206	AGUUAUAUUAGUAACAUGUCUUC		2184	2206
AD-2005954	AAAGAGUAGAGGAUGUGUAAU		2214	2234	AUUACACAUCCUCUACUCUUUUA		2212	2234
AD-2006018	GUGUAAUUAACCAUAUCUUCU		2228	2248	AGAAGAUAUGGUUAAUUACACAU		2226	2248
AD-2006035	UUCUAAAACAUGGUUACUAAU		2245	2265	AUUAGUAACCAUGUUUUAGAAGA		2243	2265
AD-2006046	UUACUAAAAGAAUAUGUAACU		2258	2278	AGUUACAUAUUCUUUUAGUAACC		2256	2278

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2006056	AUGUAACAUCAAUAUUGACCU		2271	2291	AGGUCAAUAUUGAUGUUACAUAU		2269	2291
AD-2006119	AUUGACCUUGGUUUCUUACAU		2284	2304	AUGUAAGAAACCAAGGUCAAUAU		2282	2304
AD-2006132	UCUUACACAAGUGUUGCUAAU		2297	2317	AUUAGCAACACUUGUGUAAGAAA		2295	2317
AD-2006150	AACUCAAUAGUGAAGGAGACU		2315	2335	AGUCUCCUUCACUAUUGAGUUAG		2313	2335
AD-2006164	GGAGACACUAUUAAAUUUUCU		2329	2349	AGAAAAUUUAAUAGUGUCUCCUU		2327	2349
AD-2006234	GAACCCAUGAGAGAUACUAGU		2350	2370	ACUAGUAUCUCUCAUGGGUUCAG		2348	2370
AD-2006242	AGUGGAAAGUGUUUGGUUCAU		2378	2398	AUGAACCAAACACUUUCCACUCC		2376	2398
AD-2006259	UCAGGGAUAUCUGAAGAACAU		2395	2415	AUGUUCUUCAGAUAUCCCUGAAC		2393	2415
AD-2006334	GCAGAGAUUUCUUAAGUGACU		2420	2440	AGUCACUUAAGAAAUCUCUGCCC		2418	2440
AD-2006347	AAGUGACGCCUCAUCUACAAU		2433	2453	AUUGUAGAUGAGGCGUCACUUAA		2431	2453
AD-2006362	UACAAGCUGGAAAUUCCUAAU		2448	2468	AUUAGGAAUUUCCAGCUUGUAGA		2446	2468
AD-2006437	AGUAGAAAGCUUAUAAACAAU		2473	2493	AUUGUUUAUAAGCUUUCUACUUG		2471	2493
AD-2006451	AAACAACAGGUGAUACACUCU		2487	2507	AGAGUGUAUCACCUGUUGUUUAU		2485	2507
AD-2006525	CACUGGUUUUAGUAAAUUACU		2511	2531	AGUAAUUUACUAAAACCAGUGAG		2509	2531
AD-2006548	CAGAAAGUAUCCCUAGUCUUU		2536	2556	AAAGACUAGGGAUACUUUCUGUA		2534	2556
AD-2006626	GUGGAAAAUUUGAACUGAUUU		2564	2584	AAAUCAGUUCAAAUUUUCCACUU		2562	2584
AD-2006640	CUGAUUAGUCAUAUUCCUUUU		2578	2598	AAAAGGAAUAUGACUAAUCAGUU		2576	2598
AD-2006658	UUGAUUACACUGUUUGUUACU		2596	2616	AGUAACAAACAGUGUAAUCAAAG		2594	2616
AD-2006731	UCUCAGUAAACAGAAAUAACU		2624	2644	AGUUAUUUCUGUUUACUGAGAAA		2622	2644
AD-2006743	UCUUCAUUCUUUGAUAGAAAU		2656	2676	AUUUCUAUCAAAGAAUGAAGAAC		2654	2676
AD-2006751	AAAUCUUAUUCUGUGAGGAUU		2679	2699	AAUCCUCACAGAAUAAGAUUUUA		2677	2699
AD-2006764	UGAGGAUUACAGAAUACUAUU		2692	2712	AAUAGUAUUCUGUAAUCCUCACA		2690	2712
AD-2006839	CUUCAUCAUAAAGUGUAAAGU		2754	2774	ACUUUACACUUUAUGAUGAAGAA		2752	2774
AD-2006862	AUGCUCAAAUAUGUUCUACUU		2820	2840	AAGUAGAACACAUAUUUGAGCAUUU		2818	2840
AD-2006926	UCUACUAUAGAAUAAGUUCUU		2834	2854	AAGAACUUAUUCUAUAGUAGAAC		2832	2854
AD-2006935	UUCUUAUCUUAAUUUACAGGU		2850	2870	ACCUGUAAAUUAAGAUAAGAACU		2848	2870

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_002667.5	
			Start	End			Start	End
AD-2006965	UUAAUGUUGCCUUUUAUAUUU		2894	2914	AAAUAUAAAAGGCAACAUUAAGC		2892	2914
AD-2007034	AAAUCAGAAUCACUAUAUUAU		2947	2967	AUAAUAUAGUGAUUCUGAUUUGC		2945	2967

Table 40. Modified Sense and Antisense Strand Sequences of PLN dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2004072	gsgsugacAfgAfGfUfcagaaaacuuL96		asAfsguuUfuCfUfgacuCfuGfucaccscsa		UGGGUGACAGAGUCAGAAAACUC	
AD-2004080	asasacacCfcGfUfAfagacuucauuL96		asAfsugaAfgUfCfuuacGfgGfuguuusasg		CUAAACACCCGUAAGACUUCAUA	
AD-2004095	ususcauaCfaAfCfAfcaauacucuuL96		asAfsgagUfaUfUfguguUfgUfaugaasgsu		ACUUCAUACAACACAAUACUCUA	
AD-2004113	csusauacUfgUfGfAfugaucacaguL96		asCfsuguGfaUfCfaucaCfaGfuauagsasg		CUCUAUACUGUGAUGAUCACAGC	
AD-2004142	csusaccuAfaAfAfGfaagacaguuuL96		asAfsacuGfuCfUfucuuUfuAfgguagscsc		GGCUACCUAAAAGAAGACAGUUA	
AD-2004156	ascsaguuAfuCfUfCfauauuuggcuL96		asGfsccaAfaUfAfugagAfuAfacuguscsu		AGACAGUUAUCUCAUAUUUGGCU	
AD-2004176	usgsccagCfuUfUfUfuaucuuucuuL96		asAfsgaaAfgAfUfaaaaAfgCfuggcasgsc		GCUGCCAGCUUUUUAUCUUUCUC	
AD-2004189	uscsuuucUfcUfCfGfaccacuuaauL96		asUfsuaaGfuGfGfucgaGfaGfaaagasusa		UAUCUUUCUCUCGACCACUUAAA	
AD-2004202	csascuuaAfaAfCfUfucagacuucuL96		asGfsaagUfcUfGfaaguUfuUfaagugsgsu		ACCACUUAAAACUUCAGACUUCC	
AD-2004238	asusggagAfaAfGfUfccaauaccuuL96		asAfsgguAfuUfGfgacuUfuCfuccausgsa		UCAUGGAGAAAGUCCAAUACCUC	
AD-2004260	csuscgcuCfaGfCfUfauaagaagauL96		asUfscuuCfuUfAfuagcUfgAfgcgagsusg		CACUCGCUCAGCUAUAAGAAGAG	
AD-2004284	csasaccaUfuGfAfAfaugccucaauL96		asUfsugaGfgCfAfuuucAfaUfgguugsasg		CUCAACCAUUGAAAUGCCUCAAC	
AD-2004301	csasacaaGfcAfCfGfucaaaagcuuL96		asAfsgcuUfuUfGfacguGfcUfuguugsasg		CUCAACAAGCACGUCAAAAGCUA	
AD-2004319	csusacagAfaUfCfUfauuuaucaauL96		asUfsugaUfaAfAfuagaUfuCfuguagscsu		AGCUACAGAAUCUAUUUAUCAAU	
AD-2004329	usasucaaUfuUfCfUfgucucaucuuL96		asAfsgauGfaGfAfcagaAfaUfugauasasa		UUUAUCAAUUUCUGUCUCAUCUU	
AD-2004345	asuscuuaAfuAfUfGfucucuugcuuL96		asAfsgcaAfgAfGfacauAfuUfaagausgsa		UCAUCUUAAUAUGUCUCUUGCUG	
AD-2004360	ususgcugAfuCfUfGfuaucaucguuL96		asAfscgaUfgAfUfacagAfuCfagcaasgsa		UCUUGCUGAUCUGUAUCAUCGUG	
AD-2004373	uscsaucgUfgAfUfGfcuucucugauL96		asUfscagAfgAfAfgcauCfaCfgaugasusa		UAUCAUCGUGAUGCUUCUCUGAA	
AD-2004386	uscsucugAfaGfUfUfcugcuacaauL96		asUfsuguAfgCfAfgaacUfuCfagagasasg		CUUCUCUGAAGUUCUGCUACAAC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2004400	csusacaaCfcUfCfUfagaucugcauL96		asUfsgcaGfaUfCfuagaGfgUfuguagscsa		UGCUACAACCUCUAGAUCUGCAG	
AD-2004428	csasucagCfuUfAfAfaaucugucauL96		asUfsgacAfgAfUfuuuaAfgCfugaugsusg		CACAUCAGCUUAAAAUCUGUCAU	
AD-2004458	ascsaggaAfaAfCfAfauauuguauuL96		asAfsuacAfaUfAfuuguUfuUfccugucscsu		AGACAGGAAAACAAUAUUGUAUA	
AD-2004468	asusuguaUfaAfCfAfgaccacuucuL96		asGfsaagUfgGfUfcuguUfaUfacaausasu		AUAUUGUAUAACAGACCACUUCC	
AD-2004481	cscsacuuCfcUfGfAfguagaagagguL96		asCfsucuUfcUfUfAfcucaGfgAfaguggsusc		GACCACUUCCUGAGUAGAAGAGU	
AD-2004495	gsasagagUfuUfCfUfuugugaaaauL96		asUfsuuuCfaCfAfaagaAfaCfucucusa		UAGAAGAGUUUCUUUGUGAAAAG	
AD-2004518	csasagauUfaAfGfAfcuaaaacuuuL96		asAfsaguUfuUfAfgucuUfaAfucuugsasc		GUCAAGAUUAAGACUAAAACUUA	
AD-2004539	gsusuaccAfuAfUfGfuuaucaucuuL96		asAfsgauGfaAfUfacauAfuGfguaacsasa		UUGUUACCAUAUGUAUUCAUCUG	
AD-2004555	asuscuguUfgGfAfUfcuuguaaacuL96		asGfsuuuAfcAfAfgaucCfaAfcagausgsa		UCAUCUGUUGGAUCUUGUAAACA	
AD-2004575	asusgaaaAfgGfGfCfuuuauuucuL96		asGfsaaaAfuAfAfAfagccCfuUfuucausgsu		ACAUGAAAAGGGCUUUAUUUUCA	
AD-2004594	gsusguauAfaAfAfUfgcaacuguuuL96		asAfsacaGfuUfGfcauuUfuAfuacacsusu		AAGUGUAUAAAAUGCAACUGUUG	
AD-2004608	ascsuguuGfaUfUfUfccucaacauuL96		asAfsuguUfgAfGfgaaaUfcAfacagususg		CAACUGUUGAUUUCCUCAACAUG	
AD-2004632	csascaaaUfuUfCfUfaucccaaauuL96		asAfsuuuGfgGfAfuagaAfaUfuugugsasg		CUCACAAAUUUCUAUCCCAAAUC	
AD-2004646	cscsaaauCfuUfUfUfcugaagauguL96		asCfsaucUfuCfAfgaaaAfgAfuuuggsgsa		UCCCAAAUCUUUUCUGAAGAUGA	
AD-2004660	asasgaugAfaGfAfGfuuuaguuuuuL96		asAfsaaaCfuAfAfAfacucUfuCfaucuuscsa		UGAAGAUGAAGAGUUUAGUUUUA	
AD-2004673	asgsuuuuAfaAfAfAfCfugcacugccuL96		asGfsgcaGfuGfCfUfaguuUfuAfaaacusasa		UUAGUUUUAAAACUGCACUGCCA	
AD-2004689	usgsccaaCfaAfGfUfUfucacuucaauL96		asAfsugaAfgUfGfaacuUfgUfuggcasgsu		ACUGCCAACAAGUUCACUUCAUA	
AD-2004718	csusuuugAfgGfUfGfaauauaauuuL96		asAfsauuAfuAfUfUfucacCfuCfaaagsasg		CUCUUUUGAGGUGAAUAUAAUUU	
AD-2004747	asasguauUfuUfUfCfaggucuucauL96		asUfsgaaGfaCfCfUfugaaAfaAfuacuusasg		CUAAGUAUUUUUCAGGUCUUCAC	
AD-2004763	ususcaccAfaGfUfUfAfucaaaguaauL96		asUfsuacUfuUfUfGfauacUfuGfgugaasgsa		UCUUCACCAAGUAUCAAAGUAAU	
AD-2004777	asasguaaUfaAfCfAfcaaaugaaguL96		asCfsuucAfuUfUfUfguguUfaUfuacuususg		CAAAGUAAUAACACAAAUGAAGU	
AD-2004790	asasugaaGfuGfUfCfauuauucaauL96		asUfsugaAfuAfAfAfugacAfcUfucauususg		CAAAUGAAGUGUCAUUAUUCAAA	
AD-2004806	uscsaaaaUfaGfUfCfcacugacucuL96		asGfsaguCfaGfUfUfggacUfaUfuuugasasu		AUUCAAAAUAGUCCACUGACUCC	
AD-2004825	cscsucacAfuCfUfGfuuaucuuauuL96		asAfsuaaGfaUfAfacagAfuGfugaggsasg		CUCCUCACAUCUGUUAUCUUAUU	
AD-2004837	asascuauUfuGfUfAfguaacuauccuL96		asGfsauaGfuUfAfcuacAfaAfuaguuscsu		AGAACUAUUUGUAGUAACUAUCA	
AD-2004853	usasucagAfaUfCfUfacauucuaauL96		asUfsuagAfaUfGfuagaUfuCfugauasgsu		ACUAUCAGAAUCUACAUUCUAAA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2004872	ususguauUfuUfUfUfcuaugccacuL96		asGfsuggCfaUfAfgaaaAfaAfuacaasusu		AAUUGUAUUUUUUCUAUGCCACA	
AD-2004885	asusgccaCfaUfUfAfacaucuuuuuuL96		asAfsaaaGfaUfGfuuaaUfgUfggcausasg		CUAUGCCACAUUAACACAUCUUUUA	
AD-2004896	uscsuuuuAfaAfGfUfugaugagaauL96		asUfsucuCfaUfCfaaccUfuAfaaagasusg		CAUCUUUUAAAGUUGAUGAGAAU	
AD-2004909	asusgagaAfuCfAfAfguauggaaauL96		asUfsuucCfaUfAfcuugAfuUfcucauscsa		UGAUGAGAAUCAAGUAUGGAAAA	
AD-2004922	asusggaaAfaGfUfAfaggccauacuL96		asGfsuauGfgCfCfuuacUfuUfuccausasc		GUAUGGAAAAGUAAGGCCAUACU	
AD-2004935	gscscauaCfuCfUfUfacauaauaaauL96		asUfsuauUfaUfGfuaagAfgUfauggcscsu		AGGCCAUACUCUUACAUAAUAAA	
AD-2004950	gsasaucaCfaGfAfAfuucuaguacuL96		asGfsuacUfaGfAfauucUfgUfgauucsusu		AAGAAUCACAGAAUUCUAGUACA	
AD-2004970	asusguagGfuAfAfAfucauaaaaucuL96		asGfsauuUfaUfGfauuuAfcCfuacausgsu		ACAUGUAGGUAAAUCAUAAAUCU	
AD-2004989	csusguucUfaAfGfAfcauaugaucuL96		asGfsaucAfuAfUfgucuUfaGfaacagsasu		AUCUGUUCUAAGACAUAUGAUCA	
AD-2005002	usasugauCfaAfCfAfgaugagaacuL96		asGfsuucUfcAfUfcuguUfgAfucauasusg		CAUAUGAUCAACAGAUGAGAACU	
AD-2005020	ascsugguGfgUfUfAfauaugugacuL96		asGfsucaCfaUfAfauaaCfcAfccagususc		GAACUGGUGGUUAAUAUGUGACA	
AD-2005033	asusgugaCfaGfUfGfagauuagucuL96		asGfsacuAfaUfCfucacUfgUfcacausasu		AUAUGUGACAGUGAGAUUAGUCA	
AD-2005051	csasuaucAfcUfAfAfauauacuaacuL96		asGfsuuaGfuAfUfauuaGfuGfauaugsasc		GUCAUAUCACUAAUAUACUAACA	
AD-2005067	usasacaaCfaGfAfAfucuaaucuuuL96		asAfsagaUfuAfGfauucUfgUfuguuasgsu		ACUAACAACAGAAUCUAAUCUUC	
AD-2005082	uscsuucaUfuUfAfAfggcacuguauL96		asUfsacaGfuGfCfcuuaAfaUfgaagasusu		AAUCUUCAUUUAAGGCACUGUAG	
AD-2005095	csascuguAfgUfGfAfauaucugauL96		asUfscagAfuAfAfauucaCfuAfcagugscsc		GGCACUGUAGUGAAUUAUCUGAG	
AD-2005108	usasucugAfgCfUfAfgaguaccuuL96		asAfsgguAfaCfUfcuagCfuCfagauasasu		AUUAUCUGAGCUAGAGUUACCUA	
AD-2005129	gscsuuacCfaUfAfCfuauaucuuuuL96		asAfsaagAfuAfUfaguaUfgGfuaagcsusa		UAGCUUACCAUACUAUAUCUUUG	
AD-2005146	ususuggaAfuCfAfUfgaaaccuuauL96		asUfsaagGfuUfUfcaugAfuUfccaaasgsa		UCUUUGGAAUCAUGAAACCUUAA	
AD-2005164	usasagacUfuCfAfGfaaugauuuuuL96		asAfsaaaUfcAfUfucugAfaGfucuuasasg		CUUAAGACUUCAGAAUGAUUUUG	
AD-2005179	asusuuugCfaGfGfUfugucuuccauL96		asUfsggaAfgAfCfaaccUfgCfaaaauscsa		UGAUUUUGCAGGUUGUCUUCCAU	
AD-2005193	csusuccaUfuCfCfAfgccuaacauuL96		asAfsuguUfaGfGfcuggAfaUfggaagsasc		GUCUUCCAUUCCAGCCUAACAUC	
AD-2005221	gsgscaagGfaAfAfAfuaaaagauuuuL96		asAfsaucUfuUfUfauuuUfcCfuugccsusg		CAGGCAAGGAAAAUAAAAGAUUU	
AD-2005234	asasgauuUfcCfAfGfugacagaaauL96		asUfsuucUfgUfCfacugGfaAfaucuususu		AAAAGAUUUCCAGUGACAGAAAA	
AD-2005250	usasugaaUfuCfUfCfucuccaaauuL96		asAfsuuuGfgAfGfagagAfaUfucauasusa		UAUAUGAAUUCUCUCUCCCAAAUA	
AD-2005274	gsasacuuGfuUfGfGfcccaucuauuL96		asAfsuagAfuGfGfgccaAfcAfaguucsasu		AUGAACUUGUUGGCCCAUCUAUU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2005287	csasucuaUfuAfCfAfucuacagcuuL96		asAfsgcuGfuAfUfaguAfaUfagaugsgsg		CCCAUCUAUUACACUCUACAGCUG	
AD-2005300	usascagcUfgAfCfCfCfcuugaacauuL96		asAfsuguUfcAfAfggguCfaGfcuguasgsa		UCUACAGCUGACCCUUGAACAUG	
AD-2005325	csasaaauCfuUfAfAfcuaccuaauuL96		asAfsuuaGfgUfAfguuaAfgAfuuuugscsg		CGCAAAAUCUUAACUACCUAAUA	
AD-2005350	ascsuauuGfaCfCfAfuaaaccuuauL96		asUfsaagGfuUfUfauggUfcAfauagusasg		CUACUAUUGACCAUAAACCUUAC	
AD-2005365	cscsuuacUfgAfUfAfacauaaacauL96		asUfsguuUfaUfGfuuauCfaGfuaaggsusu		AACCUUACUGAUAACAUAAACAG	
AD-2005384	ascsacauAfuUfUfUfgcguguauuuL96		asAfsuaaCfaCfGfcaaaAfuAfugugususa		UAACACAUAUUUUGCGUGUUAUA	
AD-2005396	gsusguuaUfaUfGfUfauauacacuL96		asGfsuguAfuAfAfuacaUfaUfaacacsgsc		GCGUGUUAUAUGUAUUAUACACU	
AD-2005409	csusauauUfcCfUfAfcaauaaaguuL96		asAfscuuUfaUfUfUfguagGfaAfuauaggsusg		CACUAUAUUCCUACAAUAAAGUA	
AD-2005429	asasgcuaGfaGfAfAfaauguuauuuL96		asAfsauaAfcAfUfuuucUfcUfagcuusasc		GUAAGCUAGAGAAAAUGUUAUUU	
AD-2005447	csusauucAfuUfAfAfauggaaguguL96		asCfsacuUfcCfAfuuuaAfuGfaauagsusa		UACUAUUCAUUAAAUGGAAGUGG	
AD-2005471	asascauaAfaAfGfUfUfcuucauucuuL96		asAfsgaaUfgAfAfgacuUfuUfauguusgsa		UCAACAUAAAAGUCUUCAUUCUC	
AD-2005485	csasuucuCfaUfUfGfucuucacauuL96		asAfsuguGfaAfGfacaaUfgAfgaaugsasa		UUCAUUCUCAUUGUCUUCACAUU	
AD-2005527	gsgsucuuGfcAfGfUfUfcuugucuuauL96		asUfsaagAfcAfAfgacuGfcAfagaccsasa		UUGGUCUUGCAGUCUUGUCUUAG	
AD-2005530	asasagaaUfaUfUfCfUfauguauaaguL96		asCfsuuaUfaCfAfugaaUfaUfucuuuscsc		GGAAAGAAUAUUCAUGUAUAAGU	
AD-2005557	ususgcaaUfuCfAfAfgcccuuguuuL96		asAfsacaAfgGfGfcuugAfaUfugcaasgsg		CCUUGCAAUUCAAGCCCUUGUUG	
AD-2005575	ususguucAfaGfGfGfGfucaacuguauL96		asUfsacaGfuUfGfacccUfuGfaacaascsa		UGUUGUUCAAGGGUCAACUGUAA	
AD-2005588	asascuguAfaUfAfGfgauauagcuuL96		asAfsgcuAfuAfUfccuaUfuAfcaguusgsa		UCAACUGUAAUAGGAUAUAGCUA	
AD-2005604	csusauuuUfuCfUfUfccucuaucauL96		asUfsgauAfgAfGfgaagAfaAfaauagscsu		AGCUAUUUUUCUUCCUCUAUCAA	
AD-2005617	uscsuaucAfaCfCfAfaaugguaagUL96		asCfsuuaCfcAfUfuuggUfuGfauagasgsg		CCUCUAUCAAACCAAAUGGUAAGC	
AD-2005638	asuscuauUfuUfGfCfCfaguccacucuL96		asGfsaguGfgAfCfugcaAfaAfuagausgsc		GCAUCUAUUUUGCAGUCCACUCU	
AD-2005658	usascugaGfcUfAfAfaauuagauuL96		asAfsucuAfuAfAfauuuaGfcUfcaguasgsa		UCUACUGAGCUAAAUUAUAGAUC	
AD-2005678	cscsagcuAfuGfCfUfauuuauaauuL96		asAfsuuaUfaAfAfuagcAfuAfgcuggsasu		AUCCAGCUAUGCUAUUUAUAAUU	
AD-2005687	ususuucaAfuUfUfCfuccucugacuL96		asGfsucaGfaGfGfGfagaaAfuUfgaaaasusu		AAUUUUCAAUUUCUCCUCUGACC	
AD-2005704	gsasccauUfuCfAfGfaacaucuucuL96		asGfsaagAfuGfUfucugAfaAfuggucsasg		CUGACCAUUUCAGAACAUCUUCC	
AD-2005720	csusuccaAfuAfAfCfucauaaaacuL96		asGfsuuuUfaUfGfaguuAfuUfggaagsasu		AUCUUCCAAUAACUCAUAAAACA	
AD-2005737	csasacugAfaGfUfAfaaauugaguuL96		asAfscucAfaUfUfuuacUfuCfaguugsusu		AACAACUGAAGUAAAAUUGAGUG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2005760	gsgsaaaaUfaUfAfUfucaccaaaccuL96		asGfsuuuGfgUfGfaauaUfaUfuuuccsasg		CUGGAAAAUAUAUUCACCAAACU	
AD-2005826	asasacuuUfgGfUfAfauuuaaguuuL96		asAfsacuUfaAfAfuuacCfaAfaguuusgsg		CCAAACUUUGGUAAUUUAAGUUG	
AD-2005849	asasauuaGfuUfUfAfcaccuauacuL96		asGfsuauAfgGfUfguaaAfcUfauuuusasg		CUAAAAUAGUUUACACCUAUACU	
AD-2005862	cscsuauaCfuGfCfAfuaauccaacuL96		asGfsuugGfaUfUfaugcAfgUfauaggsusg		CACCUAUACUGCAUAAUCCAACA	
AD-2005923	asascaauUfuUfAfAfauuuaAfaAfuugusgsg		asCfsaacUfgAfAfauuuaAfaAfuuguusgsg		CCAACAAUUUUAAUUUCAGUUGA	
AD-2005942	asgsacauGfuUfAfCfuauauaacuL96		asGfsuuaUfaUfUfaguaAfcAfugucususc		GAAGACAUGUUACUAAAUAUAACU	
AD-2005954	asasagagUfaGfAfGfgauguaaauL96		asUfsuacAfcAfUfccucUfaCfucuuususa		UAAAAGAGUAGAGGAUGUGUAAU	
AD-2006018	gsusguaaUfuAfAfCfcauaucuucuL96		asGfsaagAfuAfUfgguuAfaUfuacacsasu		AUGUGUAAUUAACCAUAUCUUCU	
AD-2006035	ususcuaaAfaCfAfUfgguuacuaauL96		asUfsuagUfaAfCfcaugUfuUfuagaasgsa		UCUUCUAAAACAUGGUUACUAAA	
AD-2006046	ususacuaAfaAfGfAfauauguaacuL96		asGfsuuaCfaUfAfuucuUfuUfaguaascsc		GGUUACUAAAAGAAUAUGUAACA	
AD-2006056	asusguaaCfaUfCfAfauauugaccuL96		asGfsgucAfaUfUfAfuugaUfgUfuacausasu		AUAUGUAACAUCAAUAUUGACCU	
AD-2006119	asusugacCfuUfGfGfuuucuuacauL96		asUfsguaAfgAfAfaccaAfgGfucaausasu		AUAUUGACCUUGGUUUCUUACAC	
AD-2006132	uscsuuacAfcAfAfGfuguugcuaauL96		asUfsuagCfaAfCfacuuGfuGfuaagasasa		UUUCUUACACAAGUGUUGCUAAC	
AD-2006150	asascucaAfuAfGfUfgaaggagacuL96		asGfsucuCfcUfUfcacuAfuUfgaguusasg		CUAACUCAAUAGUGAAGGAGACA	
AD-2006164	gsgsagacAfcUfAfUfuaaauuuucuL96		asGfsaaaAfuUfUfaauaGfuGfucuccsusu		AAGGAGACACUAUUAAAUUUUCU	
AD-2006234	gsasacccAfuGfAfGfagauacuaguL96		asCfsuagUfaUfCfucucAfuGfgguucsasg		CUGAACCCAUGAGAGAUACUAGA	
AD-2006242	asgsuggaAfaGfUfGfuuugguucauL96		asUfsgaaCfcAfAfacacUfuUfccacuscsc		GGAGUGGAAAGUGUUUGGUUCAG	
AD-2006259	uscsagggAfuAfUfCfugaagaacauL96		asUfsguuCfuUfCfagauAfuCfccugasasc		GUUCAGGGAUAUCUGAAGAACAG	
AD-2006334	gscsagagAfuUfUfCfuuaagugacuL96		asGfsucaCfuUfAfagaaAfuCfucugcscsc		GGGCAGAGAUUUCUUAAGUGACG	
AD-2006347	asasgugaCfgCfCfUfcaucuacaauL96		asUfsuguAfgAfUfgaggCfgUfcacuusasa		UUAAGUGACGCCUCAUCUACAAG	
AD-2006362	usascaagCfuGfGfAfaauuccuaauL96		asUfsuagGfaAfUfuuccAfgCfuuguasgsa		UCUACAAGCUGGAAAUUCCUAAA	
AD-2006437	asgsuagaAfaGfCfUfuauaaacaauL96		asUfsugUfuAfUfaagcUfuUfucuacususg		CAAGUAGAAAGCUUAUAAACAAC	
AD-2006451	asasacaaCfaGfGfUfgauacacucuL96		asGfsaguGfuAfUfcaccUfgUfuguuusasu		AUAAACAACAGGUGAUACACUCA	
AD-2006525	csascuggUfuUfUfAfguaaauuacuL96		asGfsuaaUfuUfAfcuaaAfaCfcagugsasg		CUCACUGGUUUUAGUAAAUUACC	
AD-2006548	csasgaaaGfuAfUfCfccuagucuuuL96		asAfsagaCfuAfGfggauAfcUfuucugsusa		UACAGAAAGUAUCCCUAGUCUUA	
AD-2006626	gsusggaaAfaUfUfUfgaacugauuuL96		asAfsaucAfgUfUfcaaaUfuUfuccacsusu		AAGUGGAAAAUUUGAACUGAUUA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2006640	csusgauuAfgUfCfAfuauuccuuuuL96		asAfsaagGfaAfUfaugaCfuAfaucagsusu		AACUGAUUAGUCAUAUUCCUUUG	
AD-2006658	ususgauuAfcAfCfUfguuuguuacuL96		asGfsuaaCfaAfAfcaguGfuAfaucaasasg		CUUUGAUUACACUGUUUGUUACA	
AD-2006731	uscsucagUfaAfAfCfagaaauaacuL96		asGfsuuaUfuUfCfuguuUfaCfugagasasa		UUUCUCAGUAAACAGAAAUAACU	
AD-2006743	uscsuucaUfuCfUfUfugauagaaauL96		asUfsuucUfaUfCfaaagAfaUfgaagasasc		GUUCUUCAUUCUUUGAUAGAAAU	
AD-2006751	asasaucuUfaUfUfCfugugaggauuL96		asAfsuccUfcAfCfagaaUfaAfgauuususa		UAAAAUCUUAUUCUGUGAGGAUU	
AD-2006764	usgsaggaUfuAfCfAfgaauacuauuL96		asAfsuagUfaUfUfcuguAfaUfccucascsa		UGUGAGGAUUACAGAAUACUAUA	
AD-2006839	csusucauCfaUfAfAfaguguaaaguL96		asCfsuuuAfcAfCfuuuaUfgAfugaagsasa		UUCUUCAUCAUAAAGUGUAAAGA	
AD-2006862	asusgcucAfaAfUfAfuguucuacuuL96		asAfsguaGfaAfCfauauUfuGfagcaususu		AAAUGCUCAAAUAUGUUCUACUA	
AD-2006926	uscsuacuAfuAfGfAfauaaguucuuL96		asAfsgaaCfuUfAfuucuAfuAfguagasasc		GUUCUACUAUAGAAUAAGUUCUU	
AD-2006935	ususcuuaUfcUfUfAfauuuacagguL96		asCfscugUfaAfAfuuaaGfaUfaagaascsu		AGUUCUUAUCUUAAUUUACAGGG	
AD-2006965	ususaaugUfuGfCfCfuuuuauauuuL96		asAfsauaUfaAfAfaggcAfaCfauuaasgsc		GCUUAAUGUUGCCUUUUAUAUUU	
AD-2007034	asasaucaGfaAfUfCfacuauauuauL96		asUfsaauAfuAfGfugauUfcUfgauuusgsc		GCAAAUCAGAAUCACUAUAUUAA	

Table 41. Unmodified Sense and Antisense Strand Sequences of CAMK2D dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011305	UAGCACUUUUGAUCAUCUUCU		3221	3241	AGAAGAUGAUCAAAAGUGCUAGU		3219	3241
AD-2011306	CCUCAUCAAUAAAAUGCUUAU		1411	1431	AUAAGCAUUUUAUUGAUGAGGUC		1409	1431
AD-2011307	UCCAUCAUUGUUAUUUUAACU		3763	3783	AGUUAAAAUAACAAUGAUGGAGA		3761	3783
AD-2011308	UCCACACUAUUAUUCUAAACU		2001	2021	AGUUUAGAAUAAUAGUGUGGAUU		1999	2021
AD-2011309	CUCCUAAAAGCUUCUCUAAAU		4614	4634	AUUUAGAGAAGCUUUUAGGAGUC		4612	4634
AD-2011310	GGUCUCAUUUAGUACAUAACU		4491	4511	AGUUAUGUACUAAAUGAGACCAG		4489	4511
AD-2011311	CAGACUUAAGAACUAUUGUUU		2620	2640	AAACAAUAGUUCUUAAGUCUGUU		2618	2640
AD-2011312	CAUCAAGUUUCUCUGUUAAUU		2588	2608	AAUUAACAGAGAAACUUGAUGAA		2586	2608

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011313	AUUCUCUAUAUUCUACUUGUU		1277	1297	AACAAGUAGAAUAUAGAGAAUGA		1275	1297
AD-2011314	CUGCUUGAAGAAAUUUAAUGU		1537	1557	ACAUUAAAUUUCUUCAAGCAGUC		1535	1557
AD-2011315	CACUGACAUUAAUAUUCCUAU		5434	5454	AUAGGAAUAUUAAUGUCAGUGAU		5432	5454
AD-2011316	AACCACACAUUGUUUUGAAAAUU		5033	5053	AAUUUUCAAAACAAUGUGGUUGA		5031	5053
AD-2011317	UCAUCACUAGCUAGUUUUCUU		3492	3512	AAGAAAACUAGCUAGUGAUGAUG		3490	3512
AD-2011318	UUACUUUUUCCUAAGAUUCAU		4912	4932	AUGAAUCUUAGGAAAAAGUAAGA		4910	4932
AD-2011319	UUACCUAGAAUAAAUAGUCUU		4866	4886	AAGACUAUUUAUUCUAGGUAAGA		4864	4886
AD-2011320	GGUCUUCUAAAUUUCAACAGU		2296	2316	ACUGUUGAAAUUUAGAAGACCCA		2294	2316
AD-2011321	CCUAUACAUUGUUUACACUUU		2466	2486	AAAGUGUAAACAAUGUAUAGGAA		2464	2486
AD-2011322	UCUCUUGAAUCAGAGUAUCAU		3594	3614	AUGAUACUCUGAUUCAAGAGAGA		3592	3614
AD-2011323	GCUGAGAACAAUAUAGUUAAU		4585	4605	AUUAACUAUAUAUUGUUCUCAGCAA		4583	4605
AD-2011324	CGUCCAUCUUUAUUUCUUCAU		4407	4427	AUGAAGAAAUAAAGAUGGACGCA		4405	4427
AD-2011325	AGCUUCUUUUGAUUUGCUAAU		4792	4812	AUUAGCAAAUCAAAAGAAGCUGA		4790	4812
AD-2011326	AUCUCUUUUCUUUAUCCCUGUU		3028	3048	AACAGGAUAAAAGAAAAGAGAUCA		3026	3048
AD-2011327	CACAAAUGAAUUACACAUUUU		4261	4281	AAAAUGUGUAAUUCAUUUGUGGU		4259	4281
AD-2011328	UUUGUCCAAAAGCAAUAAACU		1978	1998	AGUUUAUUGCUUUUGGACAAAGC		1976	1998
AD-2011329	UCUGAGUUUAAUCACUUUAGU		2945	2965	ACUAAAGUGAUUAAACUCAGAAA		2943	2965
AD-2011330	CCAAGUACAACUCUUCAUCAU		2573	2593	AUGAUGAAGAGUUGUACUUGGAA		2571	2593
AD-2011331	UUCUACUUUUGAAAAUGCUUUU		1961	1981	AAAAGCAUUUUCAAAGUAGAAUC		1959	1981
AD-2011332	UAUCAUAUAUUCCUUCCUAUU		2451	2471	AAUAGGAAGGAAUAUAUGAUAAG		2449	2471
AD-2011333	CCUCUCAAAACCUACAUAAUU		4990	5010	AAUUAUGUAGGUUUUGAGAGGGA		4988	5010
AD-2011334	AGUUAAUUGUUCUCUGUGAGU		4535	4555	ACUCACAGAGAACAAUUAACUUG		4533	4555
AD-2011335	UUCUAUCAUUUUGAAACACUU		4287	4307	AAGUGUUUCAAAAUGAUAGAAGA		4285	4307
AD-2011336	CAGCUUGAACUUGAGCAUACU		4762	4782	AGUAUGCUCAAGUUCAAGCUGGG		4760	4782
AD-2011337	UCACCCAAAUUUUGAAUUUUU		5678	5698	AAAAAUUCAAAAUUUGGGUGAAG		5676	5698

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011338	GCAGUAUAACUAUUCUGAUCU		5415	5435	AGAUCAGAAUAGUUAUACUGCUA		5413	5435
AD-2011339	GGUUUUCUUUGUGCUUCUCAU		3672	3692	AUGAGAAGCACAAAGAAAACCUA		3670	3692
AD-2011340	AUGAGAAUAAACAUACCAACU		5324	5344	AGUUGGUAUGUUUAUUCUCAUUU		5322	5344
AD-2011341	GAGUGAUUUCAUUGAAUAAAU		5746	5766	AUUUAUUCAAUGAAAUCACUCGA		5744	5766
AD-2011342	GUUUCCAUAUUUGUAAAAAUGU		5307	5327	ACAUUUUACAAAUAUGGAAACUG		5305	5327
AD-2011343	CAUGCUGUUUUUGGUCAAACU		2897	2917	AGUUUGACCAAAAACAGCAUGGC		2895	2917
AD-2011344	CUCUACUGCAGUAUAAUGUCU		5072	5092	AGACAUUAUACUGCAGUAGAGGA		5070	5092
AD-2011345	UUGGUGUUUGAUUUAGUUACU		932	952	AGUAACUAAAUCAAACACCAAGU		930	952
AD-2011346	UCUUUCUCCAGAAGUUUUACU		1210	1230	AGUAAAACUUCUGGAGAAAGAUA		1208	1230
AD-2011347	AGAGUUCAAAUACAACAAUUU		1767	1787	AAAUUGUUGUAUUUGAACUCUCA		1765	1787
AD-2011348	UCUGUGUUGAUUACUAAUCAU		3885	3905	AUGAUUAGUAAUCAACACAGAGA		3883	3905
AD-2011349	UGCUUUUUAGCUUCUCUCUU		3579	3599	AAGAGAGAAGCUAAAAAAAGCACA		3577	3599
AD-2011350	CUGUGACUGCAACGUCUUACU		3371	3391	AGUAAGACGUUGCAGUCACAGGA		3369	3391
AD-2011351	CACUUCUGCAUUCUCUGUUCU		2319	2339	AGAACAGAGAAUGCAGAAGUGGC		2317	2339
AD-2011352	CUCAUUUUUCCUUUGUUGAUU		3688	3708	AAUCAACAAAGGAAAAAAUGAGAA		3686	3708
AD-2011353	CCUCAGAAGCCUAUUUUUAAU		3799	3819	AUUAAAAAAUAGGCUUCUGAGGAC		3797	3819
AD-2011354	AUAGUAAUUCACAGUCCUCAU		3784	3804	AUGAGGACUGUGAAUUACUAUCG		3782	3804
AD-2011355	UUUCUCAAAUCUGUGAUCUCU		3013	3033	AGAGAUCACAGAUUUGAGAAAGA		3011	3033
AD-2011356	GCUGCCAAAAUUAUCAACACU		791	811	AGUGUUGAUAAUUUUGGCAGCAU		789	811
AD-2011357	GACUGAUUGAUAUAUUUAAGU		4355	4375	ACUUAAAUAUAUCAAUCAGUCCU		4353	4375
AD-2011358	UACACUUUUAACUUAGCAUGU		4164	4184	ACAUGCUAAGUUAAAAAGUGUAAA		4162	4184
AD-2011359	UCUGAGCAAGUUACCUCUUCU		5274	5294	AGAAGAGGUAACUUGCUCAGAGC		5272	5294
AD-2011360	GCCCUCUUUAUGUAGGUUUAU		5508	5528	AUAAACCUACAUAAAGAGGGCAG		5506	5528
AD-2011361	CAAGGAAUAUUAAUCUUCACU		5662	5682	AGUGAAGAUUAAUAUUCCUUGAA		5660	5682
AD-2011362	AUGUUGUUUAUGUUUCUUACU		4850	4870	AGUAAGAAACAUAAACAACAUGA		4848	4870

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011363	AUACUCGGACUCAAAUGUCUU		5545	5565	AAGACAUUUGAGUCCGAGUAUAU		5543	5565
AD-2011364	UGAAGCUUUUCUGAUAAUUAU		3944	3964	AUAAUUAUCAGAAAAGCUUCAAC		3942	3964
AD-2011365	GAACUCUUUGCUGUUAAUCUU		2678	2698	AAGAUUAACAGCAAAGAGUUCUA		2676	2698
AD-2011366	UUGAAAGUUUUGCUGAUUAAU		2796	2816	AUUAAUCAGCAAAACUUUCAAUG		2794	2816
AD-2011367	AUGCUAGAAGAAAACUAAAGU		1554	1574	ACUUUAGUUUUCUUCUAGCAUUA		1552	1574
AD-2011368	CCUCUUCAUGCAUGUUUCUGU		2378	2398	ACAGAAACAUGCAUGAAGAGGAG		2376	2398
AD-2011369	UACUGCUUUUGAACCUGAAGU		1903	1923	ACUUCAGGUUCAAAAGCAGUAAG		1901	1923
AD-2011370	CAACGUUCUACUGUUGCUUCU		1490	1510	AGAAGCAACAGUAGAACGUUGAC		1488	1510
AD-2011371	CAGAUAGUCCUUGUUUUUAAUU		5225	5245	AAUUAAAACAAGGACUAUCUGGG		5223	5245
AD-2011372	ACACUGUAUAUAUUUCUUGCU		4322	4342	AGCAAGAAAUAUAUACAGUGUCC		4320	4342
AD-2011373	AACUUAAUAAAGAUAUUGUGU		5341	5361	ACACAAUAUCUUUAUUAAGUUGG		5339	5361
AD-2011374	UUUCUAUUUGUGUCUCCUCUU		5056	5076	AAGAGGAGACACAAAUAGAAAUA		5054	5076
AD-2011375	GCUUACAAAUGUUUGCCAUUU		3524	3544	AAAUGGCAAACAUUUGUAAGCCU		3522	3544
AD-2011376	CUGUGAUUUUUGUUCUCUUCU		3715	3735	AGAAGAGAACAAAAAUCACACGGA		3713	3735
AD-2011377	UGAUCGAAGCUAUCAACAAUU		1842	1862	AAUUGUUGAUAGCUUCGAUCAGU		1840	1862
AD-2011378	AUGCCUGUUUAUGCUGUUCAU		4831	4851	AUGAACAGCAUAAACAGGCAUGU		4829	4851
AD-2011379	AGAAUUUCCCAGUUUAAAACU		3634	3654	AGUUUUAAACUGGGAAAUUCUAA		3632	3654
AD-2011380	GUAGAUCAAGUUUGUCUUCCU		4202	4222	AGGAAGACAAACUUGAUCUACAG		4200	4222
AD-2011381	ACAACCCUUCCUCUUUUCUCU		3853	3873	AGAGAAAAGAGGAAGGGUUGUUU		3851	3873
AD-2011382	AGUGAAAUUUGCAUAAUGAAU		3162	3182	AUUCAUUAUGCAAAUUUCACUUC		3160	3182
AD-2011383	UUAGGAUAUAUUCACAUUGUU		2961	2981	AACAAUGUGAAUAUAUCCUAAAG		2959	2981
AD-2011384	UCUUAGAUAUUAUUGCUAGUU		3905	3925	AACUAGCAAUAAUAUCUAAGAUG		3903	3925
AD-2011385	CUGGUUAAAAUGGAUGAUUUU		2772	2792	AAAAUCAUCCAUUUUAACCAGGA		2770	2792
AD-2011386	CUUCCCUGAAGUGCUUUACAU		3731	3751	AUGUAAAGCACUUCAGGGAAGAG		3729	3751
AD-2011387	CACCACUGUCUUGAUGCUCUU		5257	5277	AAGAGCAUCAAGACAGUGGUGAG		5255	5277

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011388	ACAACUCUUGUUUUGCUGUUU		5483	5503	AAACAGCAAAACAAGAGUUGUAU		5481	5503
AD-2011389	CCUUUCGUUUGCUUUCUUAUU		3057	3077	AAUAAGAAAGCAAACGAAAGGAA		3055	3077
AD-2011390	GGGCAAAAUCACUUAUGAAAU		2838	2858	AUUUCAUAAGUGAUUUUGCCCAC		2836	2858
AD-2011391	AACACUUUGAUGUUAUCAUUU		4703	4723	AAAUGAUAACAUCAAAGUGUUAC		4701	4723
AD-2011392	UUCCUCCAAGGAGUUAGAAUU		3619	3639	AAUUCUAACUCCUUGGAGGAAGA		3617	3639
AD-2011393	AAGUGUUUUGAACUUGAUCUU		3549	3569	AAGAUCAAGUUCAAAACACUUAU		3547	3569
AD-2011394	UGGCCUCAUUUUUCUCUUUUU		4060	4080	AAAAAGAGAAAAAUGAGGCCAUU		4058	4080
AD-2011395	AGGUAUUUGCAUUGUUUAAAU		3328	3348	AUUUAAACAAUGCAAAUACCUCC		3326	3348
AD-2011396	CCUAAUAUUGUGCGACUUCAU		881	901	AUGAAGUCGCACAAUAUUAGGGU		879	901
AD-2011397	ACACCAAAUGAAGUGGUCAUU		5582	5602	AAUGACCACUUCAUUUGGUGUCU		5580	5602
AD-2011398	GUAGCAAAUCAGUAUAUUCUU		5631	5651	AAGAAUAUACUGAUUUGCUACUA		5629	5651
AD-2011399	CGAAAGCAAGAGAUUAUCAAU		1808	1828	AUUGAUAAUCUCUUGCUUUCGUG		1806	1828
AD-2011400	CCAACACAGACUCUAUCAGCU		1324	1344	AGCUGAUAGAGUCUGUGUUGGUC		1322	1344
AD-2011401	GACUAGAAUGAUAUUUGAUAU		5381	5401	AUAUCAAAUAUCAUUCUAGUCAC		5379	5401
AD-2011402	GCCUGAGAAUUUGCUUUUAGU		1084	1104	ACUAAAAGCAAAUUCUCAGGCUU		1082	1104
AD-2011403	CAAAACAAUGGUCUCUUCUGU		4555	4575	ACAGAAGAGACCAUUGUUUUGCU		4553	4575
AD-2011404	GAUAAACAACAAAGCCAACGU		1654	1674	ACGUUGGCUUUGUUGUUUAUCUU		1652	1674
AD-2011405	UUUUUUAAUACGAACCUGUCU		4678	4698	AGACAGGUUCGUAUUAAAAAAAA		4676	4698
AD-2011406	GGUUUAUAAUUCUGCUUAAGU		3273	3293	ACUUAAGCAGAAUUAUAAACCUU		3271	3293
AD-2011407	AGGAUAAAAGCAAGUUCCUCU		3351	3371	AGAGGAACUUGCUUUUAUCCUAA		3349	3371
AD-2011408	UGUAGAGUGUUUUUUUUUACAU		4148	4168	AUGUAAAAAAAAAACACUCUACAUC		4146	4168
AD-2011409	UUCUCUGAAUGACAGUUGUAU		2638	2658	AUACAACUGUCAUUCAGAGAACA		2636	2658
AD-2011410	AUUGUAUUUACCUCUCCUGUU		5012	5032	AACAGGAGAGGUAAAUACAAUCU		5010	5032
AD-2011411	UGGCAUACAUGUAUUUAAAGU		4115	4135	ACUUUAAAUACAUGUAUGCCACG		4113	4135
AD-2011412	UUGUCAAGAUUUUAAAGGUUU		3257	3277	AAACCUUUAAAAAUCUUGACAACA		3255	3277

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011413	UAUGAAAAAUUCCUACUGGACU		763	783	AGUCCAGUAGGAAUUUUCAUACA		761	783
AD-2011414	CCUCUUUGUGGCAAAACAUCU		2250	2270	AGAUGUUUUGCCACAAAGAGGUG		2248	2270
AD-2011415	CAGUCAGAAGAGACUCGUGUU		2114	2134	AACACGAGUCUCUUCUGACUGCA		2112	2134
AD-2011416	AAGUCACUGAACAACUGAUCU		1827	1847	AGAUCAGUUGUUCAGUGACUUUG		1825	1847
AD-2011417	GGAGCUUAUGAUUUUCCAUCU		1355	1375	AGAUGGAAAAAUCAUAAGCUCCAG		1353	1375
AD-2011418	GUGCGGAUCGUUUCGCAACUU		278	298	AAGUUGCGAAACGAUCCGCACUG		276	298
AD-2011419	AAUCUGCCGUCUUUUGAAGCU		859	879	AGCUUCAAAAGACGGCAGAUUCU		857	879
AD-2011420	UACUUCUUUUCCCGACUUCUU		4893	4913	AAGAAGUCGGGAAAAGAAGUAGG		4891	4913
AD-2011421	CUACUGUAAUCCACAACCCUU		1725	1745	AAGGGUUGUGGAUUACAGUAGUU		1723	1745
AD-2011422	UUUGUUCCAUUCUUUUCUUAU		3083	3103	AUAAGAAAAGAAUGGAACAAAAG		3081	3103
AD-2011423	GAAGAUGUGAAAGCACGAAAU		1793	1813	AUUUCGUGCUUUCACAUCUUCAU		1791	1813
AD-2011424	CAGUAAAUAUAUUGAGCCAUU		4007	4027	AAUGGCUCAAUAUAUUUACUGUA		4005	4027
AD-2011425	AAUGGGAAAGAAAACUUCUCU		2222	2242	AGAGAAGUUUUUCUUUCCCAUUUG		2220	2242
AD-2011426	GGCAGAAUGUUCAUUUUCAUU		2157	2177	AAUGAAAAAUGAACAUUCUGCCAC		2155	2177
AD-2011427	CGCCUGCAUAGCAUAUAUUAU		2050	2070	AUAAUAUAUGCUAUGCAGGCGGC		2048	2070
AD-2011428	CUGGCUGUUUUUCCAUUUCCU		318	338	AGGAAAUGGAAAAACAGCCAGGC		316	338
AD-2011429	AGGGAAAUAAAAAGUCUUUUGU		3188	3208	ACAAAAGACUUUUAUUUCCCUUU		3186	3208
AD-2011430	CCACCCUUUCUGGUCAUCUCU		481	501	AGAGAUGACCAGAAAGGGUGGCG		479	501
AD-2011431	UGCCAUAAUGGUAAAGGACUU		4339	4359	AAGUCCUUUACCAUUAUGGCAAG		4337	4359
AD-2011432	CAAACUGUGUAAACUGGAAAU		2912	2932	AUUUCCAGUUUACACAGUUUGAC		2910	2932
AD-2011433	UAAACCCUCAUGUACAUCUGU		2016	2036	ACAGAUGUACAUGAGGGUUUAGA		2014	2036
AD-2011434	UUUUGUGAAGCAGCUAUACGU		4095	4115	ACGUAUAGCUGCUUCACAAAAGG		4093	4115
AD-2011435	AUGGAGAUAUCAUUGAUAAAU		5151	5171	AUUUAUCAAUGAUAUCUCCAUUC		5149	5171
AD-2011436	GUGUAUUCAUCAUUGCAUUCU		5108	5128	AGAAUGCAAUGAUGAAUACACAG		5106	5128
AD-2011437	GAAGCUUUGGGUAAUUUAGUU		1919	1939	AACUAAAUUACCCAAAGCUUCAG		1917	1939

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011438	GCUGUGAAAUUGGCAGACUUU		1124	1144	AAAGUCUGCCAAUUUCACAGCUG		1122	1144
AD-2011439	UAACAAUUAAAGUGGGAUGAU		2813	2833	AUCAUCCCACUUUAAUUGUUAAU		2811	2833
AD-2011440	GCUGGCUACAAGGAAUUUCUU		1594	1614	AAGAAAUUCCUUGUAGCCAGCAU		1592	1614
AD-2011441	UGUUAUUAUUCUUUGCUCACU		5455	5475	AGUGAGCAAAGAAUAAUAACAAU		5453	5475
AD-2011442	AUAACAAUUUGCACUUGGUGU		4506	4526	ACACCAAGUGCAAAUUGUUAUGU		4504	4526
AD-2011443	CUUCCGCACUAAGAUGUGAGU		4217	4237	ACUCACAUCUUAGUGCGGAAGAC		4215	4237
AD-2011444	CUCUCAUCUCACCUCUCUGUU		3870	3890	AACAGAGAGGUGAGAUGAGAGAA		3868	3890
AD-2011445	CACCCAUGGAUCUGUCAACGU		1475	1495	ACGUUGACAGAUCCAUGGGUGCU		1473	1495
AD-2011446	AUAAAAUUCUACUGACUUCUU		5761	5781	AAGAAGUCAGUAGAAUUUUAUUC		5759	5781
AD-2011447	UGCCAUCUUGACAACUAUGCU		1576	1596	AGCAUAGUUGUCAAGAUGGCACC		1574	1596
AD-2011448	ACUUAGGUAUCCUAACUAUGU		3304	3324	ACAUAGUUAGGAUACCUAAGUCC		3302	3324
AD-2011449	CUGCUUCUACUCCUCCUGCUU		255	275	AAGCAGGAGGAGUAGAAGCAGAG		253	275
AD-2011450	UGAGGAAAUUGUGAUUUGUUU		4233	4253	AAACAAAUCACAAUUUCCUCACA		4231	4253
AD-2011451	GACGAGUAUCAGCUUUUCGAU		704	724	AUCGAAAAGCUGAUACUCGUCCG		702	724
AD-2011452	UCCCUCUGACCCUCAGUUUCU		5292	5312	AGAAACUGAGGGUCAGAGGGAAG		5290	5312
AD-2011453	GCCCUUUUGAUAACAGAAGCU		5192	5212	AGCUUCUGUUAUCAAAAGGGCUU		5190	5212
AD-2011454	AGAAAUUAAAUGGUAGCAGUU		5400	5420	AACUGCUACCAUUUAAUUUCUAU		5398	5420
AD-2011455	GAUGGAUUUUCACCGAUUCUU		1945	1965	AAGAAUCGGUGAAAAUCCAUCCC		1943	1965
AD-2011456	ACAUCACACUUGCUCACAUGU		4814	4834	ACAUGUGAGCAAGUGUGAUGUUU		4812	4834
AD-2011457	UCUAAGGCCUGAAAACCAUUU		2268	2288	AAAUGGUUUUUCAGGCCUUAGAUG		2266	2288
AD-2011458	UUACGUAAAGAUCCUUAUGGU		1226	1246	ACCAUAAGGAUCUUUACGUAAAA		1224	1246
AD-2011459	UACAGUGAAGCUGAUGCCAGU		992	1012	ACUGGCAUCAGCUUCACUGUAGU		990	1012
AD-2011460	AUUUGCUGAAUUGAAUUGUUU		2989	3009	AAACAAUUCAAUUCAGCAAAUUC		2987	3009
AD-2011461	UAAGCUCUAGUUUUGGACUUAU		3289	3309	AUAAGUCCAAACUAGAGCUUAAG		3287	3309
AD-2011462	CUGGCUAGUAGUGUGUGAGAU		3132	3152	AUCUCACACACUACUAGCCAGCC		3130	3152

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2011463	AUGUGAUGCAUCAUCUUAUCU		2435	2455	AGAUAAGAUGAUGCAUCACAUAU		2433	2455
AD-2011464	CCUACAUGUAAUGCAUAUGUU		2419	2439	AACAUAUGCAUUACAUGUAGGAC		2417	2439
AD-2011465	CAGCAGCCAAGAGUUUGUUGU		1614	1634	ACAACAAACUCUUGGCUGCUGAG		1612	1634
AD-2011466	UCCAAUCCAGCCUUCACAUGU		4642	4662	ACAUGUGAAGGCUGGAUUGGAGG		4640	4662
AD-2011467	ACCAACAGUACCCAUCAAGCU		2188	2208	AGCUUGAUGGGUACUGUUGGUGA		2186	2208
AD-2011468	CUGCUAGGGAUCAUCAGAAAU		822	842	AUUUCUGAUGAUCCCUAGCAGAA		820	842
AD-2011469	GGUUUGCUACCACAUAAAGCU		2877	2897	AGCUUUAUGUGGUAGCAAACCAA		2875	2897
AD-2011470	AGUGUACAGCAUCAUGCUCCU		4931	4951	AGGAGCAUGAUGCUGUACACUGA		4929	4951
AD-2011471	UGGCUUCGACCACAACCUGCU		672	692	AGCAGGUUGUGGUCGAAGCCAUC		670	692
AD-2011472	CCAGUCAUUGCAUUCAGCAGU		1008	1028	ACUGCUGAAUGCAAUGACUGGCA		1006	1028
AD-2011473	CUUCCAUGAUGCACAGACAGU		1506	1526	ACUGUCUGUGCAUCAUGGAAGCA		1504	1526
AD-2011474	CAGGAGGAUACCAACUUGAUU		3407	3427	AAUCAAGUUGGUAUCCUCCUGGC		3405	3427
AD-2011475	UGAUGGAAACAAGGAGUCAAU		1744	1764	AUUGACUCCUUGUUUCCAUCAGG		1742	1764
AD-2011476	AAGCAAGAAUCAGUUGGUUUU		2862	2882	AAAACCAACUGAUUCUUGCUUCU		2860	2882
AD-2011477	CAACUGCUUGCCACUCGUCCU		293	313	AGGACGAGUGGCAAGCAGUUGCG		291	313
AD-2011478	GUGUUGAAGUAUUACUGUAGU		4186	4206	ACUACAGUAAUACUUCAACACCA		4184	4206
AD-2011479	UUACUGUUGGCAAAACAAUAU		2517	2537	AUAUUGUUUUGCCAACAGUAAUU		2515	2537
AD-2011480	CAGUGUGUCCUACUCUGGUCU		4475	4495	AGACCAGAGUAGGACACACUGCC		4473	4495
AD-2011481	AGAAACCAGAUGGAGUAAAGU		1635	1655	ACUUUACUCCAUCUGGUUUCUUC		1633	1655
AD-2011482	GCUCCACAGCAAACCUUCCUU		4946	4966	AAGGAAGGUUUGCUGUGGAGCAU		4944	4966
AD-2011483	GACUUUGAAGCCUACACAAAU		1865	1885	AUUUGUGUAGGCUUCAAAGUCCC		1863	1885

Table 42. Modified Sense and Antisense Strand Sequences of CAMK2D dsRNA Agents Comprising an Unsaturated C22 Hydrocarbon Chain Conjugated to Position 6 on the Sense Strand, Counting from the 5'-end of the Sense Strand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011304	cscsaca(Cda)AfaAfCfUfuaaauuacuuL96		asAfsguaAfuUfUfaaguUfuGfuguggsasa		UUCCACACAAACUUAAAUUACUG	
AD-2011305	usasgca(Cda)UfuUfUfGfaucaucuucuL96		asGfsaagAfuGfAfucaaAfaGfugcuasgsu		ACUAGCACUUUUGAUCAUCUUCA	
AD-2011306	cscsuca(Uda)CfaAfUfAfaaaugcuuauL96		asUfsaagCfaUfUfuuauUfgAfugaggsusc		GACCUCAUCAAUAAAAUGCUUAC	
AD-2011307	uscscau(Cda)AfuUfGfUfuauuuuaacuL96		asGfsuuaAfaAfUfaacaAfuGfauggasgsa		UCUCCAUCAUUGUUAUUUUAACG	
AD-2011308	uscscac(Ada)CfuAfUfUfauucuaaacuL96		asGfsuuuAfgAfAfuaauAfgUfguggasusu		AAUCCACACUAUUAUUCUAAACC	
AD-2011309	csusccu(Ada)AfaAfGfCfuucucuaaauL96		asUfsuuaGfaGfAfagcuUfuUfaggagsusc		GACUCCUAAAAGCUUCUCUAAAC	
AD-2011310	gsgsucu(Cda)AfuUfUfAfguacauaacuL96		asGfsuuaUfgUfAfcuaaAfuGfagaccsasg		CUGGUCUCAUUUAGUACAUAACA	
AD-2011311	csasgac(Uda)UfaAfGfAfacuauuguuuL96		asAfsacaAfuAfGfuucuUfaAfgucugsusu		AACAGACUUAAGAACUAUUGUUC	
AD-2011312	csasuca(Ada)GfuUfUfCfucuguuaauuL96		asAfsuuaAfcAfGfagaaAfcUfugaugsasa		UUCAUCAAGUUUCUCUGUUAAUG	
AD-2011313	asusucu(Cda)UfaUfAfUfucuacuuguuL96		asAfscaaGfuAfGfaauaUfaGfagaausgsa		UCAUUCUCUAUAUUCUACUUGUG	
AD-2011314	csusgcu(Uda)GfaAfGfAfaauuuaauguL96		asCfsauuAfaAfUfuucuUfcAfagcagsusc		GACUGCUUGAAGAAAUUUAAUGC	
AD-2011315	csascug(Ada)CfaUfUfAfauauuccuauL96		asUfsaggAfaUfAfuuaaUfgUfcagugsasu		AUCACUGACAUUAAUAUUCCUAU	
AD-2011316	asascca(Cda)AfuUfGfUfuuugaaaauuL96		asAfsuuuUfcAfAfaacaAfuGfugguusgsa		UCAACCACAUUGUUUUGAAAAUA	
AD-2011317	uscsauc(Ada)CfuAfGfCfuaguuuucuuL96		asAfsgaaAfaCfUfagcuAfgUfgaugasusg		CAUCAUCACUAGCUAGUUUUCUA	
AD-2011318	ususacu(Uda)UfuUfCfCfuaagauucauL96		asUfsgaaUfcUfUfaggaAfaAfaguaasgsa		UCUUACUUUUUCCUAAGAUUCAG	
AD-2011319	ususacc(Uda)AfgAfAfUfaaauagucuuL96		asAfsgacUfaUfUfuauuCfuAfgguaasgsa		UCUUACCUAGAAUAAAUAGUCUC	
AD-2011320	gsgsucu(Uda)CfuAfAfAfuuucaacaguL96		asCfsuguUfgAfAfauuuAfgAfagaccscsa		UGGGUCUUCUAAAUUUCAACAGU	
AD-2011321	cscsuau(Ada)CfaUfUfGfuuuacacuuuL96		asAfsaguGfuAfAfacaaUfgUfauaggsasa		UUCCUAUACAUUGUUUACACUUC	
AD-2011322	uscsucu(Uda)GfaAfUfCfagaguaucauL96		asUfsgauAfcUfCfugauUfcAfagagasgsa		UCUCUCUUGAAUCAGAGUAUCAU	
AD-2011323	gscsuga(Gda)AfaCfAfAfuauaguuaauL96		asUfsuaaCfuAfUfauugUfuCfucagcsasa		UUGCUGAGAACAAUAUAGUUAAC	
AD-2011324	csgsucc(Ada)UfcUfUfUfauuucuucauL96		asUfsgaaGfaAfAfuaaaGfaUfggacgscsa		UGCGUCCAUCUUUAUUUCUUCAG	
AD-2011325	asgscuu(Cda)UfuUfUfGfauuugcuaauL96		asUfsuagCfaAfAfucaaAfaGfaagcusgsa		UCAGCUUCUUUUGAUUUGCUAAA	
AD-2011326	asuscuc(Uda)UfuUfCfUfuuauccuguuL96		asAfscagGfaUfAfaagaAfaAfgagauscsa		UGAUCUCUUUUCUUUAUCCUGUU	
AD-2011327	csascaa(Ada)UfgAfAfUfuacacauuuuL96		asAfsaauGfuGfUfaauuCfaUfuugugsgsu		ACCACAAAUGAAUUACACAUUUA	
AD-2011328	ususugu(Cda)CfaAfAfAfgcaauaaacuL96		asGfsuuuAfuUfGfcuuuUfgGfacaaasgsc		GCUUUGUCCAAAAGCAAUAAACC	
AD-2011329	uscsuga(Gda)UfuUfAfAfucacuuuaguL96		asCfsuaaAfgUfGfauuaAfaCfucagasasa		UUUCUGAGUUUAAUCACUUUAGG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011330	cscsaag(Uda)AfcAfAfCfucuucaucauL96		asUfsgauGfaAfGfaguuGfuAfcuuggsasa		UUCCAAGUACAACUCUUCAUCAA	
AD-2011331	ususcua(Cda)UfuUfGfAfaaaugcuuuuL96		asAfsaagCfaUfUfuucaAfaGfuagaasusc		GAUUCUACUUUGAAAAUGCUUUG	
AD-2011332	usasuca(Uda)AfuAfUfUfccuuccuauuL96		asAfsuagGfaAfGfgauuAfuAfugauasasg		CUUAUCAUAUAUUCCUUCCUAUA	
AD-2011333	cscsucu(Cda)AfaAfAfCfcuacauaaauuL96		asAfsuuaUfgUfAfgguuUfuGfagagggsga		UCCCUCUCAAAACCUACAUAAUA	
AD-2011334	asgsuua(Ada)UfuGfUfUfcucugugaguL96		asCfsucaCfaGfAfgaacAfaUfuaacususg		CAAGUUAAUUGUUCUCUGUGAGC	
AD-2011335	ususcua(Uda)CfaUfUfUfugaaaacacuuL96		asAfsgugUfuUfCfaaaaUfgAfuagaasgsa		UCUUCUAUCAUUUUGAAACACUG	
AD-2011336	csasgcu(Uda)GfaAfCfUfugagcauacuL96		asGfsuauGfcUfCfaaguUfcAfagcugsgsg		CCCAGCUUGAACUUGAGCAUACA	
AD-2011337	uscsacc(Cda)AfaAfUfUfuugaauuuuuL96		asAfsaaaUfuCfAfaaauUfuGfggugasasg		CUUCACCCAAAUUUUGAAUUUUU	
AD-2011338	gscsagu(Ada)UfaAfCfUfauucugaucuL96		asGfsaucAfgAfAfuaguUfaUfacugcsusa		UAGCAGUAUAACUAUUCUGAUCA	
AD-2011339	gsgsuuu(Uda)CfuUfUfGfugcuucucauL96		asUfsgagAfaGfGfCfacaaAfgAfaaaccsusa		UAGGUUUUCUUUGUGCUUCUCAU	
AD-2011340	asusgag(Ada)AfuAfAfAfAfcauaccaacuL96		asGfsuugGfuAfUfguuuAfuUfcucaususu		AAAUGAGAAUAAACAUACCAACU	
AD-2011341	gsasgug(Ada)UfuUfCfAfuugaauaaauL96		asUfsuuaUfuCfAfaugaAfaUfcacucsgsa		UCGAGUGAUUUCAUUGAAUAAAA	
AD-2011342	gsusuuc(Cda)AfuAfUfUfuguaaaauguL96		asCfsauuUfuAfCfaaauAfuGfgaaacsusg		CAGUUUCCAUAUUUGUAAAAUGA	
AD-2011343	csasugc(Uda)GfuUfUfUfugucaaacuL96		asGfsuuuGfaCfCfaaaaAfcAfgcaugsgsc		GCCAUGCUGUUUUUGGUCAAACU	
AD-2011344	csuscua(Cda)UfgCfAfGfuauaugucuL96		asGfsacaUfuAfUfacugCfaGfuagagsgsa		UCCUCUACUGCAGUAUAAUGUCU	
AD-2011345	ususggu(Gda)UfuUfGfAfuuuaguuacuL96		asGfsuaaCfuAfAfaucaAfaCfaccaasgsu		ACUUGGUGUUUGAUUUAGUUACU	
AD-2011346	uscsuuu(Cda)UfcCfAfGfaaguuuuacuL96		asGfsuaaAfaCfUfucugGfaGfaaagasusa		UAUCUUUCUCCAGAAGUUUUACG	
AD-2011347	asgsagu(Uda)CfaAfAfUfacaacaauuuL96		asAfsauuGfuUfGfuauuUfgAfacucuscsa		UGAGAGUUCAAAUACAACAAUUG	
AD-2011348	uscsugu(Gda)UfuGfAfUfuacuaaucauL96		asUfsgauUfaGfUfaaucAfaCfacagasgsa		UCUCUGUGUUGAUUACUAAUCAU	
AD-2011349	usgscuu(Uda)UfuUfAfGfcuucucucuuL96		asAfsgagAfgAfAfgcuaAfaAfaagcascsa		UGUGCUUUUUUAGCUUCUCUCUU	
AD-2011350	csusgug(Ada)CfuGfCfAfacgucuuacuL96		asGfsuaaGfaCfCfguugcAfgUfcacaggsga		UCCUGUGACUGCAACGUCUUACU	
AD-2011351	csascuu(Cda)UfgCfAfUfucucuguucuL96		asGfsaacAfgAfGfaaugCfaGfaagugsgsc		GCCACUUCUGCAUUCUCUGUUCU	
AD-2011352	csuscau(Uda)UfuUfCfCfuuuguugauuL96		asAfsucaAfcAfAfaggaAfaAfaugagsasa		UUCUCAUUUUUCCCUUUGUUGAUU	
AD-2011353	cscsuca(Gda)AfaGfCfCfuauuuuaaauL96		asUfsuaaAfaAfUfaggcUfuCfugaggsasc		GUCCUCAGAAGCCUAUUUUUAAA	
AD-2011354	asusagu(Ada)AfuUfCfAfcaguccucauL96		asUfsgagGfaCfUfgugaAfuUfacuauscsg		CGAUAGUAAUUCACAGUCCUCAG	
AD-2011355	ususucu(Cda)AfaAfUfCfugugaucucuL96		asGfsagaUfcAfCfagauUfuGfagaaasgsa		UCUUUCUCAAAUCUGUGAUCUCU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011356	gscsugc(Cda)AfaAfAfUfuaucaacacuL96		asGfsuguUfgAfUfaauuUfuGfgcagcsasu		AUGCUGCCAAAAUUAUCAACACC	
AD-2011357	gsascug(Ada)UfuGfAfUfauauuuaaguL96		asCfsuuaAfaUfAfuaucAfaUfcagucscsu		AGGACUGAUUGAUAUAUUUAAGA	
AD-2011358	usascac(Uda)UfuUfAfAfcuuagcauguL96		asCfsaugCfuAfAfguuaAfaAfguguasasa		UUUACACUUUUAACUUAGCAUGU	
AD-2011359	uscsuga(Gda)CfaAfGfUfuaccucuucuL96		asGfsaagAfgGfUfaacuUfgCfucagasgsc		GCUCUGAGCAAGUUACCUCUUCC	
AD-2011360	gscsccu(Cda)UfuUfAfUfguagguuuauL96		asUfsaaaCfcUfAfcauaAfaGfagggcsasg		CUGCCCUCUUUAUGUAGGUUUAC	
AD-2011361	csasagg(Ada)AfuAfUfUfaaucuucacuL96		asGfsugaAfgAfUfuaauAfuUfccuugsasa		UUCAAGGAAUAUUAAUCUUCACC	
AD-2011362	asusguu(Gda)UfuUfAfUfguuucuuacuL96		asGfsuaaGfaAfAfcauaAfaCfaacausgsa		UCAUGUUGUUUAUGUUUCUUACC	
AD-2011363	asusacu(Cda)GfgAfCfUfcaaaugucuuL96		asAfsgacAfuUfUfgaguCfcGfaguausasu		AUAUACUCGGACUCAAAUGUCUC	
AD-2011364	usgsaag(Cda)UfuUfUfCfugauaauuauL96		asUfsaauUfaUfCfagaaAfaGfcuucasasc		GUUGAAGCUUUUCUGAUAAUUAU	
AD-2011365	gsasacu(Cda)UfuUfGfCfuguuaaucuuL96		asAfsgauUfaAfCfagcaAfaGfaguucsusa		UAGAACUCUUUGCUGUUAAUCUG	
AD-2011366	ususgaa(Ada)GfuUfUfUfgcugauuaauL96		asUfsuaaUfcAfGfcaaaAfcUfuucaasusg		CAUUGAAAGUUUUGCUGAUUAAC	
AD-2011367	asusgcu(Ada)GfaAfGfAfaaacuaaaguL96		asCfsuuuAfgUfUfuucuUfcUfagcaususa		UAAUGCUAGAAGAAAACUAAAGG	
AD-2011368	cscsucu(Uda)CfaUfGfCfauguuucuguL96		asCfsagaAfaCfAfugcaUfgAfagaggsasg		CUCCUCUUCAUGCAUGUUUCUGA	
AD-2011369	usascug(Cda)UfuUfUfGfaaccugaaguL96		asCfsuucAfgGfUfucaaAfaGfcaguasasg		CUUACUGCUUUUGAACCUGAAGC	
AD-2011370	csasacg(Uda)UfcUfAfCfuguugcuucuL96		asGfsaagCfaAfCfaguaGfaAfcguugsasc		GUCAACGUUCUACUGUUGCUUCC	
AD-2011371	csasgau(Ada)GfuCfCfUfuguuuuaauuL96		asAfsuuaAfaAfCfaaggAfcUfaucugsgsg		CCCAGAUAGUCCUUGUUUUAAUG	
AD-2011372	ascsacu(Gda)UfaUfAfUfauuucuugcuL96		asGfscaaGfaAfAfuauaUfaCfaguguscsc		GGACACUGUAUAUAUUUCUUGCC	
AD-2011373	asascuu(Ada)AfuAfAfAfgauauuguguL96		asCfsacaAfuAfUfcuuuAfuUfaaguusgsg		CCAACUUAAUAAAGAUAUUGUGA	
AD-2011374	ususucu(Ada)UfuUfGfUfgucuccucuuL96		asAfsgagGfaGfAfcacaAfaUfagaaasusa		UAUUUCUAUUUGUGUCUCCUCUA	
AD-2011375	gscsuua(Cda)AfaAfUfGfuuugccauuuL96		asAfsaugGfcAfAfacauUfuGfuaagcscsu		AGGCUUACAAAUGUUUGCCAUUC	
AD-2011376	csusgug(Ada)UfuUfUfUfguucucuucuL96		asGfsaagAfgAfAfcaaaAfaUfcacagsgsa		UCCUGUGAUUUUUGUUCUCUUCC	
AD-2011377	usgsauc(Gda)AfaGfCfUfaucaacaauuL96		asAfsuugUfuGfAfuagcUfuCfgaucasgsu		ACUGAUCGAAGCUAUCAACAAUG	
AD-2011378	asusgcc(Uda)GfuUfUfAfugcuguucauL96		asUfsgaaCfaGfCfauaaAfcAfggcausgsu		ACAUGCCUGUUUAUGCUGUUCAU	
AD-2011379	asgsaau(Uda)UfcCfCfAfguuuaaaacuL96		asGfsuuuUfaAfAfcuggGfaAfauucusasa		UUAGAAUUUCCCAGUUUAAAACA	
AD-2011380	gsusaga(Uda)CfaAfGfUfuugucuuccuL96		asGfsgaaGfaCfAfaacuUfgAfucuacsasg		CUGUAGAUCAAGUUUGUCUUCCG	
AD-2011381	ascsaac(Cda)CfuUfCfCfucuuuucucuL96		asGfsagaAfaAfGfaggaAfgGfguugususu		AAACAACCCUUCCUCUUUUCUCU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011382	asgsuga(Ada)AfuUfUfGfcauaaugaauL96		asUfsucaUfuAfUfUfgcaaAfuUfucacususc		GAAGUGAAAUUUGCAUAAUGAAU	
AD-2011383	ususagg(Ada)UfaUfAfUfucacauuguuL96		asAfscaaUfgUfUfGfaauaUfaUfccuaasasg		CUUUAGGAUAUAUUCACAUUGUU	
AD-2011384	uscsuua(Gda)AfuAfUfUfauugcuaguuL96		asAfscuaGfcAfAfuaauAfuCfuaagasusg		CAUCUUAGAUAUUAUUGCUAGUG	
AD-2011385	csusggu(Uda)AfaAfAfUfggaugauuuuL96		asAfsaauCfaUfUfCfcauuUfuAfaccagsgsa		UCCUGGUUAAAAUGGAUGAUUUU	
AD-2011386	csusucc(Cda)UfgAfAfGfUfugcuuuacauL96		asUfsguaAfaGfUfCfacuuCfaGfggaagsasg		CUCUUCCCUGAAGUGCUUUACAG	
AD-2011387	csascca(Cda)UfgUfCfUfUfgaugcucuuL96		asAfsgagCfaUfUfCfaagaCfaGfuggugsasg		CUCACCACUGUCUUGAUGCUCUG	
AD-2011388	ascsaac(Uda)CfuUfGfUfUfuuugcuguuuL96		asAfsacaGfcAfAfaacaAfgAfguugusasu		AUACAACUCUUGUUUUGCUGUUG	
AD-2011389	cscsuuu(Cda)GfuUfUfGfUfcuuucuuauuL96		asAfsuaaGfaAfAfgcaaAfcGfaaaggsasa		UUCCUUUCGUUUGCUUUCUUAUU	
AD-2011390	gsgsggca(Ada)AfaUfCfAfcuuaugaaauL96		asUfsuucAfuAfAfgugaUfuUfugcccsasc		GUGGGCAAAAUCACUUAUGAAAG	
AD-2011391	asascac(Uda)UfuGfAfUfguuaucauuuL96		asAfsaugAfuAfAfcaucAfaAfgguusasc		GUAACACUUUGAUGUUAUCAUUU	
AD-2011392	ususccu(Cda)CfaAfGfGfgfaguuagaauuL96		asAfsuucUfaAfCfuccuUfgGfaggaasgsa		UCUUCCUCCAAGGAGUUAGAAUU	
AD-2011393	asasgug(Uda)UfuUfGfAfacuugaucuuL96		asAfsgauCfaAfGfuucaAfaAfcacuusasu		AUAAGUGUUUUGAACUUGAUCUU	
AD-2011394	usgsgcc(Uda)CfaUfUfUfUfuucuuuuuuL96		asAfsaaaGfaGfAfaaaaUfgAfggccasusu		AAUGGCCUCAUUUUUCUCUUUUU	
AD-2011395	asgsgua(Uda)UfuGfCfAfuuguuuaaauL96		asUfsuuaAfaCfAfaugcAfaAfuaccuscsc		GGAGGUAUUUGCAUUGUUUAAAG	
AD-2011396	cscsuaa(Uda)AfuUfGfUfgcgacuucauL96		asUfsgaaGfuCfGfcacaAfuAfuuaggsgsu		ACCCUAAUAUUGUGCGACUUCAU	
AD-2011397	ascsacc(Ada)AfaUfGfAfaguggucauuL96		asAfsugaCfcAfCfuucaUfuUfggugusccsu		AGACACCAAAUGAAGUGGUCAUC	
AD-2011398	gsusagc(Ada)AfaUfCfAfguauauucuuL96		asAfsgaaUfaUfAfcugaUfuUfgcuacsusa		UAGUAGCAAAUCAGUAUAUUCUA	
AD-2011399	csgsaaa(Gda)CfaAfGfAfgauuaucaauL96		asUfsugaUfaAfUfcucuUfgCfuuucgsusg		CACGAAAGCAAGAGAUUAUCAAA	
AD-2011400	cscsaac(Ada)CfaGfAfCfucuaucagcuL96		asGfscugAfuAfGfagucUfgUfguuggsusc		GACCAACACAGACUCUAUCAGCA	
AD-2011401	gsascua(Gda)AfaUfGfAfuauuugauauL96		asUfsaucAfaAfUfaucaUfuCfuagucsasc		GUGACUAGAAUGAUAUUUGAUAG	
AD-2011402	gscscug(Ada)GfaAfUfUfugcuuuuaguL96		asCfsuaaAfaGfGfCfaaauUfcUfcaggcsusu		AAGCCUGAGAAUUUGCUUUUAGC	
AD-2011403	csasaaa(Cda)AfaUfGfGfucucuucuguL96		asCfsagaAfgAfGfaccaUfuGfuuuugscsu		AGCAAAACAAUGGUCUCUUCUGG	
AD-2011404	gsasuaa(Ada)CfaAfCfAfaagccaacguL96		asCfsguuGfgCfUfuuguUfgUfuuaucsusu		AAGAUAAACAACAAAGCCAACGU	
AD-2011405	ususuuu(Uda)AfaUfAfCfgaaccugucuL96		asGfsacaGfgUfUfCfguaUfuAfaaaaasasa		UUUUUUUUAAUACGAACCUGUCC	
AD-2011406	gsgsuuu(Ada)UfaAfUfUfCfugcuuaaguL96		asCfsuuaAfgCfAfgaauUfaUfaaaccsusu		AAGGUUUAUAAUUCCUGCUUAAGC	
AD-2011407	asgsgau(Ada)AfaAfGfCfCfaaguuccucuL96		asGfsaggAfaCfCfUfugcuUfuUfauccusasa		UUAGGAUAAAAGCAAGUUCCUCC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011408	usgsuag(Ada)GfuGfUfUfuuuuuacauL96		asUfsguaAfaAfAfaaacAfcUfcuacasusc		GAUGUAGAGUGUUUUUUUACAC	
AD-2011409	ususcuc(Uda)GfaAfUfGfacaguuguauL96		asUfsacaAfcUfGfucauUfcAfgagaascsa		UGUUCUCUGAAUGACAGUUGUAA	
AD-2011410	asusugu(Ada)UfuUfAfCfcucuccuguuL96		asAfscagGfaGfAfgguaAfaUfacaauscsu		AGAUUGUAUUUACCUCUCCUGUC	
AD-2011411	usgsgca(Uda)AfcAfUfGfuauuuaaguL96		asCfsuuuAfaAfUfacauGfuAfugccascsg		CGUGGCAUACAUGUAUUUAAAGA	
AD-2011412	ususguc(Ada)AfgAfUfUfuaaagguuuL96		asAfsaccUfuUfAfaaauCfuUfgacaascsa		UGUUGUCAAGAUUUUAAAGGUUU	
AD-2011413	usasuga(Ada)AfaUfUfCfcuacuggacuL96		asGfsuccAfgUfAfggaaUfuUfucauascsa		UGUAUGAAAAUUCCUACUGGACA	
AD-2011414	cscsucu(Uda)UfgUfGfGfcaaaacaucuL96		asGfsaugUfuUfUfgccaCfaAfagaggsusg		CACCUCUUUGUGGCAAAACAUCU	
AD-2011415	csasguc(Ada)GfaAfGfAfgacucguguuL96		asAfscacGfaGfUfcucuUfcUfgacugscsa		UGCAGUCAGAAGAGACUCGUGUG	
AD-2011416	asasguc(Ada)CfuGfAfAfcaacugaucuL96		asGfsaucAfgUfUfUfguucAfgUfgacuususg		CAAAGUCACUGAACAACUGAUCG	
AD-2011417	gsgsagc(Uda)UfaUfGfAfuuuuccaucuL96		asGfsaugGfaAfAfaucaUfaAfgcuccsasg		CUGGAGCUUAUGAUUUUCCAUCA	
AD-2011418	gsusgcg(Gda)AfuCfGfUfuucgcaacuuL96		asAfsguuGfcGfAfaacgAfuCfcgcacsusg		CAGUGCGGAUCGUUUCGCAACUG	
AD-2011419	asasucu(Gda)CfcGfUfCfuuuugaagcuL96		asGfscuuCfaAfAfagacGfgCfagauuscsu		AGAAUCUGCCGUCUUUUGAAGCA	
AD-2011420	usascuu(Cda)UfuUfUfCfccgacuucuuL96		asAfsgaaGfuCfGfggaaAfaGfaaguasgsg		CCUACUUCUUUUUCCCGACUUCUU	
AD-2011421	csusacu(Gda)UfaAfUfCfcacaacccuuL96		asAfsgggUfuGfUfggauUfaCfaguagsusu		AACUACUGUAAUCCACACAACCCUG	
AD-2011422	ususugu(Uda)CfcAfUfUfcuuucuuauuL96		asUfsaagAfaAfAfgaauGfgAfacaaasasg		CUUUUGUUCCAUUCUUUUUCUUAC	
AD-2011423	gsasaga(Uda)GfuGfAfAfagcacgaaauL96		asUfsuucGfuGfCfuuucAfcAfucuucsasu		AUGAAGAUGUGAAAGCACGAAAG	
AD-2011424	csasgua(Ada)AfuAfUfAfuugagccauuL96		asAfsuggCfuCfAfauauAfuUfuacugsusa		UACAGUAAAUAUAUUUGAGCCAUG	
AD-2011425	asasugg(Gda)AfaAfGfAfaaacuucucuL96		asGfsagaAfgUfUfuucuUfuCfccauususg		CAAAUGGGAAAGAAAACUUCUCA	
AD-2011426	gsgscag(Ada)AfuGfUfUfcauuuucauuL96		asAfsugaAfaAfUfUfgaacAfuUfUfcugccsasc		GUGGCAGAAUGUUCAUUUUCAUC	
AD-2011427	csgsccu(Gda)CfaUfAfGfcauauauuauL96		asUfsaauAfuAfUfUfgcuaUfgCfaggcgsgsc		GCCGCCUGCAUAGCAUAUAUUAG	
AD-2011428	csusggc(Uda)GfuUfUfUfuccauuuccuL96		asGfsgaaAfuGfGfGfaaaaAfcAfgccagsgsc		GCCUGGCUGUUUUUCCAUUUCCC	
AD-2011429	asgsgga(Ada)AfuAfAfAfagucuuuuguL96		asCfsaaaAfgAfCfUfuuuuAfuUfUfcccususu		AAAGGGAAAUAAAAAGUCUUUUGA	
AD-2011430	cscsacc(Cda)UfuUfCfCfUfggucaucucuL96		asGfsagaUfgAfCfCfagaAfaGfGfgguggscsg		CGCCACCCUUUCUGGUCAUCUCC	
AD-2011431	usgscca(Uda)AfaUfGfGfGfuaaaggacuuL96		asAfsgucCfuUfUfUfaccaUfuAfuggcasasg		CUUGCCAUAAUGGUAAAGGACUG	
AD-2011432	csasaac(Uda)GfuGfUfAfaacuggaaauL96		asUfsuucCfaGfUfUfuacAfcAfguuugsasc		GUCAAACUGUGUAAACUGGAAAA	
AD-2011433	usasaac(Cda)CfuCfAfUfguacaucuguL96		asCfsagaUfgUfAfcaugAfgGfguuuasgsa		UCUAAACCCUCAUGUACAUCUGG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011434	ususuug(Uda)GfaAfGfCfagcuauacguL96		asCfsguaUfaGfCfugcuUfcAfcaaasgsg		CCUUUUGUGAAGCAGCUAUACGU	
AD-2011435	asusgga(Gda)AfuAfUfCfauugauaaauL96		asUfsuuaUfcAfAfugauAfuCfuccauususcc		GAAUGGAGAUAUCAUUGAUAAAU	
AD-2011436	gsusgua(Uda)UfcAfUfCfauugcauucuL96		asGfsaauGfcAfAfugauGfaAfuacacsasg		CUGUGUAUUCAUCAUUGCAUUCC	
AD-2011437	gsasagc(Uda)UfuGfGfGfuaauuuaguuL96		asAfscuaAfaUfUfUfaccAfaAfgcuucsasg		CUGAAGCUUUGGGUAAUUUAGUG	
AD-2011438	gscsugu(Gda)AfaAfUfUfggcagacuuuL96		asAfsaguCfuGfCfcaauUfuCfacagcsusg		CAGCUGUGAAAUUGGCAGACUUU	
AD-2011439	usasaca(Ada)UfuAfAfAfgugggaugauL96		asUfscauCfcCfAfcuuuAfaUfuguuasasu		AUUAACAAUUAAAGUGGGAUGAU	
AD-2011440	gscsugg(Cda)UfaCfAfAfggaauuucuuL96		asAfsgaaAfuUfCfcuugUfaGfccagcsasu		AUGCUGGCUACAAGGAAUUUCUC	
AD-2011441	usgsuua(Uda)UfaUfUfCfuuugcucacuL96		asGfsugaGfcAfAfagaaUfaAfuaacasasu		AUUGUUAUUAUUCUUUGCUCACG	
AD-2011442	asusaac(Ada)AfuUfUfGfcacuugguguL96		asCfsaccAfaGfUfUfgcaaAfuUfguuausgsu		ACAUAACAAUUUGCACUUGGUGA	
AD-2011443	csusucc(Gda)CfaCfUfAfagaugugagL96		asCfsucaCfaUfCfuuagUfgCfggaagsasc		GUCUUCCGCACUAAGAUGUGAGG	
AD-2011444	csuscuc(Ada)UfcUfCfAfccucucugguL96		asAfscagAfgAfGfgugaGfaUfgagagsasa		UUCUCUCAUCUCACCUCUCUGUG	
AD-2011445	csasccc(Ada)UfgGfAfUfcugucaacguL96		asCfsguuGfaCfAfgaucCfaUfggugugsusu		AGCACCCAUGGAUCUGUCAACGU	
AD-2011446	asusaaa(Ada)UfuCfUfAfcugacuucuuL96		asAfsgaaGfuCfAfguagAfaUfuuuaususc		GAAUAAAAUUCUACUGACUUCUA	
AD-2011447	usgscca(Uda)CfuUfGfAfcaacuaugcuL96		asGfscauAfgUfUfgucaAfgAfuggcascsc		GGUGCCAUCUUGACAACUAUGCU	
AD-2011448	ascsuua(Gda)GfuAfUfCfcuaacuaugL96		asCfsauaGfuUfAfggauAfcCfuaagusccsc		GGACUUAGGUAUCCUAACUAUGU	
AD-2011449	csusgcu(Uda)CfuAfCfUfCfccuccugcuuL96		asAfsgcaGfgAfGfgaguAfgAfagcagsasg		CUCUGCUUCUACUCCUCCUGCUC	
AD-2011450	usgsagg(Ada)AfaUfUfGfugauuuguuuL96		asAfsacaAfaUfCfacaaUfuUfccucascsa		UGUGAGGAAAUUGUGAUUUGUUC	
AD-2011451	gsascga(Gda)UfaUfCfAfgcuuuucgauL96		asUfscgaAfaAfGfcugaUfaCfucgucscsg		CGGACGAGUAUCAGCUUUUCGAG	
AD-2011452	uscsccu(Cda)UfgAfCfCfcucaguuuucuL96		asGfsaaaCfuGfAfggguCfaGfagggasasg		CUUCCCUCUGACCCUCAGUUUCC	
AD-2011453	gscsccu(Uda)UfuGfAfUfaacagaagcuL96		asGfscuuCfuGfUfuaucAfaAfagggcsusu		AAGCCCUUUUGAUAACAGAAGCC	
AD-2011454	asgsaaa(Uda)UfaAfAfUfgguagcaguuL96		asAfscugCfuAfCfcauuUfaAfuuucusasu		AUAGAAAUUAAAUGGUAGCAGUA	
AD-2011455	gsasugg(Ada)UfuUfUfCfaccgauucuuL96		asAfsgaaUfcGfGfUfugaaAfaUfccaucscsc		GGGAUGGAUUUUCACCGAUUCUA	
AD-2011456	ascsauc(Ada)CfaCfUfUfUfgcucacauguL96		asCfsaugUfgAfGfcaagUfgUfgaugususu		AAACAUCACACUUGCUCACACAUGC	
AD-2011457	uscsuaa(Gda)GfcCfUfGfUfaaaccauuuL96		asAfsaugGfuUfUfucagGfcCfuuagasusg		CAUCUAAGGCCUGAAAACCAUUC	
AD-2011458	ususacg(Uda)AfaAfGfAfuccuuauggguL96		asCfscauAfaGfGfaucuUfuAfcguaasasa		UUUUACGUAAAGAUCCUUAUGGA	
AD-2011459	usascag(Uda)GfaAfGfCfUfgaugccaguL96		asCfsuggCfaUfCfagcuUfcAfcuguasgsu		ACUACAGUGAAGCUGAUGCCAGU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2011460	asusuug(Cda)UfgAfAfUfugaauuguuuL96		asAfsacaAfuUfCfaauuCfaGfcaaaususc		GAAUUUGCUGAAUUGAAUUGUUU	
AD-2011461	usasagc(Uda)CfuAfGfUfuuggacuuauL96		asUfsaagUfcCfAfaacuAfgAfgcuuasasg		CUUAAGCUCUAGUUUGGACUUAG	
AD-2011462	csusggc(Uda)AfgUfAfGfugugugagauL96		asUfscucAfcAfCfacuaCfuAfgccagscsc		GGCUGGCUAGUAGUGUGUGAGAA	
AD-2011463	asusgug(Ada)UfgCfAfUfcaucuuaucuL96		asGfsauaAfgAfUfgaugCfaUfcacausasu		AUAUGUGAUGCAUCAUCUUAUCA	
AD-2011464	cscsuac(Ada)UfgUfAfAfugcauauguuL96		asAfscauAfuGfCfauuaCfaUfguaggsasc		GUCCUACAUGUAAUGCAUAUGUG	
AD-2011465	csasgca(Gda)CfcAfAfGfaguuguuguL96		asCfsaacAfaAfCfucuuGfgCfugcugsasg		CUCAGCAGCCAAGAGUUUGUUGA	
AD-2011466	uscscaa(Uda)CfcAfGfCfcuucacauguL96		asCfsaugUfgAfAfggcuGfgAfuuggasgsg		CCUCCAAUCCAGCCUUCACAUGG	
AD-2011467	ascscaa(Cda)AfgUfAfCfccaucaagcuL96		asGfscuuGfaUfGfgguaCfuGfuuggusgsa		UCACCAACAGUACCCAUCAAGCC	
AD-2011468	csusgcu(Ada)GfgGfAfUfcaucagaaauL96		asUfsuucUfgAfUfgaucCfcUfagcagsasa		UUCUGCUAGGGAUCAUCAGAAAC	
AD-2011469	gsgsuuu(Gda)CfuAfCfCfacauaaagcuL96		asGfscuuUfaUfGfugguAfgCfaaaccsasa		UUGGUUUGCUACCACAUAAAGCC	
AD-2011470	asgsugu(Ada)CfaGfCfAfucaugcuccuL96		asGfsgagCfaUfGfaugcUfgUfacacusgsa		UCAGUGUACAGCAUCAUGCUCCA	
AD-2011471	usgsgcu(Uda)CfgAfCfCfacaaccugcuL96		asGfscagGfuUfGfugguCfgAfagccasusc		GAUGGCUUCGACCACAACCUGCA	
AD-2011472	cscsagu(Cda)AfuUfGfCfauucagcaguL96		asCfsugcUfgAfAfugcaAfuGfacuggscsa		UGCCAGUCAUUGCAUUCAGCAGA	
AD-2011473	csusucc(Ada)UfgAfUfGfcacagacaguL96		asCfsuguCfuGfUfgcauCfaUfggaagscsa		UGCUUCCAUGAUGCACAGACAGG	
AD-2011474	csasgga(Gda)GfaUfAfCfcaacuugauuL96		asAfsucaAfgUfUfgguaUfcCfuccugsgsc		GCCAGGAGGAUACCAACUUGAUA	
AD-2011475	usgsaug(Gda)AfaAfCfAfaggagucaauL96		asUfsugaCfuCfCfuuguUfuCfcaucasgsg		CCUGAUGGAAACAAGGAGUCAAC	
AD-2011476	asasgca(Ada)GfaAfUfCfaguugguuuuL96		asAfsaacCfaAfCfugauUfcUfugcuuscsu		AGAAGCAAGAAUCAGUUGGUUUG	
AD-2011477	csasacu(Gda)CfuUfGfCfcacucguccuL96		asGfsgacGfaGfUfggcaAfgCfaguugscsg		CGCAACUGCUUGCCACUCGUCCC	
AD-2011478	gsusguu(Gda)AfaGfUfAfuuacuguaguL96		asCfsuacAfgUfAfauacUfuCfaacacscsa		UGGUGUUGAAGUAUUACUGUAGA	
AD-2011479	ususacu(Gda)UfuGfGfCfaaaacaauauL96		asUfsauuGfuUfUfugccAfaCfaguaasusu		AAUUACUGUUGGCAAAACAAUAG	
AD-2011480	csasgug(Uda)GfuCfCfUfacucuggucuL96		asGfsaccAfgAfGfuaggAfcAfcacugscsc		GGCAGUGUGUCCUACUCUGGUCU	
AD-2011481	asgsaaa(Cda)CfaGfAfUfggaguaaaguL96		asCfsuuuAfcUfCfcaucUfgGfuuucususc		GAAGAAACCAGAUGGAGUAAAGA	
AD-2011482	gscsucc(Ada)CfaGfCfAfaaccuuccuuL96		asAfsggaAfgGfUfuugcUfgUfggagcsasu		AUGCUCCACAGCAAACCUUCCUA	
AD-2011483	gsascuu(Uda)GfaAfGfCfcuacacaaauL96		asUfsuugUfgUfAfggcuUfcAfaagucscsc		GGGACUUUGAAGCCUACACAAAA	

Table 43. Unmodified Sense and Antisense Strand Sequences of CAMK2D dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2005899	GUGCGGAUCGUUUCGCAACUU		278	298	AAGUUGCGAAACGAUCCGCACUG		276	298
AD-2005914	CAACUGCUUGCCACUCGUCCU		293	313	AGGACGAGUGGCAAGCAGUUGCG		291	313
AD-2005989	CUGGCUGUUUUUCCCAUUUCCU		318	338	AGGAAAUGGAAAAACAGCCAGGC		316	338
AD-2006091	CCACCCUUUCUGGUCAUCUCU		481	501	AGAGAUGACCAGAAAGGGUGGCG		479	501
AD-2006275	UGGCUUCGACCACAACCUGCU		672	692	AGCAGGUUGUGGUCGAAGCCAUC		670	692
AD-2006307	GACGAGUAUCAGCUUUUUCGAU		704	724	AUCGAAAAGCUGAUACUCGUCCG		702	724
AD-2006395	UAUGAAAAAUUCCUACUGGACU		763	783	AGUCCAGUAGGAAUUUUUCAUACA		761	783
AD-2006473	GCUGCCAAAAUUAUCAACACU		791	811	AGUGUUGAUAAUUUUGGCAGCAU		789	811
AD-2006501	CUGCUAGGGAUCAUCAGAAAU		822	842	AUUUCUGAUGAUCCCUAGCAGAA		820	842
AD-2006588	AAUCUGCCGUCUUUUGAAGCU		859	879	AGCUUCAAAAGACGGCAGAUUCU		857	879
AD-2006610	CCUAAUAUUGUGCGACUUCAU		881	901	AUGAAGUCGCACAAUAUUAGGGU		879	901
AD-2006710	UUGGUGUUUUGAUUUAGUUACU		932	952	AGUAACUAAAUCAAACACCAAGU		930	952
AD-2006870	UACAGUGAAGCUGAUGCCAGU		992	1012	ACUGGCAUCAGCUUCACUGUAGU		990	1012
AD-2006886	CCAGUCAUUGCAUUCAGCAGU		1008	1028	ACUGCUGAAUGCAAUGACUGGCA		1006	1028
AD-2007012	GCCUGAGAAUUUGCUUUUAGU		1084	1104	ACUAAAAGCAAAUUCUCAGGCUU		1082	1104
AD-2007079	GCUGUGAAAUUGGCAGACUUU		1124	1144	AAAGUCUGCCAAUUUCACAGCUG		1122	1144
AD-2007144	UCUUUCUCCCAGAAGUUUUACU		1210	1230	AGUAAAACUUCUGGAGAAAGAUA		1208	1230
AD-2007160	UUACGUAAAGAUCCCUAUGGU		1226	1246	ACCAUAAGGAUCUUUACGUAAAA		1224	1246
AD-2007211	AUUCUCUAUAUUCUACUUGUU		1277	1297	AACAAGUAGAAUAUAGAGAAUGA		1275	1297
AD-2007238	CCAACACAGACUCUAUCAGCU		1324	1344	AGCUGAUAGAGUCUGUGUUGGUC		1322	1344
AD-2007269	GGAGCUUAUGAUUUUCCAUCU		1355	1375	AGAUGGAAAAUCAUAAGCUCCAG		1353	1375
AD-2007325	CCUCAUCAAUAAAAUGCUUAU		1411	1431	AUAAGCAUUUUAUUGAUGAGGUC		1409	1431
AD-2007389	CACCCAUGGAUCUGUCAACGU		1475	1495	ACGUUGACAGAUCCAUGGGUGCU		1473	1495
AD-2007404	CAACGUUCUACUGUUGCUUCU		1490	1510	AGAAGCAACAGUAGAACGUUGAC		1488	1510
AD-2007420	CUUCCAUGAUGCACAGACAGU		1506	1526	ACUGUCUGUGCAUCAUGGAAGCA		1504	1526
AD-2007451	CUGCUUGAAGAAAUUUAAUGU		1537	1557	ACAUUAAAUUUCUUCAAGCAGUC		1535	1557
AD-2007468	AUGCUAGAAGAAAACUAAAGU		1554	1574	ACUUUAGUUUUCUUCUAGCAUUA		1552	1574
AD-2007490	UGCCAUCUUGACAACUAUGCU		1576	1596	AGCAUAGUUGUCAAGAUGGCACC		1574	1596
AD-2007508	GCUGGCUACAAGGAAUUUCUU		1594	1614	AAGAAAUUCCUUGUAGCCAGCAU		1592	1614
AD-2007528	CAGCAGCCAAGAGUUUGUUGU		1614	1634	ACAACAAACUCUUGGCUGCUGAG		1612	1634

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2007549	AGAAACCAGAUGGAGUAAAGU		1635	1655	ACUUUACUCCAUCUGGUUUCUUC		1633	1655
AD-2007568	GAUAAACAACAAAGCCAACGU		1654	1674	ACGUUGGCUUUGUUUGUUUAUCUU		1652	1674
AD-2007585	CUACUGUAAUCCACAACCCUU		1725	1745	AAGGGUUGUGGAUUACAGUAGUU		1723	1745
AD-2007604	UGAUGGAAACAAGGAGUCAAU		1744	1764	AUUGACUCCUUGUUUCCAUCAGG		1742	1764
AD-2007627	AGAGUUCAAAUACAACAAUUU		1767	1787	AAAUUGUUGUAUUUGAACUCUCA		1765	1787
AD-2007653	GAAGAUGUGAAAGCACGAAAU		1793	1813	AUUUCGUGCUUUCACAUCUUCAU		1791	1813
AD-2007668	CGAAAGCAAGAGAUUAUCAAU		1808	1828	AUUGAUAAAUCUCUUGCUUUCGUG		1806	1828
AD-2007687	AAGUCACUGAACAACUGAUCU		1827	1847	AGAUCAGUUGUUCAGUGACUUUG		1825	1847
AD-2007702	UGAUCGAAGCUAUCAACAAUU		1842	1862	AAUUGUUGAUAGCUUCGAUCAGU		1840	1862
AD-2007705	GACUUUGAAGCCUACACAAAU		1865	1885	AUUUGUGUAGGCUUCAAAGUCCC		1863	1885
AD-2007743	UACUGCUUUUGAACCUGAAGU		1903	1923	ACUUCAGGUUCAAAAGCAGUAAG		1901	1923
AD-2007759	GAAGCUUUGGGUAAUUUAGUU		1919	1939	AACUAAAUUACCCAAAGCUUCAG		1917	1939
AD-2007785	GAUGGAUUUUCACCGAUUCUU		1945	1965	AAGAAUCGGUGAAAAUCCAUCCC		1943	1965
AD-2007801	UUCUACUUUGAAAAUGCUUUU		1961	1981	AAAAGCAUUUUCAAAGUAGAAUC		1959	1981
AD-2007818	UUUGUCCAAAAGCAAUAAACU		1978	1998	AGUUUAUUGCUUUUGGACAAAGC		1976	1998
AD-2007841	UCCACACUAUUAUUCUAAACU		2001	2021	AGUUUAGAAAUAAUAGUGUGGAUU		1999	2021
AD-2007856	UAAACCCUCAUGUACAUCUGU		2016	2036	ACAGAUGUACAUGAGGGUUUAGA		2014	2036
AD-2007870	CGCCUGCAUAGCAUAUAUUAU		2050	2070	AUAAUAUAUGCUAUGCAGGCGGC		2048	2070
AD-2007934	CAGUCAGAAGAGACUCGUGUU		2114	2134	AACACGAGUCUCUUCUGACUGCA		2112	2134
AD-2007974	GGCAGAAUGUUCAUUUUCAUU		2157	2177	AAUGAAAAUGAACAUUCUGCCAC		2155	2177
AD-2007985	ACCAACAGUACCCAUCAAGCU		2188	2208	AGCUUGAUGGGUACUGUUGGUGA		2186	2208
AD-2008019	AAUGGGAAAGAAAAACUUCUCU		2222	2242	AGAGAAGUUUUUCUUUUCCCAUUUG		2220	2242
AD-2008047	CCUCUUUGUGGCAAAACAUCU		2250	2270	AGAUGUUUUUGCCACAAAGAGGUG		2248	2270
AD-2008065	UCUAAGGCCUGAAAACCAUUU		2268	2288	AAAUGGUUUUUCAGGCCUUAGAUG		2266	2288
AD-2008093	GGUCUUCUAAAUUUUCAACAGU		2296	2316	ACUGUUUGAAAAUUUAGAAGACCCA		2294	2316
AD-2008116	CACUUCUGCAUUCUCUGUUCU		2319	2339	AGAACAGAGAAUGCAGAAGUGGC		2317	2339
AD-2008170	CCUCUUCAUGCAUGUUUCUGU		2378	2398	ACAGAAACAUGCAUGAAGAGGAG		2376	2398
AD-2008211	CCUACAUGUAAUGCAUAUGUU		2419	2439	AACAUAUGCAUUACAUGUAGGAC		2417	2439
AD-2008227	AUGUGAUGCAUCAUCUUAUCU		2435	2455	AGAUAAGAUGAUGCAUCACACAUAU		2433	2455
AD-2008242	UAUCAUAUAUUCCUUCCUAUU		2451	2471	AAUAGGAAGGAAUAUAUGAUAAAG		2449	2471

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2008257	CCUAUACAUUGUUUACACUUU		2466	2486	AAAGUGUAAACAAUGUAUAGGAA		2464	2486
AD-2008273	CCACACAAACUUAAAUUACUU		2502	2522	AAGUAAUUUAAGUUUGUGUGGAA		2500	2522
AD-2008288	UUACUGUUGGCAAAACAAUAU		2517	2537	AUAUUGUUUUGCCAACAGUAAUU		2515	2537
AD-2008310	CCAAGUACAACUCUUCAUCAU		2573	2593	AUGAUGAAGAGUUGUACUUGGAA		2571	2593
AD-2008325	CAUCAAGUUUCUCUGUUAAUU		2588	2608	AAUUAACAGAGAAACUUGAUGAA		2586	2608
AD-2008357	CAGACUUAAGAACUAUUGUUU		2620	2640	AAACAAUAGUUCUUAAGUCUGUU		2618	2640
AD-2008375	UUCUCUGAAUGACAGUUGUAU		2638	2658	AUACAACUGUCAUUCAGAGAACA		2636	2658
AD-2008402	GAACUCUUUGCUGUUAAUCUU		2678	2698	AAGAUUAACAGCAAAGAGUUCUA		2676	2698
AD-2008453	CUGGUUAAAAUGGAUGAUUUU		2772	2792	AAAAUCAUCCAUUUUAACCAGGA		2770	2792
AD-2008474	UUGAAAGUUUUGCUGAUUAAU		2796	2816	AUUAAUCAGCAAAACUUUCAAUG		2794	2816
AD-2008491	UAACAAUUAAAGUGGGAUGAU		2813	2833	AUCAUCCCACUUUAAUUGUUAAU		2811	2833
AD-2008516	GGGCAAAAUCACUUAUGAAAU		2838	2858	AUUUCAUAAGUGAUUUUGCCCAC		2836	2858
AD-2008540	AAGCAAGAAUCAGUUGGUUUU		2862	2882	AAAACCAACUGAUUCUUGCUUCU		2860	2882
AD-2008555	GGUUUGCUACCACAUAAAGCU		2877	2897	AGCUUUAUGUGGUAGCAAACCAA		2875	2897
AD-2008575	CAUGCUGUUUUUGGUCAAACU		2897	2917	AGUUUGACCAAAAACAGCAUGGC		2895	2917
AD-2008590	CAAACUGUGUAAACUGGAAAU		2912	2932	AUUUCCAGUUUACACAGUUUGAC		2910	2932
AD-2008623	UCUGAGUUUAAUCACUUUAGU		2945	2965	ACUAAAGUGAUUAAACUCAGAAA		2943	2965
AD-2008639	UUAGGAUAUAUUCACAUUGUU		2961	2981	AACAAUGUGAAUAUAUCCUAAAG		2959	2981
AD-2008666	AUUUGCUGAAUUGAAUUGUUU		2989	3009	AAACAAUUCAAUUCAGCAAAUUC		2987	3009
AD-2008687	UUUCUCAAAUCUGUGAUCUCU		3013	3033	AGAGAUCACAGAUUUGAGAAAGA		3011	3033
AD-2008701	AUCUCUUUUCUUUAUCCUGUU		3028	3048	AACAGGAUAAAGAAAAGAGAUCA		3026	3048
AD-2008708	CCUUUCGUUUGCUUUCUUAUU		3057	3077	AAUUAAGAAAGCAAACGAAAGGAA		3055	3077
AD-2008714	UUUGUUCCAUUCUUUUCUUAU		3083	3103	AUAAGAAAAGAAUGGAACAAAAG		3081	3103
AD-2008720	CUGGCUAGUAGUGUGUGAGAU		3132	3152	AUCUCACACACUACUAGCCAGCC		3130	3152
AD-2008746	AGUGAAAUUUGCAUAAUGAAU		3162	3182	AUUCAUUAUGCAAAUUUCACUUC		3160	3182
AD-2008767	AGGGAAAUAAAAGUCUUUUGU		3188	3208	ACAAAAGACUUUUAUUUCCCUUU		3186	3208
AD-2008799	UAGCACUUUUGAUCAUCUUCU		3221	3241	AGAAGAUGAUCAAAAAGUGCUAGU		3219	3241
AD-2008835	UUGUCAAGAUUUUAAAGGUUU		3257	3277	AAACCUUUAAAAUCUUGACAACA		3255	3277
AD-2008844	GGUUUAUAAUUCUGCUUAAGU		3273	3293	ACUUAAGCAGAAUUAUAAACCUU		3271	3293
AD-2008860	UAAGCUCUAGUUUGGACUUAU		3289	3309	AUAAGUCCAAACUAGAGCUUAAG		3287	3309

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2008875	ACUUAGGUAUCCUAACUAUGU		3304	3324	ACAUAGUUAGGAUACCUAAGUCC		3302	3324
AD-2008899	AGGUAUUUGCAUUGUUUAAAU		3328	3348	AUUUAAACAAUGCAAAUACCUCC		3326	3348
AD-2008921	AGGAUAAAAGCAAGUUCCUCU		3351	3371	AGAGGAACUUGCUUUUAUCCUAA		3349	3371
AD-2008941	CUGUGACUGCAACGUCUUACU		3371	3391	AGUAAGACGUUGCAGUCACAGGA		3369	3391
AD-2008977	CAGGAGGAUACCAACUUGAUU		3407	3427	AAUCAAGUUGGUAUCCUCCUGGC		3405	3427
AD-2009021	UCAUCACUAGCUAGUUUUCUU		3492	3512	AAGAAAACUAGCUAGUGAUGAUG		3490	3512
AD-2009053	GCUUACAAAUGUUUUGCCAUUU		3524	3544	AAAUGGCAAACAUUUGUAAGCCU		3522	3544
AD-2009078	AAGUGUUUUUGAACUUGAUCUU		3549	3569	AAGAUCAAGUUCAAAACACUUAU		3547	3569
AD-2009108	UGCUUUUUUAGCUUCUCUCUU		3579	3599	AAGAGAGAAGCUAAAAAAAGCACA		3577	3599
AD-2009122	UCUCUUGAAUCAGAGUAUCAU		3594	3614	AUGAUACUCUGAUUCAAGAGAGA		3592	3614
AD-2009147	UUCCUCCAAGGAGUUAGAAUU		3619	3639	AAUUCUAACUCCUUGGAGGAAGA		3617	3639
AD-2009162	AGAAUUUCCCAGUUUAAAACU		3634	3654	AGUUUUAAACUGGGAAAUUCUAA		3632	3654
AD-2009197	GGUUUUCUUUGUGCUUCUCAU		3672	3692	AUGAGAAGCACAAAGAAAACCUA		3670	3692
AD-2009211	CUCAUUUUUCCUUUGUUGAUU		3688	3708	AAUCAACAAAGGAAAAAAUGAGAA		3686	3708
AD-2009238	CUGUGAUUUUUUGUUCUCUUCU		3715	3735	AGAAGAGAACAAAAAAUCACAGGA		3713	3735
AD-2009253	CUUCCCUGAAGUGCUUUACAU		3731	3751	AUGUAAAGCACUUCAGGGAAGAG		3729	3751
AD-2009285	UCCAUCAUUGUUAUUUUUAACU		3763	3783	AGUUAAAAUAACAAUGAUGGAGA		3761	3783
AD-2009302	AUAGUAAUUCACAGUCCUCAU		3784	3804	AUGAGGACUGUGAAUUACUAUCG		3782	3804
AD-2009317	CCUCAGAAGCCUAUUUUUUAAU		3799	3819	AUUAAAAAAUAGGCUUCUGAGGAC		3797	3819
AD-2009344	ACAACCCUUCCUCUUUUCUCU		3853	3873	AGAGAAAAGAGGAAGGGUUGUUU		3851	3873
AD-2009354	CUCUCAUCUCACCUCUCUGUU		3870	3890	AACAGAGAGGUGAGAUGAGAGAA		3868	3890
AD-2009369	UCUGUGUUGAUUACUAAUCAU		3885	3905	AUGAUUAGUAAUCAACACAGAGA		3883	3905
AD-2009386	UCUUAGAUAUUAUUGCUAGUU		3905	3925	AACUAGCAAUAAUAUCUAAGAUG		3903	3925
AD-2009425	UGAAGCUUUUCUGAUAAUUAU		3944	3964	AUAAUUAUCAGAAAAGCUUCAAC		3942	3964
AD-2009437	CAGUAAAUAUAUUGAGCCAUU		4007	4027	AAUGGCUCAAUAUAUUUACUGUA		4005	4027
AD-2009490	UGGCCUCAUUUUUCUCUUUUU		4060	4080	AAAAAGAGAAAAAUGAGGCCAUU		4058	4080
AD-2009505	UUUUGUGAAGCAGCUAUACGU		4095	4115	ACGUAUAGCUGCUUCACAAAAGG		4093	4115
AD-2009525	UGGCAUACAUGUAUUUAAAGU		4115	4135	ACUUUAAAUACAUGUAUGCCACG		4113	4135
AD-2009537	UGUAGAGUGUUUUUUUUACAU		4148	4168	AUGUAAAAAAAAACACUCUACAUC		4146	4168
AD-2009546	UACACUUUUAACUUAGCAUGU		4164	4184	ACAUGCUAAGUUAAAAAGUGUAAA		4162	4184

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2009568	GUGUUGAAGUAUUACUGUAGU		4186	4206	ACUACAGUAAUACUUCAACACCA		4184	4206
AD-2009584	GUAGAUCAAGUUUGUCUUCCU		4202	4222	AGGAAGACAAACUUGAUCUACAG		4200	4222
AD-2009599	CUUCCGCACUAAGAUGUGAGU		4217	4237	ACUCACAUCUUAGUGCGGAAGAC		4215	4237
AD-2009615	UGAGGAAAUUGUGAUUUGUUU		4233	4253	AAACAAAUCACAAUUUCCUCACA		4231	4253
AD-2009643	CACAAAUGAAUUACACAUUUU		4261	4281	AAAAUGUGUAAUUCAUUUGUGGU		4259	4281
AD-2009655	UUCUAUCAUUUUGAAACACUU		4287	4307	AAGUGUUUCAAAAUGAUAGAAGA		4285	4307
AD-2009690	ACACUGUAUAUAUUUCUUGCU		4322	4342	AGCAAGAAAUAUAUACAGUGUCC		4320	4342
AD-2009707	UGCCAUAAAUGGUAAAGGACUU		4339	4359	AAGUCCUUUACCAUUAUGGCAAG		4337	4359
AD-2009722	GACUGAUUGAUAUAUUUAAGU		4355	4375	ACUUAAAUAUAUCAAUCAGUCCU		4353	4375
AD-2009755	CGUCCAUCUUUAUUUCUUCAU		4407	4427	AUGAAGAAAUAAAGAUGGACGCA		4405	4427
AD-2009823	CAGUGUGUCCUACUCUGGUCU		4475	4495	AGACCAGAGUAGGACACACUGCC		4473	4495
AD-2009839	GGUCUCAUUUAGUACAUAACU		4491	4511	AGUUAUGUACUAAAUGAGACCAG		4489	4511
AD-2009852	AUAACAAUUUGCACUUGGUGU		4506	4526	ACACCAAGUGCAAAUUGUUAUGU		4504	4526
AD-2009881	AGUUAAUUGUUCUCUGUGAGU		4535	4555	ACUCACAGAGAACAAUUAACUUG		4533	4555
AD-2009901	CAAAACAAUGGUCUCUUCUGU		4555	4575	ACAGAAGAGACCAUUGUUUUGCU		4553	4575
AD-2009931	GCUGAGAACAAUAUAGUUAAU		4585	4605	AUUAACUAUAUUGUUCUCAGCAA		4583	4605
AD-2009959	CUCCUAAAAGCUUCUCUAAAU		4614	4634	AUUUAGAGAAGCUUUUAGGAGUC		4612	4634
AD-2009987	UCCAAUCCAGCCUUCACAUGU		4642	4662	ACAUGUGAAGGCUGGAUUGGAGG		4640	4662
AD-2010007	UUUUUUAAUACGAACCUGUCU		4678	4698	AGACAGGUUCGUAUUAAAAAAAA		4676	4698
AD-2010032	AACACUUUGAUGUUAUCAUUU		4703	4723	AAAUGAUAACAUCAAAGUGUUAC		4701	4723
AD-2010054	CAGCUUGAACUUGAGCAUACU		4762	4782	AGUAUGCUCAAGUUCAAGCUGGG		4760	4782
AD-2010084	AGCUUCUUUUGAUUUGCUAAU		4792	4812	AUUAGCAAAUCAAAAGAAGCUGA		4790	4812
AD-2010106	ACAUCACACUUGCUCACAUGU		4814	4834	ACAUGUGAGCAAGUGUGAUGUUU		4812	4834
AD-2010123	AUGCCUGUUUAUGCUGUUCAU		4831	4851	AUGAACAGCAUAAACAGGCAUGU		4829	4851
AD-2010142	AUGUUGUUUAUGUUUCUUACU		4850	4870	AGUAAGAAACAUAAACAACAUGA		4848	4870
AD-2010158	UUACCUAGAAUAAAUAGUCUU		4866	4886	AAGACUAUUUUAUUCUAGGUAAGA		4864	4886
AD-2010165	UACUUCUUUUCCCGACUUCUU		4893	4913	AAGAAGUCGGGAAAAGAAGUAGG		4891	4913
AD-2010184	UUACUUUUUCCUAAGAUUCAU		4912	4932	AUGAAUCUUAGGAAAAAGUAAGA		4910	4932
AD-2010203	AGUGUACAGCAUCAUGCUCCU		4931	4951	AGGAGCAUGAUGCUGUACACUGA		4929	4951
AD-2010218	GCUCCACAGCAAACCUUCCUU		4946	4966	AAGGAAGGUUUGCUGUGGAGCAU		4944	4966

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2		Antisense Strand Sequence 5' to 3'	SEQ ID NO.	Range in NM_001321571.2	
			Start	End			Start	End
AD-2010262	CCUCUCAAAACCUACAUAAUU		4990	5010	AAUUAUGUAGGUUUUGAGAGGGA		4988	5010
AD-2010280	AUUGUAUUUACCUCUCCUGUU		5012	5032	AACAGGAGAGGUAAAUACAAUCU		5010	5032
AD-2010301	AACCACAUUGUUUUUGAAAAUU		5033	5053	AAUUUUCAAAACAAUGUGGUUGA		5031	5053
AD-2010309	UUUCUAUUUUGUGUCUCCUCUU		5056	5076	AAGAGGAGACACAAAUAGAAAUA		5054	5076
AD-2010325	CUCUACUGCAGUAUAAUGUCU		5072	5092	AGACAUUAUACUGCAGUAGAGGA		5070	5092
AD-2010361	GUGUAUUCAUCAUUGCAUUCU		5108	5128	AGAAUGCAAUGAUGAAUACACAG		5106	5128
AD-2010404	AUGGAGAUAUCAUUGAUAAAU		5151	5171	AUUUAUCAAUGAUAUCUCCAUUC		5149	5171
AD-2010441	GCCCUUUUGAUAACAGAAGCU		5192	5212	AGCUUCUGUUAUCAAAAGGGCUU		5190	5212
AD-2010454	CAGAUAGUCCUUGUUUUAAUU		5225	5245	AAUUAAAACAAGGACUAUCUGGG		5223	5245
AD-2010486	CACCACUGUCUUGAUGCUCUU		5257	5277	AAGAGCAUCAAGACAGUGGUGAG		5255	5277
AD-2010503	UCUGAGCAAGUUACCUCUUCU		5274	5294	AGAAGAGGUAACUUGCUCAGAGC		5272	5294
AD-2010521	UCCCUCUGACCCUCAGUUUCU		5292	5312	AGAAACUGAGGGUCAGAGGGAAG		5290	5312
AD-2010535	GUUUCCAUAUUUGUAAAAUGU		5307	5327	ACAUUUUACAAAUAUGGAAACUG		5305	5327
AD-2010545	AUGAGAAUAAACAUACCAACU		5324	5344	AGUUGGUAUGUUUAUUCUCAUUU		5322	5344
AD-2010559	AACUUAAUAAAGAUAUUGUGU		5341	5361	ACACAAUAUCUUUAUUAAGUUGG		5339	5361
AD-2010598	GACUAGAAUGAUAUUUGAUAU		5381	5401	AUAUCAAAUAUCAUUCUAGUCAC		5379	5401
AD-2010609	AGAAAUUAAAUGGUAGCAGUU		5400	5420	AACUGCUACCAUUUAAUUUCUAU		5398	5420
AD-2010624	GCAGUAUAACUAUUCUGAUCU		5415	5435	AGAUCAGAAUAGUUAUACUGCUA		5413	5435
AD-2010643	CACUGACAUUAAUAUUCCUAU		5434	5454	AUAGGAAUAUUAAUGUCAGUGAU		5432	5454
AD-2010655	UGUUAUUAUCUCUUUGCUCACU		5455	5475	AGUGAGCAAAGAAUAAUAACAAU		5453	5475
AD-2010683	ACAACUCUUGUUUUGCUGUUU		5483	5503	AAACAGCAAAACAAGAGUUGUAU		5481	5503
AD-2010708	GCCCUCUUUAUGUAGGUUUAU		5508	5528	AUAAACCUACAUAAAGAGGGCAG		5506	5528
AD-2010745	AUACUCGGACUCAAAUGUCUU		5545	5565	AAGACAUUUGAGUCCGAGUAUAU		5543	5565
AD-2010782	ACACCAAAUGAAGUGGUCAUU		5582	5602	AAUGACCACUUCAUUUGGUGUCU		5580	5602
AD-2010830	GUAGCAAAUCAGUAUAUUCUU		5631	5651	AAGAAUAUACUGAUUUGCUACUA		5629	5651
AD-2010855	CAAGGAAUAUUAAUCUUCACU		5662	5682	AGUGAAGAUUAAUAUUCCUUGAA		5660	5682
AD-2010871	UCACCCAAAUUUUGAAUUUUU		5678	5698	AAAAAUUCAAAAUUUGGGUGAAG		5676	5698
AD-2010911	GAGUGAUUUCAUUGAAUAAAU		5746	5766	AUUUAUUCAAUGAAAUCACUCGA		5744	5766
AD-2010919	AUAAAAUUCUACUGACUUCUU		5761	5781	AAGAAGUCAGUAGAAUUUUAUUUC		5759	5781

Table 44. Modified Sense and Antisense Strand Sequences of CAMK2D dsRNA Agents Comprising a GalNAc Conjugate Targeting Ligand

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2005876	csusgcuuCfuAfCfUfccuccugcuuL96		asAfsgcaGfgAfGfgaguAfgAfagcagsasg		CUCUGCUUCUACUCCUCCUGCUC	
AD-2005899	gsusgcggAfuCfGfUfuucgcaacuuL96		asAfsguuGfcGfAfaacgAfuCfcgcacsusg		CAGUGCGGAUCGUUUCGCAACUG	
AD-2005914	csasacugCfuUfGfCfcacucguccuL96		asGfsgacGfaGfUfggcaAfgCfaguugscsg		CGCAACUGCUUGCCACUCGUCCC	
AD-2005989	csusggcuGfuUfUfUfuccauuuccuL96		asGfsgaaAfuGfGfaaaaAfcAfgccagsgsc		GCCUGGCUGUUUUUCCAUUUCCC	
AD-2006091	cscsacccUfuUfCfUfggucaucucuL96		asGfsagaUfgAfCfcagaAfaGfgguggscsg		CGCCACCCUUUCUGGUCAUCUCC	
AD-2006275	usgsgcuuCfgAfCfCfacaaccugcuL96		asGfscagGfuUfGfugguCfgAfagccasusc		GAUGGCUUCGACCACAACCUGCA	
AD-2006307	gsascgagUfaUfCfAfgcuuuucgauL96		asUfscgaAfaAfGfcugaUfaCfucgucscsg		CGGACGAGUAUCAGCUUUUCGAG	
AD-2006395	usasugaaAfaUfUfCfcuacuggacuL96		asGfsuccAfgUfAfggaaUfuUfucauascsa		UGUAUGAAAAUUCCUACUGGACA	
AD-2006473	gscsugccAfaAfAfUfuaucaacacuL96		asGfsuguUfgAfUfaauuUfuGfgcagcsasu		AUGCUGCCAAAAUUAUCAACACC	
AD-2006501	csusgcuaGfgGfAfUfcaucagaaauL96		asUfsuucUfgAfUfgaucCfcUfagcagsasa		UUCUGCUAGGGAUCAUCAGAAAC	
AD-2006588	asasucugCfcGfUfCfuuuugaagcuL96		asGfscuuCfaAfAfagacGfgCfagauuscsu		AGAAUCUGCCGUCUUUUGAAGCA	
AD-2006610	cscsuaauAfuUfGfUfgcgacuucauL96		asUfsgaaGfuCfGfcacaAfuAfuuaggsgsu		ACCCUAAUAUUGUGCGACUUCAU	
AD-2006710	ususggugUfuUfGfAfuuuaguuacuL96		asGfsuaaCfuAfAfaucaAfaCfaccaasgsu		ACUUGGUGUUUGAUUUAGUUACU	
AD-2006870	usascaguGfaAfGfCfugaugccaguL96		asCfsuggCfaUfCfagcuUfcAfcuguasgsu		ACUACAGUGAAGCUGAUGCCAGU	
AD-2006886	cscsagucAfuUfGfCfauucagcaguL96		asCfsugcUfgAfAfugcaAfuGfacuggscsa		UGCCAGUCAUUGCAUUCAGCAGA	
AD-2007012	gscscugaGfaAfUfUfugcuuuuaguL96		asCfsuaaAfaGfCfaaauUfcUfcaggcsusu		AAGCCUGAGAAUUUGCUUUUAGC	
AD-2007079	gscsugugAfaAfUfUfggcagacuuuL96		asAfsaguCfuGfCfcaauUfuCfacagcsusg		CAGCUGUGAAAUUGGCAGACUUU	
AD-2007144	uscsuuucUfcCfAfGfaaguuuuacuL96		asGfsuaaAfaCfUfucugGfaGfaaagasusa		UAUCUUUCUCCAGAAGUUUUACG	
AD-2007160	ususacguAfaAfGfAfuccuuaugguL96		asCfscauAfaGfGfaucuUfuAfcguaasasa		UUUUACGUAAAGAUCCUUAUGGA	
AD-2007211	asusucucUfaUfAfUfucuacuuguuL96		asAfscaaGfuAfGfaauaUfaGfagaausgsa		UCAUUCUCUAUAUUCUACUUGUG	
AD-2007238	cscsaacaCfaGfAfCfucuaucagcuL96		asGfscugAfuAfGfagucUfgUfguuggsusc		GACCAACACAGACUCUAUCAGCA	
AD-2007269	gsgsagcuUfaUfGfAfuuuuccaucuL96		asGfsaugGfaAfAfaucaUfaAfgcuccsasg		CUGGAGCUUAUGAUUUUCCAUCA	
AD-2007325	cscsucauCfaAfUfAfaaaugcuuauL96		asUfsaagCfaUfUfuuauUfgAfugaggsusc		GACCUCAUCAAUAAAAUGCUUAC	
AD-2007389	csascccaUfgGfAfUfcugucaacguL96		asCfsguuGfaCfAfgaucCfaUfgggugscsu		AGCACCCAUGGAUCUGUCAACGU	
AD-2007404	csasacguUfcUfAfCfuguugcuucuL96		asGfsaagCfaAfCfaguaGfaAfcguugsasc		GUCAACGUUCUACUGUUGCUUCC	
AD-2007420	csusuccaUfgAfUfGfcacagacaguL96		asCfsuguCfuGfUfgcauCfaUfggaagscsa		UGCUUCCAUGAUGCACAGACAGG	
AD-2007451	csusgcuuGfaAfGfAfaauuuaauguL96		asCfsauuAfaAfUfuucuUfcAfagcagsusc		GACUGCUUGAAGAAAUUUAAUGC	
AD-2007468	asusgcuaGfaAfGfAfaaacuaaaguL96		asCfsuuuAfgUfUfuucuUfcUfagcaususa		UAAUGCUAGAAGAAAACUAAAGG	
AD-2007490	usgsccauCfuUfGfAfcaacuaugcuL96		asGfscauAfgUfUfgucaAfgAfuggcascsc		GGUGCCAUCUUGACAACUAUGCU	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2007508	gscsuggcUfaCfAfAfggaauucuuL96		asAfsgaaAfuUfCfcuugUfaGfccagcsasu		AUGCUGGCUACAAGGAAUUUCUC	
AD-2007528	csasgcagCfcAfAfGfagauuguugL96		asCfsaacAfaAfCfucuuCfgCfugcugsasg		CUCAGCAGCCAAGAGUUUGUUGA	
AD-2007549	asgsaaccCfaGfAfUfggaguaaagL96		asCfsuuuAfcUfCfcaucUfgGfuuucususc		GAAGAAACCAGAUGGAGUAAAGA	
AD-2007568	gsasuaaaCfaAfCfAfaagccaacgL96		asCfsguuGfgCfUfuuguUfgUfuaucsusu		AAGAUAAACAACAAAGCCAACGU	
AD-2007585	csusacugUfaAfUfCfcacaacccuuL96		asAfsgggUfuGfUfggauUfaCfaguagsusu		AACUACUGUAAUCCACAACCCUG	
AD-2007604	usgsauggAfaAfCfAfaggagucaauL96		asUfsugaCfuCfCfuuguUfuCfcaucasgsg		CCUGAUGGAAACAAGGAGUCAAC	
AD-2007627	asgsaguuCfaAfAfUfacaacaauuuL96		asAfsauuGfuUfGfuauuUfgAfacucuscsa		UGAGAGUUCAAAUACAACAAUUG	
AD-2007653	gsasagauGfuGfAfAfagcacgaaauL96		asUfsuucGfuGfCfuuucAfcAfucuucsasu		AUGAAGAUGUGAAAGCACGAAAG	
AD-2007668	csgsaaagCfaAfGfAfgauuaucaauL96		asUfsugaUfaAfUfcucuUfgCfuuucgsusg		CACGAAAGCAAGAGAUUAUCAAA	
AD-2007687	asasgucaCfuGfAfAfcaacugaucuL96		asGfsaucAfgUfUfguucAfgUfgacuususg		CAAAGUCACUGAACAACUGAUCG	
AD-2007702	usgsaucgAfaGfCfUfaucaacaauuL96		asAfsuugUfuGfAfuagcUfuCfgaucasgsu		ACUGAUCGAAGCUAUCAACAAUG	
AD-2007705	gsascuuuGfaAfGfCfCfcuacacaaauL96		asUfsuugUfgUfAfggcuUfcAfaagucscsc		GGGACUUUGAAGCCUACACAAAA	
AD-2007743	usascugcUfuUfUfGfaaccugaaguL96		asCfsuucAfgGfUfucaaAfaGfcaguasasg		CUUACUGCUUUUGAACCUGAAGC	
AD-2007759	gsasagcuUfuGfGfGfuaauuuaguuL96		asAfscuaAfaUfUfUfacccAfaAfgcuucsasg		CUGAAGCUUUGGGUAAUUUAGUG	
AD-2007785	gsasuggaUfuUfUfCfaccgauucuuL96		asAfsgaaUfcGfGfGfugaaAfaUfccaucscsc		GGGAUGGAUUUUUCACCGAUUCUA	
AD-2007801	ususcuacUfuUfGfAfaaugcuuuuL96		asAfsaagCfaUfUfUfucaAfaGfuagaasusc		GAUUCUACUUUGAAAAUGCUUUG	
AD-2007818	ususugucCfaAfAfAfgcaauaaacuL96		asGfsuuuAfuUfGfCfcuuUfgGfacaaasgsc		GCUUUGUCCAAAGCAAUAAACC	
AD-2007841	uscscacaCfuAfUfUfauucuaaacuL96		asGfsuuuAfgAfAfuaauAfgUfguggasusu		AAUCCACACUAUUAUUCUAAACC	
AD-2007856	usasaaccCfuCfAfUfguacaucuguL96		asCfsagaUfgUfAfcaugAfgGfguuuasgsa		UCUAAACCCUCAUGUACAUCUGG	
AD-2007870	csgsccugCfaUfAfGfcauauauuauL96		asUfsaauAfuAfUfgcuaUfgCfaggcgsgsc		GCCGCCUGCAUAGCAUAUAUUAG	
AD-2007934	csasgucaGfaAfGfAfgacucguguuL96		asAfscacGfaGfUfcucuUfcUfgacugscsa		UGCAGUCAGAAGAGACUCGUGUG	
AD-2007974	gsgscagaAfuGfUfUfcauuuucauuL96		asAfsugaAfaAfUfgaacAfuUfcugccsasc		GUGGCAGAAUGUUCAUUUUCAUC	
AD-2007985	ascscaacAfgUfAfCfccaucaagcuL96		asGfscuuGfaUfGfgguaCfuGfuuggusgsa		UCACCAACAGUACCCAUCAAGCC	
AD-2008019	asasuggcAfaAfGfAfaaacuucucuL96		asGfsagaAfgUfUfuucuUfuCfccauususg		CAAAUGGGAAAGAAAACUUCUCA	
AD-2008047	cscsucuuUfgUfGfGfcaaaacaucuL96		asGfsaugUfuUfUfGfgccaCfaAfagaggsusg		CACCUCUUUGUGGCAAAACAUCU	
AD-2008065	uscsuaagGfcCfUfGfaaaaccauuuL96		asAfsaugGfuUfUfUfucagGfcCfuuagasusg		CAUCUAAGGCCUGAAAAACCAUUC	
AD-2008093	gsgsucuuCfuAfAfAfuuucaacaguL96		asCfsuguUfgAfAfauuuAfgAfagacccsca		UGGGUCUUCUAAAUUUCAACAGU	
AD-2008116	csascuucUfgCfAfUfucuguucuL96		asGfsaacAfgAfGfaaugCfaGfaaguggsgsc		GCCACUUCUGCAUUCUCUGUUCU	
AD-2008170	cscsucuuCfaUfGfCfCfauguuucugL96		asCfsagaAfaCfAfugcaUfgAfagaggsasg		CUCCUCUUCAUGCAUGUUUCUGA	
AD-2008211	cscsuacaUfgUfAfAfugcauaugguL96		asAfscauAfuGfCfauuaCfaUfguaggsasc		GUCCUACAUGUAAUGCAUAUGUG	
AD-2008227	asusgugaUfgCfAfUfcaucuuaucuL96		asGfsauaAfgAfUfgaugCfaUfcacausasu		AUAUGUGAUGCAUCAUCUUAUCA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2008242	usasucauAfuAfUfUfccuuccuauuL96		asAfsuagGfaAfGfgaauAfuAfugauasasg		CUUAUCAUAUAUUCCUUCCUAUA	
AD-2008257	cscsuauaCfaUfUfGfuuuacacuuuL96		asAfsaguGfuAfAfacaaUfgUfauaggsasa		UUCCUAUACAUUGUUUACACUUC	
AD-2008273	cscsacacAfaAfCfUfuaaauuacuuL96		asAfsguaAfuUfUfaaguUfuGfuguggsasa		UUCCACACAAACUUAAAUUACUG	
AD-2008288	ususacugUfuGfGfCfaaaacaauauL96		asUfsauuGfuUfUfugccAfaCfaguaasusu		AAUUACUGUUGGCAAAACAAUAG	
AD-2008310	cscsaaguAfcAfAfCfucuucaucauL96		asUfsgauGfaAfGfaguuGfuAfcuuggsasa		UUCCAAGUACAACUCUUCAUCAA	
AD-2008325	csasucaaGfuUfUfCfucuguuaauuL96		asAfsuuaAfcAfGfagaaAfcUfugaugsasa		UUCAUCAAGUUUCUCUGUUAAUG	
AD-2008357	csasgacuUfaAfGfAfacuauuguuuL96		asAfsacaAfuAfGfuucuUfaAfgucugsusu		AACAGACUUAAGAACUAUUGUUC	
AD-2008375	ususcucuGfaAfUfGfacaguuguauL96		asUfsacaAfcUfGfucauUfcAfgagaascsa		UGUUCUCUGAAUGACAGUUGUAA	
AD-2008402	gsasacucUfuUfGfCfuguuaaucuuL96		asAfsgauUfaAfCfagcaAfaGfaguucsusa		UAGAACUCUUUGCUGUUAAUCUG	
AD-2008453	csusgguuAfaAfAfUfggaugauuuuL96		asAfsaauCfaUfCfcauuUfuAfaccagsgsa		UCCUGGUUAAAAUGGAUGAUUUU	
AD-2008474	ususgaaaGfuUfUfUfgcugauuaauL96		asUfsuaaUfcAfGfcaaaAfcUfuucaasusg		CAUUGAAAGUUUUGCUGAUUAAC	
AD-2008491	usasacaaUfuAfAfAfgugggaugauL96		asUfscauCfcCfAfcuuuAfaUfuguuasasu		AUUAACAAUUAAAGUGGGAUGAU	
AD-2008516	gsgsgcaaAfaUfCfAfcuuaugaaauL96		asUfsuucAfuAfAfgugaUfuUfugcccsasc		GUGGGCAAAAUCACUUAUGAAAG	
AD-2008540	asasgcaaGfaAfUfCfaguugguuuuL96		asAfsaacCfaAfCfugauUfcUfugcuuscsu		AGAAGCAAGAAUCAGUUGGUUUG	
AD-2008555	gsgsuuugCfuAfCfCfacauaaagcuL96		asGfscuuUfaUfGfugguAfgCfaaaccsasa		UUGGUUUGCUACCACAUAAAGCC	
AD-2008575	csasugcuGfuUfUfUfuggucaaacuL96		asGfsuuuGfaCfCfaaaaAfcAfgcaugsgsc		GCCAUGCUGUUUUUGGUCAAACU	
AD-2008590	csasaacuGfuGfUfAfaacuggaaauL96		asUfsuucCfaGfUfuuacAfcAfguuugsasc		GUCAAACUGUGUAAACUGGAAAA	
AD-2008623	uscsugagUfuUfAfAfucacuuuaguL96		asCfsuaaAfgUfGfauuaAfaCfucagasasa		UUUCUGAGUUUAAUCACUUUAGG	
AD-2008639	ususaggaUfaUfAfUfucacauuguuL96		asAfscaaUfgUfGfaauaUfaUfccuaasasg		CUUUAGGAUAUAUUCACAUUGUU	
AD-2008666	asusuugcUfgAfAfUfugaauuguuuL96		asAfsacaAfuUfCfaauuCfaGfcaaaususc		GAAUUUGCUGAAUUGAAUUGUUU	
AD-2008687	ususucucAfaAfUfCfugugaucucuL96		asGfsagaUfcAfCfagauUfuGfagaaasgsa		UCUUUCUCAAAUCUGUGAUCUCU	
AD-2008701	asuscucuUfuUfCfUfuuauccuguuL96		asAfscagGfaUfAfaagaAfaAfgagauscsa		UGAUCUCUUUUCUUUAUCCUGUU	
AD-2008708	cscsuuucGfuUfUfGfcuuucuuauuL96		asAfsuaaGfaAfAfgcaaAfcGfaaaggsasa		UUCCUUUCGUUUGCUUUCUUAUU	
AD-2008714	ususuguuCfcAfUfUfcuuuucuuauL96		asUfsaagAfaAfAfgaauGfgAfacaaasasg		CUUUUGUUCCAUUCUUUUCUUAC	
AD-2008720	csusggcuAfgUfAfGfugugugagauL96		asUfscucAfcAfCfacuaCfuAfgccagscsc		GGCUGGCUAGUAGUGUGUGAGAA	
AD-2008746	asgsugaaAfuUfUfGfcauaaugaauL96		asUfsucaUfuAfUfgcaaAfuUfucacususc		GAAGUGAAAUUUGCAUAAUGAAU	
AD-2008767	asgsggaaAfuAfAfAfagucuuuuguL96		asCfsaaaAfgAfCfuuuuAfuUfucccususu		AAAGGGAAAUAAAAGUCUUUUGA	
AD-2008799	usasgcacUfuUfUfGfaucaucuucuL96		asGfsaagAfuGfAfucaaAfaGfugcuasgsu		ACUAGCACUUUUGAUCAUCUUCA	
AD-2008835	ususgucaAfgAfUfUfuuaaaggguuuL96		asAfsaccUfuUfAfaaauCfuUfgacaascsa		UGUUGUCAAGAUUUUAAAGGUUU	
AD-2008844	gsgsuuuaUfaAfUfUfcugcuuaaguL96		asCfsuuaAfgCfAfgaauUfaUfaaaccsusu		AAGGUUUAUAAUUCUGCUUAAGC	
AD-2008860	usasagcuCfuAfGfUfuuggacuuauL96		asUfsaagUfcCfAfaacuAfgAfgcuuasasg		CUUAAGCUCUAGUUUGGACUUAG	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2008875	ascsuuagGfuAfUfCfcuaacuaugL96		asCfsauaGfuUfAfggauAfcCfuaaguscsc		GGACUUAGGUAUCCUAACUAUGU	
AD-2008899	asgsguauUfuGfCfAfauguuuaaauL96		asUfsuuaAfaCfAfaugcAfaAfuaccuscsc		GGAGGUAUUUGCAUUGUUUAAAG	
AD-2008921	asgsgauuAfaAfGfCfaaguuccucuL96		asGfsaggAfaCfUfugcuUfuUfauccusasa		UUAGGAUAAAAGCAAGUUCCUCC	
AD-2008941	csusgugaCfuGfCfAfacgcuuuacuL96		asGfsuaaGfaCfGfuugcAfgUfcacagsgsa		UCCUGUGACUGCAACGUCUUACU	
AD-2008977	csasggagGfaUfAfCfcaacuugauuL96		asAfsucaAfgUfUfgguaUfcCfuccugsgsc		GCCAGGAGGAUACCAACUUGAUA	
AD-2009021	uscsaucaCfuAfGfCfUfagcuuucuuL96		asAfsgaaAfaCfUfagcuAfgUfgaugasusg		CAUCAUCACUAGCUAGUUUUCUA	
AD-2009053	gscsuuacAfaAfUfGfUfuugccauuuL96		asAfsaugGfcAfAfacauUfuGfuaagcscsu		AGGCUUACAAAUGUUUGCCAUUC	
AD-2009078	asasguguUfuUfGfAfacuugaucuuL96		asAfsgauCfaAfGfuucaAfaAfcacuusasu		AUAAGUGUUUUGAACUUGAUCUU	
AD-2009108	usgscuuuUfuUfAfGfcucucucuuL96		asAfsgagAfgAfAfgcuaAfaAfaagcascsa		UGUGCUUUUUUAGCUUCUCUCUU	
AD-2009122	uscsucuuGfaAfUfCfagaguaucauL96		asUfsgauAfcUfCfugauUfcAfagagasgsa		UCUCUCUUGAAUCAGAGUAUCAU	
AD-2009147	ususccucCfaAfGfGfaguuagaauuL96		asAfsuucUfaAfCfuccuUfgGfaggaasgsa		UCUUCCUCCAAGGAGUUAGAAUU	
AD-2009162	asgsaauuUfcCfCfAfguuuaaaacuL96		asGfsuuuUfaAfAfcuggGfaAfauucusasa		UUAGAAUUUCCCAGUUUAAAACA	
AD-2009197	gsgsuuuuCfuUfUfGfugcucucauuL96		asUfsgagAfaGfCfacaaAfgAfaaaccsusa		UAGGUUUUCUUUGUGCUUCUCAU	
AD-2009211	csuscauuUfuUfCfCfuuuguugauuL96		asAfsucaAfcAfAfagaaAfaAfaugagsasa		UUCUCAUUUUUCCUUUGUUGAUU	
AD-2009238	csusgugaUfuUfUfUfgucucuucuuL96		asGfsaagAfgAfAfcaaaAfaUfcacagsgsa		UCCUGUGAUUUUUGUUCUCUUCC	
AD-2009253	csusucccUfgAfAfGfugcuuuacauL96		asUfsguaAfaGfCfacuuCfaGfggaagsasg		CUCUUCCCUGAAGUGCUUUACAG	
AD-2009285	uscscaucAfuUfGfUfuauuuaaccuL96		asGfsuuaAfaAfUfUfaacaAfuGfauggasgsa		UCUCCAUCAUUGUUAUUUUAACG	
AD-2009302	asusaguaAfuUfCfAfcaguccucauL96		asUfsgagGfaCfUfgugaAfuUfacuauscsg		CGAUAGUAAUUCACAGUCCUCAG	
AD-2009317	cscsucagAfaGfCfCfuauuuuaaauL96		asUfsuaaAfaAfUfaggcUfuCfugaggsasc		GUCCUCAGAAGCCUAUUUUUAAA	
AD-2009344	ascsaaccCfuUfCfCfucuuuucucuL96		asGfsagaAfaAfGfaggaAfgGfguugususu		AAACAACCCUUCCUCUUUUCUCU	
AD-2009354	csuscucaUfcUfCfAfccucucugguL96		asAfscagAfgAfGfgugaGfaUfgagagsasa		UUCUCUCAUCUCACCUCUCUGUG	
AD-2009369	uscsugugUfuGfAfUfuacuaaucauL96		asUfsgauUfaGfUfUfaaucAfaCfacagasgsa		UCUCUGUGUUGAUUACUAAUCAU	
AD-2009386	uscsuuagAfuAfUfUfuauugcuaguuL96		asAfscuaGfcAfAfuaauAfuUfcuaagasusg		CAUCUUAGAUAUUAUUGCUAGUG	
AD-2009425	usgsaagcUfuUfUfCfuguauaauauL96		asUfsaauUfaUfUfCfagaaAfaGfcuucasasc		GUUGAAGCUUUUUCUGAUAAUUAU	
AD-2009437	csasguaaAfuAfUfAfuugagccauuL96		asAfsuggCfuCfAfauauAfuUfuacugsusa		UACAGUAAAUAUAUUGAGCCAUG	
AD-2009490	usgsgccuCfaUfUfUfuucuuuuuuL96		asAfsaaaGfaGfAfAfaaaaUfgAfggccasusu		AAUGGCCUCAUUUUUCUCUUUUU	
AD-2009505	ususuuguGfaAfGfCfagcuauacguL96		asCfsguaUfaGfCfugcuUfcAfcaaaasgsg		CCUUUUGUGAAGCAGCUAUACGU	
AD-2009525	usgsgcauAfcAfUfGfuauuuaaaguL96		asCfsuuuAfaAfUfacauGfuAfugccascsg		CGUGGCAUACAUGUAUUUAAAGA	
AD-2009537	usgsuagaGfuGfUfUfuuuuuacauL96		asUfsguaAfaAfAfaaacAfcUfcuacasusc		GAUGUAGAGUGUUUUUUUUACAC	
AD-2009546	usascacuUfuUfAfAfcuuagcaugL96		asCfsaugCfuAfAfguuaAfaAfguguasasa		UUUACACUUUUAACUUAGCAUGU	
AD-2009568	gsusguugAfaGfUfAfuuacuguaguL96		asCfsuacAfgUfUfAfauacUfuCfaacacscsa		UGGUGUUGAAGUAUUACUGUAGA	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2009584	gsusagauCfaAfGfUfuugucuuccuL96		asGfsgaaGfaCfAfaacuUfgAfucuacsasg		CUGUAGAUCAAGUUUGUCUUCCG	
AD-2009599	csusuccgCfaCfUfAfagauguggaggUL96		asCfsucaCfaUfCfuuagUfgCfggaagsasc		GUCUUCCGCACUAAGAUGUGAGG	
AD-2009615	usgsaggaAfaUfUfGfuguauuguuuUL96		asAfsacaAfaUfUfCfacaaUfuUfccucascsa		UGUGAGGAAAUUGUGAUUUGUUC	
AD-2009643	csascaaaUfgAfAfUfUfuacacauuuuUL96		asAfsaauGfuGfUfUfaauuCfaUfuugugsgsu		ACCACAAAUGAAUUACACAUUUA	
AD-2009655	ususcuauCfaUfUfUfUfugaaaacacuuL96		asAfsgugUfuUfUfCfaaaaUfgAfuagaasgsa		UCUUCUAUCAUUUUGAAACACUG	
AD-2009690	ascsacugUfaUfAfUfUfauuucuugcuL96		asGfscaaGfaAfAfUfauuaUfaCfagugusccsc		GGACACUGUAUAUAUUUCUUGCC	
AD-2009707	usgsccauAfaUfUfGfGfuaaaggacuuL96		asAfsgucCfuUfUfUfaccaUfuAfuggcasasg		CUUGCCAUAAAUGGUAAAGGACUG	
AD-2009722	gsascugaUfuGfAfUfUfauauuuaaguL96		asCfsuuaAfaUfAfUfauucAfaUfUfcagucscsu		AGGACUGAUUGAUAUAUUUAAGA	
AD-2009755	csgsuccaUfcUfUfUfUfauuucucauuL96		asUfsgaaGfaAfAfUfuaaaGfaUfUfggacgscsa		UGCGUCCAUCUUUAUUUCUUCAG	
AD-2009823	csasguguGfuCfCfUfUfacucuggucuL96		asGfsaccAfgAfGfuaggAfcAfcacugscsc		GGCAGUGUGUCCUACUCUGGUCU	
AD-2009839	gsgsucucAfuUfUfUfAfguacauaacuL96		asGfsuuaUfgUfUfAfcuaaAfuGfagaccsasg		CUGGUCUCAUUUAGUACAUAAACA	
AD-2009852	asusaacaAfuUfUfUfGfcacuuggugUL96		asCfsaccAfaGfUfUfgcaaAfuUfguuausgsu		ACAUAACAAUUUGCACUUGGUGA	
AD-2009881	asgsuuaaUfuGfUfUfUfcucugugagUL96		asCfsucaCfaGfAfGfaacAfaUfUfuaacususg		CAAGUUAAUUGUUCUCUGUGAGC	
AD-2009901	csasaaacAfaUfGfGfGfucucuucugUL96		asCfsagaAfgAfGfaccaUfuGfuuuugscsu		AGCAAAACAAUGGUCUCUUCUGG	
AD-2009931	gscsugagAfaCfAfAfUfauaguuaauL96		asUfsuaaCfuAfUfUfauugUfuCfucagcsasa		UUGCUGAGAACAAUAUAGUUAAC	
AD-2009959	csuscccuAfaAfGfCfUfucucuaaaauL96		asUfsuuaGfaGfAfagcuUfuUfaggagsusc		GACUCCCUAAAAGCUUCUCUAAAC	
AD-2009987	uscscaauCfcAfGfCfCfuucacauguL96		asCfsaugUfgAfAfGfgcuGfgAfuuggasgsg		CCUCCAAUCCAGCCUUCACAUGG	
AD-2010007	ususuuuuAfaUfAfCfCfgaaccugucuL96		asGfsacaGfgUfUfCfguaUfuAfaaaaasasa		UUUUUUUUAAUACGAACCUGUCC	
AD-2010032	asascacuUfuGfAfUfUfguuaucauuuL96		asAfsaugAfuAfAfCfaucAfaAfUfguguusasc		GUAACACUUUGAUGUUAUCAUUU	
AD-2010054	csasgcuuGfaAfCfUfUfugagcauaacuL96		asGfsuauGfcUfCfaaguUfcAfagcugsgsg		CCCAGCUUGAACUUGAGCAUACA	
AD-2010084	asgscuucUfuUfUfGfUfauuugcuaauL96		asUfsuagCfaAfAfuucaaAfaGfaagcusgsa		UCAGCUUCUUUUGAUUUGCUAAA	
AD-2010106	ascsaucaCfaCfUfUfUfgcucacaugUL96		asCfsaugUfgAfGfcaagUfgUfgaugususu		AAACAUCACACUUGCUCACAUGC	
AD-2010123	asusgccuGfuUfUfAfAfugcuguucauL96		asUfsgaaCfaGfCfauaaAfcAfggcausgsu		ACAUGCCUGUUUAUGCUGUUCAU	
AD-2010142	asusguugUfuUfAfUfguuucuuacuL96		asGfsuaaGfaAfAfcauaAfaCfaacausgsa		UCAUGUUGUUUAUGUUUCUUACC	
AD-2010158	ususaccuAfgAfAfUfUfaaauagucuuL96		asAfsgacUfuAfUfUfuauuCfuAfgguaasgsa		UCUUACCUAGAAUAAAUAAGUCUC	
AD-2010165	usascuucUfuUfUfUfCfccgacuucuuL96		asAfsgaaGfuCfGfggaaAfaGfaaguasgsg		CCUACUUCUUUUUCCCGACUUCUU	
AD-2010184	ususacuuUfuUfUfCfCfuaagauucauL96		asUfsgaaUfcUfUfUfaggaAfaAfaguaasgsa		UCUUACUUUUUCCUAAGAUUCAG	
AD-2010203	asgsuguaCfaGfCfAfUfcaugcuccuL96		asGfsgagCfaUfUfGfaugcUfgUfacacusgsa		UCAGUGUACAGCAUCAUGCUCCA	
AD-2010218	gscsuccaCfaGfCfAfaaccuuccuuL96		asAfsggaAfgGfUfuugcUfgUfggagcsasu		AUGCUCCACAGCAAACCUUCCUA	
AD-2010262	cscsucucAfaAfAfCfCfucacauaauuL96		asAfsuuaUfgUfAfgguuUfuGfagaggsgsa		UCCCUCUCAAAACCUACACAUAAUA	
AD-2010280	asusuguaUfuUfAfCfCfucuccuguuL96		asAfscagGfaGfAfgguaAfaUfacaauscsu		AGAUUGUAUUUACCUCCUCCUGUC	

Duplex Name	Sense Strand Sequence 5' to 3'	SEQ ID NO.	Antisense Strand Sequence 5' to 3'	SEQ ID NO.	mRNA target sequence	SEQ ID NO.
AD-2010301	asasccacAfuUfGfUfuuugaaaauuL96		asAfsuuuUfcAfAfaacaAfuGfugguusgsa		UCAACCACAUUGUUUUGAAAAUA	
AD-2010309	ususucuaUfuUfGfUfgucuccucuuL96		asAfsgagGfaGfAfcacaAfaUfagaaasusa		UAUUUCUAUUUGUGUCUCCUCUA	
AD-2010325	csuscuacUfgCfAfGfuauaaugucuL96		asGfsacaUfuAfUfacugCfaGfuagagsgsa		UCCUCUACUGCAGUAUAAUGUCU	
AD-2010361	gsusguauUfcAfUfCfauugcauucuL96		asGfsaauGfcAfAfugauGfaAfuacacsasg		CUGUGUAUUCAUCAUUGCAUUCC	
AD-2010404	asusggagAfuAfUfCfauugauaaauL96		asUfsuuaUfcAfAfugauAfuCfuccaususc		GAAUGGAGAUAUCAUUGAUAAAU	
AD-2010441	gscscccuuUfuGfAfUfaacagaagcsuL96		asGfscuuCfuGfUfuaucAfaAfagggcsusu		AAGCCCUUUUGAUAACAGAAGCC	
AD-2010454	csasgauaGfuCfCfUfuguuuuaauuL96		asAfsuuaAfaAfCfaaggAfcUfaucugsgsg		CCCAGAUAGUCCUUGUUUUAAUG	
AD-2010486	csasccacUfgUfCfUfugaugcucuuL96		asAfsgagCfaUfCfaagaCfaGfuggugsasg		CUCACCACUGUCUUGAUGCUCUG	
AD-2010503	uscsugagCfaAfGfUfuaccucuucuL96		asGfsaagAfgGfUfaacuUfgCfucagasgsc		GCUCUGAGCAAGUUACCUCUUCC	
AD-2010521	uscscccucUfgAfCfCfcucaguuucuL96		asGfsaaaCfuGfAfggguCfaGfagggasasg		CUUCCCUCUGACCCUCAGUUUCC	
AD-2010535	gsusuuccAfuAfUfUfuguaaaauguL96		asCfsauuUfuAfCfaaauAfuGfgaaacsusg		CAGUUUCCAUAUUUGUAAAAUGA	
AD-2010545	asusgagaAfuAfAfAfAfcauaccaacuL96		asGfsuugGfuAfUfUfguuuAfuUfcucaususu		AAAUGAGAAUAAACAUACCAACU	
AD-2010559	asascuuaAfuAfAfAfAfgauauuguguL96		asCfsacaAfuAfUfCfcuuuAfuUfaaguusgsg		CCAACUUAAUAAAGAUAUUGUGA	
AD-2010598	gsascuagAfaUfGfAfAfuauuugauauL96		asUfsaucAfaAfUfaucaUfuCfuagucsasc		GUGACUAGAAUGAUAUUUGAUAG	
AD-2010609	asgsaaauUfaAfAfUfgguagcaguuL96		asAfscugCfuAfCfcauuUfaAfuuucusasu		AUAGAAAUUAAAUGGUAGCAGUA	
AD-2010624	gscsaguaUfaAfCfUfauucugauccuL96		asGfsaucAfgAfAfuaguUfaUfacugcsusa		UAGCAGUAUAACUAUUCUGAUCA	
AD-2010643	csascugaCfaUfUfAfauauuccuauL96		asUfsaggAfaUfAfuuaaUfgUfcagugsasu		AUCACUGACAUUAAUAUUCCUAU	
AD-2010655	usgsuuauUfaUfUfCfuuugcucacuL96		asGfsugaGfcAfAfagaaUfaAfuaacasasu		AUUGUUAUUAUUCUUUGCUCACG	
AD-2010683	ascsaacuCfuUfGfUfuuugcuguuuL96		asAfsacaGfcAfAfaacaAfgAfguugusasu		AUACAACUCUUGUUUUGCUGUUG	
AD-2010708	gscsccucUfuUfAfUfguaggguuuaauL96		asUfsaaaCfcUfAfcauaAfaGfagggcsasg		CUGCCCUCUUUAUGUAGGGUUUAC	
AD-2010745	asusacucGfgAfCfUfcaaaugucuuL96		asAfsgacAfuUfUfgaguCfcGfaguausasu		AUAUACUCGGACUCAAAUGUCUC	
AD-2010782	ascsaccaAfaUfGfAfaguggucauuL96		asAfsugaCfcAfCfuucaUfuUfgguguscsu		AGACACCAAAUGAAGUGGUCAUC	
AD-2010830	gsusagcaAfaUfCfCfAfguauauucuuL96		asAfsgaaUfaUfAfcugaUfuUfgcuacsusa		UAGUAGCAAAUCAGUAUAUUCUA	
AD-2010855	csasaggaAfuAfUfUfUfaauucuucacuL96		asGfsugaAfgAfUfuaauAfuUfccuugsasa		UUCAAGGAAUAUUAAUCUUCACC	
AD-2010871	uscsacccAfaAfUfUfUfuugaauuuuuuL96		asAfsaaaUfuCfCfAfaaauUfuGfggugasasg		CUUCACCCAAAUUUUUGAAUUUUU	
AD-2010911	gsasgugaUfuUfCfAfUfugaauaaaauL96		asUfsuuaUfuCfAfaugaAfaUfcacucsgsa		UCGAGUGAUUUCAUUGAAUAAAA	
AD-2010919	asusaaaaUfuCfUfAfcugacuucuuL96		asAfsgaaGfuCfAfguagAfaUfuuuaususc		GAAUAAAAUUCUACUGACUUCUA	

Example 17: *In vitro* Screening of dsRNA agents targeting PLN and CAMK2D

In vitro Dual-Luciferase and Endogenous Screening Assays

Hepa1-6 cells cells were transfected by adding 50 µL of siRNA duplexes and 100 ng of a plasmid, comprising partial sequences of human CAMK2D (NM_001321571, *e.g.,* nucleotides 1-2959, or nucleotides 2659-5613), or the sequence of human PLN (NM_002667.5), per well along with 100 µL of Opti-MEM plus 0.5 µL of Lipofectamine 2000 per well (Invitrogen, Carlsbad CA. cat # 13778-150) and then incubated at room temperature for 15 minutes. Specifically, V217 plasmid comprises nucleotides 1-2959 of human CAMK2D (NM_001321571), and V218 plasmid comprises nucleotides 2969-5613 of human CAMK2D (NM_001321571). V216 plasmid comprises the sequence of human PLN (NM_002667.5). The mixture was then added to the cells which are re-suspended in 35 µL of fresh complete media. The transfected cells were incubated at 37°C in an atmosphere of 5% CO2. Single-dose experiments were performed at 10 nM.

Twenty-four hours after the siRNAs and plasmid were transfected, Firefly (transfection control) and Renilla (fused to LEP target sequence) luciferase were measured. First, media was removed from cells. Then Firefly luciferase activity was measured by adding 75 µL of Dual-Glo® Luciferase Reagent equal to the culture medium volume to each well and mix. The mixture was incubated at room temperature for 30 minutes before luminescense (500nm) was measured on a Spectramax (Molecular Devices) to detect the Firefly luciferase signal. Renilla luciferase activity was measured by adding 75 µL of room temperature of Dual-Glo® Stop & Glo® Reagent to each well and the plates were incubated for 10-15 minutes before luminescence was again measured to determine the Renilla luciferase signal. The Dual-Glo® Stop & Glo® Reagent quenches the firefly luciferase signal and sustained luminescence for the Renilla luciferase reaction. siRNA activity was determined by normalizing the Renilla (LEP) signal to the Firefly (control) signal within each well. The magnitude of siRNA activity was then assessed relative to cells that were transfected with the same vector but were not treated with siRNA or were treated with a non-targeting siRNA. All transfections were done with n=4.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H_2O per reaction were added per well. Plates were

sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

Real time PCR

Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human AGT, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: 5'-cuuAcGcuGAGuAcuucGAdTsdT-3' and antisense 5'-UCGAAGuACUcAGCGuAAGdTsdT-3'.

The results of the dual-luciferase assays of the agents targeting PLN listed in Tables 39 and 40 are provided in Table 45.

The results of the dual-luciferase assays of the agents targeting CAMK2D listed in Tables 43 and 44 are provided in Table 46.

Table 45. Single Dose Reporter Screen for Human PLN in Hepa1-6 Cells

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message remaining	SD
AD-2007034.1	74.471	1.336
AD-2006965.1	79.415	2.654
AD-2006935.1	98.367	7.093
AD-2006926.1	73.665	2.308
AD-2006862.1	71.965	3.478
AD-2006839.1	72.055	3.068
AD-2006764.1	80.783	2.256
AD-2006751.1	94.187	1.833
AD-2006743.1	78.258	2.789
AD-2006731.1	75.200	2.414
AD-2006658.1	63.797	3.636
AD-2006640.1	79.573	3.340
AD-2006626.1	89.767	1.977
AD-2006548.1	79.254	2.656
AD-2006525.1	97.983	1.930
AD-2006451.1	71.323	1.207

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message remaining	SD
AD-2006437.1	78.733	3.978
AD-2006362.1	68.489	1.876
AD-2006347.1	57.744	1.454
AD-2006334.1	90.091	3.499
AD-2006259.1	80.739	2.113
AD-2006242.1	69.201	4.156
AD-2006234.1	69.430	2.572
AD-2006164.1	85.985	2.877
AD-2006150.1	78.944	2.370
AD-2006132.1	65.844	3.148
AD-2006119.1	67.891	2.715
AD-2006056.1	79.256	3.796
AD-2006046.1	72.080	2.814
AD-2006035.1	74.951	1.204
AD-2006018.1	69.416	1.116
AD-2005954.1	71.976	1.319
AD-2005942.1	73.672	3.299
AD-2005923.1	84.576	1.308
AD-2005862.1	52.157	0.938
AD-2005849.1	88.045	2.389
AD-2005826.1	112.005	0.917
AD-2005760.1	111.182	2.680
AD-2005737.1	81.084	2.698
AD-2005720.1	67.458	0.088
AD-2005704.1	66.982	2.264
AD-2005687.1	90.730	1.279
AD-2005678.1	73.201	2.059
AD-2005658.1	81.316	2.647
AD-2005638.1	76.024	1.690
AD-2005617.1	69.415	0.770
AD-2005604.1	62.339	1.020
AD-2005588.1	73.296	3.091
AD-2005575.1	73.973	2.813
AD-2005557.1	86.620	2.652
AD-2005530.1	87.155	2.522
AD-2005527.1	66.437	3.464
AD-2005485.1	60.734	1.125
AD-2005471.1	74.209	2.816

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message remaining	SD
AD-2005447.1	55.255	1.527
AD-2005429.1	61.881	2.504
AD-2005409.1	55.910	1.679
AD-2005396.1	70.051	1.457
AD-2005384.1	59.529	1.880
AD-2005365.1	49.761	2.254
AD-2005350.1	49.862	1.778
AD-2005325.1	49.062	1.550
AD-2005300.1	72.649	3.503
AD-2005287.1	66.711	1.750
AD-2005274.1	57.620	2.185
AD-2005250.1	42.331	0.718
AD-2005234.1	36.497	1.216
AD-2005221.1	39.891	0.782
AD-2005193.1	47.444	1.638
AD-2005179.1	31.061	0.664
AD-2005164.1	38.766	1.143
AD-2005146.1	42.489	1.460
AD-2005129.1	35.148	1.370
AD-2005108.1	45.362	1.516
AD-2005095.1	44.466	0.712
AD-2005082.1	44.276	0.629
AD-2005067.1	35.862	0.419
AD-2005051.1	36.352	0.285
AD-2005033.1	35.934	1.159
AD-2005020.1	52.416	1.682
AD-2005002.1	43.338	1.433
AD-2004989.1	33.202	0.616
AD-2004970.1	39.309	0.534
AD-2004950.1	36.412	1.258
AD-2004935.1	39.050	0.834
AD-2004922.1	55.716	2.384
AD-2004909.1	37.126	0.682
AD-2004896.1	31.389	0.294
AD-2004885.1	43.271	1.438
AD-2004872.1	36.214	2.665
AD-2004853.1	39.615	1.714
AD-2004837.1	32.899	1.054

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message remaining	SD
AD-2004825.1	29.761	1.078
AD-2004806.1	32.790	1.262
AD-2004790.1	29.823	1.134
AD-2004777.1	33.226	1.762
AD-2004763.1	30.181	1.410
AD-2004747.1	28.097	0.601
AD-2004718.1	47.433	3.268
AD-2004689.1	29.233	0.737
AD-2004673.1	35.458	0.769
AD-2004660.1	29.217	0.864
AD-2004646.1	42.910	1.006
AD-2004632.1	23.375	0.587
AD-2004608.1	84.486	2.159
AD-2004594.1	10.840	0.844
AD-2004575.1	6.793	0.323
AD-2004555.1	6.065	0.193
AD-2004539.1	4.753	0.229
AD-2004518.1	4.717	0.315
AD-2004495.1	4.114	0.253
AD-2004481.1	64.414	0.700
AD-2004468.1	5.628	0.493
AD-2004458.1	7.073	0.385
AD-2004428.1	8.520	0.514
AD-2004400.1	25.806	0.707
AD-2004386.1	3.955	0.105
AD-2004373.1	7.681	0.321
AD-2004360.1	5.221	0.397
AD-2004345.1	9.997	0.707
AD-2004329.1	5.616	0.469
AD-2004319.1	4.875	0.267
AD-2004301.1	10.902	0.526
AD-2004284.1	6.918	0.616
AD-2004260.1	13.058	0.327
AD-2004238.1	7.854	0.197
AD-2004202.1	7.385	0.540
AD-2004189.1	10.326	0.405
AD-2004176.1	12.764	0.435
AD-2004156.1	11.300	1.182

Duplex Name	RLuc/FLuc	
	10 nM	
	% Message remaining	SD
AD-2004142.1	12.730	0.159
AD-2004113.1	8.975	0.279
AD-2004095.1	2.922	0.151
AD-2004080.1	59.209	1.282
AD-2004072.1	13.064	0.967
Postive control	0.538	0.095

Table 46. Single Dose Reporter Screen for Human CAMK2D in Hepa1-6 Cells

Duplex Name	RLuc/Fluc V218		RLuc/Fluc V217	
	10 nM		10 nM	
	% Message Remaining	SD	% Message Remaining	SD
AD-2010919.1	54.775	3.875		
AD-2010911.1	63.205	0.834		
AD-2010871.1	87.416	5.684		
AD-2010855.1	93.304	3.325		
AD-2010830.1	84.938	3.881		
AD-2010782.1	78.337	4.600		
AD-2010745.1	88.461	6.353		
AD-2010708.1	93.117	6.451		
AD-2010683.1	69.483	3.261		
AD-2010655.1	77.639	2.283		
AD-2010643.1	87.707	3.018		
AD-2010624.1	80.114	3.333		
AD-2010609.1	90.918	4.832		
AD-2010598.1	74.414	1.039		
AD-2010559.1	86.988	1.703		
AD-2010545.1	71.871	3.138		
AD-2010535.1	73.669	2.725		
AD-2010521.1	71.072	0.767		
AD-2010503.1	72.548	1.061		
AD-2010486.1	72.378	2.071		
AD-2010454.1	84.009	4.651		
AD-2010441.1	74.567	2.349		
AD-2010404.1	79.424	3.393		
AD-2010361.1	76.688	2.472		
AD-2010325.1	79.238	4.044		
AD-2010309.1	67.564	2.345		

Duplex Name	RLuc/Fluc V218		RLuc/Fluc V217	
	10 nM		10 nM	
	% Message Remaining	SD	% Message Remaining	SD
AD-2010301.1	59.701	2.046		
AD-2010280.1	72.425	2.744		
AD-2010262.1	81.376	4.975		
AD-2010218.1	67.650	4.358		
AD-2010203.1	67.587	2.183		
AD-2010184.1	68.670	2.029		
AD-2010165.1	76.258	1.121		
AD-2010158.1	93.459	0.881		
AD-2010142.1	79.214	1.961		
AD-2010123.1	68.582	1.827		
AD-2010106.1	68.511	1.973		
AD-2010084.1	65.765	2.122		
AD-2010054.1	55.070	1.970		
AD-2010032.1	61.999	2.320		
AD-2010007.1	80.181	2.149		
AD-2009987.1	86.662	5.464		
AD-2009959.1	52.258	1.295		
AD-2009931.1	76.826	2.364		
AD-2009901.1	79.035	4.350		
AD-2009881.1	89.987	6.509		
AD-2009852.1	85.627	5.840		
AD-2009839.1	81.043	3.015		
AD-2009823.1	92.060	3.075		
AD-2009755.1	97.673	4.670		
AD-2009722.1	7.458	0.308		
AD-2009707.1	8.020	0.492		
AD-2009690.1	15.120	0.831		
AD-2009655.1	6.171	0.266		
AD-2009643.1	13.322	0.233		
AD-2009615.1	6.271	0.252		
AD-2009599.1	26.536	0.816		
AD-2009584.1	8.085	0.842		
AD-2009568.1	6.779	0.301		
AD-2009546.1	12.216	0.260		
AD-2009537.1	7.405	0.140		
AD-2009525.1	8.702	0.322		
AD-2009505.1	15.844	0.558		
AD-2009490.1	7.311	0.324		
AD-2009437.1	10.483	0.217		

Duplex Name	RLuc/Fluc V218		RLuc/Fluc V217	
	10 nM		10 nM	
	% Message Remaining	SD	% Message Remaining	SD
AD-2009425.1	3.552	0.159		
AD-2009386.1	7.732	0.356		
AD-2009369.1	4.180	0.202		
AD-2009354.1	12.511	0.483		
AD-2009344.1	19.310	0.115		
AD-2009317.1	7.006	0.297		
AD-2009302.1	3.416	0.110		
AD-2009285.1	8.156	0.520		
AD-2009253.1	4.172	0.270		
AD-2009238.1	3.640	0.161		
AD-2009211.1	12.916	0.346		
AD-2009197.1	2.605	0.169		
AD-2009162.1	9.455	0.439		
AD-2009147.1	8.493	0.118		
AD-2009122.1	3.398	0.118		
AD-2009108.1	5.204	0.207		
AD-2009078.1	2.664	0.213		
AD-2009053.1	6.560	0.119		
AD-2009021.1	9.111	0.335		
AD-2008977.1	30.950	1.078		
AD-2008941.1	24.568	0.808		
AD-2008921.1	37.944	1.475		
AD-2008899.1	4.925	0.148		
AD-2008875.1	28.692	0.747		
AD-2008860.1	7.795	0.347		
AD-2008844.1	7.289	0.395		
AD-2008835.1	3.943	0.103		
AD-2008799.1	4.375	0.238		
AD-2008767.1	5.444	0.182		
AD-2008746.1	2.286	0.105		
AD-2008720.1	12.460	1.301		
AD-2008714.1	28.171	0.547	45.65886104	1.1035907
AD-2008708.1	33.037	0.902	61.79586966	1.5307934
AD-2008701.1	16.450	0.554	44.17280687	1.9294042
AD-2008687.1	2.892	0.059	16.20778676	0.3737978
AD-2008666.1	1.191	0.085	11.77972984	0.3063456
AD-2008639.1	1.494	0.085	16.08336566	0.8901049
AD-2008623.1	2.640	0.112	17.62525171	0.4686597
AD-2008590.1	1.714	0.101	14.38598257	0.6394634

Duplex Name	RLuc/Fluc V218		RLuc/Fluc V217	
	10 nM		10 nM	
	% Message Remaining	SD	% Message Remaining	SD
AD-2008575.1	3.631	0.077	19.99313108	1.1687672
AD-2008555.1	11.072	1.039	31.50514332	1.917178
AD-2008540.1	2.454	0.076	20.53179586	0.2485112
AD-2008516.1	1.405	0.050	21.48519022	0.3653849
AD-2008474.1			24.02537918	0.6280613
AD-2008453.1			13.73531117	0.3217469
AD-2008402.1			16.87679869	0.6515435
AD-2008375.1			16.98560683	0.498232
AD-2008357.1			14.21209621	0.3004723
AD-2008325.1			16.73471701	0.7296915
AD-2008310.1			19.32436409	0.8544198
AD-2008288.1			14.23682319	0.3745798
AD-2008273.1			71.50461253	3.2380485
AD-2008257.1			27.49900574	1.3455372
AD-2008242.1			10.76358	0.5053741
AD-2008227.1			15.28452231	0.4654998
AD-2008211.1			13.60926615	0.1644877
AD-2008170.1			25.66489061	0.7888178
AD-2008116.1			51.30787029	3.2940949
AD-2008093.1			27.98984317	0.9882612
AD-2008065.1			21.13859066	0.4752274
AD-2008047.1			48.37926908	0.6189311
AD-2008019.1			43.49619485	1.3081996
AD-2007985.1			19.09148666	0.2746135
AD-2007974.1			51.99112086	2.5656624
AD-2007934.1			33.97294662	1.0110332
AD-2007870.1			36.17811695	2.5952914
AD-2007856.1			32.58840548	0.8877862
AD-2007841.1			26.72922	1.5211521
AD-2007818.1			24.81585399	0.7996233
AD-2007801.1			10.70960323	0.5182464
AD-2007785.1			12.3854364	0.1588502
AD-2007759.1			17.54481777	0.8691434
AD-2007743.1			18.65843633	0.8691339
AD-2007705.1			20.97710268	2.0623631
AD-2007702.1			22.23837144	1.2792613
AD-2007687.1			14.28983278	0.5877183
AD-2007668.1			12.55123358	0.5519322
AD-2007653.1			16.03158735	1.0494897

Duplex Name	RLuc/Fluc V218		RLuc/Fluc V217	
	10 nM		10 nM	
	% Message Remaining	SD	% Message Remaining	SD
AD-2007627.1			8.778782361	0.5807514
AD-2007604.1			11.3277006	0.5801774
AD-2007585.1			12.12409743	0.3406555
AD-2007568.1			10.91668988	0.9796381
AD-2007549.1			28.5985825	1.8602411
AD-2007528.1			26.24339901	3.0900046
AD-2007508.1			24.20127046	2.0456574
AD-2007490.1			32.98630016	1.863752
AD-2007468.1			52.37705677	2.902928
AD-2007451.1			14.12308396	0.8174496
AD-2007420.1			12.9988447	1.0455565
AD-2007404.1			73.82535862	3.2250002
AD-2007389.1			23.12555471	0.7699604
AD-2007325.1			40.76464069	2.3558281
AD-2007269.1			13.45774901	0.9639825
AD-2007238.1			26.19603597	0.5648448
AD-2007211.1			48.54309211	2.5350914
AD-2007160.1			10.07170671	0.3209378
AD-2007144.1			12.95174443	0.6263839
AD-2007079.1			11.53212195	0.5785039
AD-2007012.1			19.95795834	0.7006737
AD-2006886.1			31.6192243	0.2459024
AD-2006870.1			27.09699643	1.5404928
AD-2006710.1			19.12964642	1.8183614
AD-2006610.1			10.23483771	0.9179456
AD-2006588.1			14.25744683	0.8293527
AD-2006501.1			36.6319817	3.3363464
AD-2006473.1			20.91053622	1.3427681
AD-2006395.1			15.13240856	1.3434645
AD-2006307.1			11.78307666	0.4056504
AD-2006275.1			18.57181389	1.7504964
AD-2006091.1			58.62441069	4.1443315
AD-2005989.1			33.17064508	1.9442207
AD-2005914.1			27.87696239	0.6755636
AD-2005899.1			36.94068638	0.6020162
AD-2005876.1			40.88704238	2.1567636
			37.2476383	0.6411107
Positive control	0.455	0.108	0.781	0.124

Informal Sequence Listing

SEQ ID NO:1
>NM_000684.3 Homo sapiens adrenoceptor beta 1 (ADRB1), mRNA
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SEQ ID NO:2
>NM_007419.3 Mus musculus adrenergic receptor, beta 1 (Adrb1), mRNA
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SEQ ID NO:3
>NM_012701.1 Rattus norvegicus adrenoceptor beta 1 (Adrb1), mRNA
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SEQ ID NO:4
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SEQ ID NO:9
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SEQ ID NO:31
>NM_000685.5 Homo sapiens angiotensin II receptor type 1 (AGTR1),
transcript variant 1, mRNA
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SEQ ID NO:32
Reverse complement of SEQ ID NO:31
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SEQ ID NO:33
>NM_177322.3 Mus musculus angiotensin II receptor, type 1a (Agtr1a), mRNA
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SEQ ID NO:34
Reverse complement of SEQ ID NO:33
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SEQ ID NO:35
>NM_030985.4 Rattus norvegicus angiotensin II receptor, type 1a (Agtr1a), mRNA
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SEQ ID NO:37
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Reverse complement of SEQ ID NO:37
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Reverse complement of SEQ ID NO:39
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SEQ ID NO:48
Reverse complement of SEQ ID NO:47
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Reverse complement of SEQ ID NO:49
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SEQ ID NO:74
Reverse complement of SEQ ID NO:73
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SEQ ID NO:75
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Reverse complement of SEQ ID NO:75
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SEQ ID NO:77
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SEQ ID NO:78
Reverse complement of SEQ ID NO:77
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SEQ ID NO:79
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SEQ ID NO:80
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SEQ ID NO:81
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SEQ ID NO:82
Reverse complement of SEQ ID NO:81
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SEQ ID NO:83
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SEQ ID NO:85
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SEQ ID NO:86
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SEQ ID NO:87
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SEQ ID NO:88
Reverse complement of SEQ ID NO:87
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SEQ ID NO:90
Reverse complement of SEQ ID NO:89
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SEQ ID NO:94
Reverse complement of SEQ ID NO:93
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SEQ ID NO:95
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SEQ ID NO:96
Reverse complement of SEQ ID NO:95
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SEQ ID NO:97
>NM_001261696.1 Macaca mulatta potassium inwardly rectifying channel subfamily J member 3 (KCNJ3), mRNA
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SEQ ID NO:98
Reverse complement of SEQ ID NO:97
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SEQ ID NO:99
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SEQ ID NO:103
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SEQ ID NO:108
Reverse complement of SEQ ID NO:107
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SEQ ID NO:110
Reverse complement of SEQ ID NO:109
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SEQ ID NO:111
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SEQ ID NO:113
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SEQ ID NO:119
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SEQ ID NO:120
Reverse complement of SEQ ID NO:119
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SEQ ID NO: 121
>NM_003676.4 Homo sapiens delta 4-desaturase, sphingolipid 1 (DEGS1),
transcript variant 1, mRNA
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SEQ ID NO: 122
Reverse complement of SEQ ID NO: 121
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SEQ ID NO: 123
>NM_007853.5 Mus musculus delta(4)-desaturase, sphingolipid 1 (Degs1),
transcript variant 1, mRNA
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SEQ ID NO: 124
Reverse complement of SEQ ID NO: 123
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SEQ ID NO: 125
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SEQ ID NO: 126
Reverse complement of SEQ ID NO: 125
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SEQ ID NO: 127
>XM_005540946.2 PREDICTED: Macaca fascicularis delta(4)-desaturase,
sphingolipid 1 (DEGS1), mRNA
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SEQ ID NO: 128
Reverse complement of SEQ ID NO: 127
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SEQ ID NO: 129
>NM_001266006.1 Macaca mulatta delta 4-desaturase, sphingolipid 1 (DEGS1),
mRNA
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SEQ ID NO: 130
Reverse complement of SEQ ID NO: 129
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SEQ ID NO: 131
>NM_000230.3 Homo sapiens leptin (LEP), mRNA
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SEQ ID NO: 132
Reverse complement of SEQ ID NO: 131
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SEQ ID NO: 133
>NM_008493.3 Mus musculus leptin (Lep), mRNA
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SEQ ID NO: 134
Reverse complement of SEQ ID NO: 133
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SEQ ID NO: 135
>NM_013076.3 Rattus norvegicus leptin (Lep), mRNA
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SEQ ID NO: 136
Reverse complement of SEQ ID NO: 135
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SEQ ID NO: 137
>XM_005550685.2 PREDICTED: Macaca fascicularis leptin (LEP), transcript variant X1, mRNA
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SEQ ID NO: 138
Reverse complement of SEQ ID NO: 137
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SEQ ID NO: 139
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SEQ ID NO: 140
Reverse complement of SEQ ID NO: 139
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SEQ ID NO: 141
>NM_144997.7 Homo sapiens folliculin (FLCN), transcript variant 1, mRNA
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SEQ ID NO: 142
Reverse complement of SEQ ID NO: 141
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SEQ ID NO: 143
>NM_001271356.1 Mus musculus folliculin (Flcn), transcript variant 1, mRNA
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SEQ ID NO: 144
Reverse complement of SEQ ID NO: 143
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SEQ ID NO: 145
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SEQ ID NO: 146
Reverse complement of SEQ ID NO: 145
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SEQ ID NO: 147
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SEQ ID NO: 148
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SEQ ID NO: 149
>NM_001266691.1 Macaca mulatta folliculin (FLCN), mRNA
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SEQ ID NO: 150
Reverse complement of SEQ ID NO: 149
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SEQ ID NO: 151
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SEQ ID NO: 154
Reverse complement of SEQ ID NO: 153
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SEQ ID NO: 155
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SEQ ID NO: 157
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SEQ ID NO: 160
Reverse complement of SEQ ID NO: 159
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SEQ ID NO: 161
>NM_001259.8 Homo sapiens cyclin dependent kinase 6 (CDK6), transcript variant 1, mRNA
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SEQ ID NO: 162
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SEQ ID NO: 163
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SEQ ID NO: 165
>NM_001191861.2 Rattus norvegicus cyclin-dependent kinase 6 (Cdk6), mRNA
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SEQ ID NO: 166
Reverse complement of SEQ ID NO: 165
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SEQ ID NO: 167
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SEQ ID NO: 168
Reverse complement of SEQ ID NO: 167
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SEQ ID NO: 169
>NM_001261307.1 Macaca mulatta cyclin dependent kinase 6 (CDK6), mRNA
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SEQ ID NO: 170
Reverse complement of SEQ ID NO: 169
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SEQ ID NO: 171
>NM_020761.3 Homo sapiens regulatory associated protein of MTOR complex 1 (RPTOR), transcript variant 1, mRNA
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SEQ ID NO: 173
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SEQ ID NO: 175
>NM_001134499.2 Rattus norvegicus regulatory associated protein of MTOR, complex 1 (Rptor), mRNA

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SEQ ID NO: 180
Reverse complement of SEQ ID NO: 179
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SEQ ID NO: 181
>NM_004958.4 Homo sapiens mechanistic target of rapamycin kinase (MTOR), transcript variant 1, mRNA
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SEQ ID NO: 183
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SEQ ID NO: 185
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SEQ ID NO: 187
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SEQ ID NO: 188
Reverse complement of SEQ ID NO: 187
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SEQ ID NO: 189
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SEQ ID NO: 191
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SEQ ID NO: 193
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(PDE3B), mRNA
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Reverse complement of SEQ ID NO: 207
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SEQ ID NO:222
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SEQ ID NO:223
>NM_010834.3 Mus musculus myostatin (Mstn), mRNA
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SEQ ID NO:224
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SEQ ID NO:225
>NM_019151.1 Rattus norvegicus myostatin (Mstn), mRNA
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SEQ IDNO:226
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SEQ ID NO:227
>NM_001287623.1 Macaca fascicularis myostatin (MSTN), mRNA
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SEQ ID NO:228
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SEQ ID NO:229
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SEQ ID NO:231
>NM_001039523.3 Homo sapiens cholinergic receptor nicotinic alpha 1 subunit (CHRNA1), transcript variant 1, mRNA
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SEQ ID NO:233
>NM_007389.5 Mus musculus cholinergic receptor, nicotinic, alpha
polypeptide 1 (muscle) (Chrna1), mRNA
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SEQ ID NO:234

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SEQ ID NO:235
>NM_024485.2 Rattus norvegicus cholinergic receptor nicotinic alpha 1 subunit (Chrna1), mRNA
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SEQ ID NO:237
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SEQ ID NO:239
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SEQ ID NO:241
>NM_000747.3 Homo sapiens cholinergic receptor nicotinic beta 1 subunit (CHRNB1), mRNA
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SEQ ID NO:243
>NM_009601.4 Mus musculus cholinergic receptor, nicotinic, beta polypeptide 1 (muscle) (Chrnb1), mRNA
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SEQ ID NO:244
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SEQ ID NO:245
>NM_001395118.1 Rattus norvegicus cholinergic receptor nicotinic beta 1 subunit (Chrnb1), transcript variant 1, mRNA
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SEQ ID NO:246
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SEQ ID NO:247
>XM_005582753.2 PREDICTED: Macaca fascicularis cholinergic receptor, nicotinic beta 1 (CHRNB1), transcript variant X1, mRNA
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SEQ ID NO:248
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SEQ ID NO:249
>XM_015118481.2 PREDICTED: Macaca mulatta cholinergic receptor nicotinic beta 1 subunit (CHRNB1), transcript variant X1, mRNA
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SEQ ID NO:250
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SEQ ID NO:251
>NM_000751.3 Homo sapiens cholinergic receptor nicotinic delta subunit (CHRND), transcript variant 1, mRNA
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SEQ ID NO:253
>NM_021600.3 Mus musculus cholinergic receptor, nicotinic, delta polypeptide (Chrnd), mRNA
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SEQ ID NO:255
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SEQ ID NO:257
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SEQ ID NO:263
>NM_009603.1 Mus musculus cholinergic receptor, nicotinic, epsilon
polypeptide (Chrne), mRNA
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SEQ ID NO:264
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SEQ ID NO:265
>NM_017194.1 Rattus norvegicus cholinergic receptor nicotinic epsilon subunit (Chrne), mRNA
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SEQ ID NO:266
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SEQ ID NO:267
>XM_015437499.1 PREDICTED: Macaca fascicularis cholinergic receptor,
nicotinic epsilon (CHRNE), transcript variant X1, mRNA
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SEQ ID NO:268
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SEQ ID NO:269
>XM_015118354.2 PREDICTED: Macaca mulatta cholinergic receptor nicotinic epsilon subunit (CHRNE), transcript variant X1, mRNA
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SEQ ID NO:270
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SEQ ID NO:271
>NM_005199.5 Homo sapiens cholinergic receptor nicotinic gamma subunit (CHRNG), mRNA
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SEQ ID NO:273
>NM_009604.3 Mus musculus cholinergic receptor, nicotinic, gamma
polypeptide (Chrng), mRNA
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SEQ ID NO:275
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SEQ ID NO:277
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SEQ ID NO:301
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SEQ ID NO:303
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SEQ ID NO:320
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SEQ ID NO:321
>NM_005055.5 Homo sapiens receptor associated protein of the synapse (RAPSN), transcript variant 1, mRNA
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SEQ ID NO:323
>NM_009023.3 Mus musculus receptor-associated protein of the synapse (Rapsn), mRNA
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SEQ ID NO:325
>NM_001108584.1 Rattus norvegicus receptor-associated protein of the synapse (Rapsn), mRNA
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SEQ ID NO:326
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GAACA

SEQ ID NO:327
>XM_015434747.1 PREDICTED: Macaca fascicularis receptor associated protein of the synapse (RAPSN), transcript variant X1, mRNA
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SEQ ID NO:328
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SEQ ID NO:329
>XM_015114296.2 PREDICTED: Macaca mulatta receptor associated protein of the synapse (RAPSN), transcript variant X1, mRNA
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SEQ ID NO:330
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SEQ ID NO:331
>NM_000334.4 Homo sapiens sodium voltage-gated channel alpha subunit 4 (SCN4A), mRNA
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SEQ ID NO:332
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SEQ ID NO:335
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SEQ ID NO:337
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SEQ ID NO:339
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SEQ ID NO:349
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SEQ ID NO:351
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SEQ ID NO:354
Reverse complement of SEQ ID NO:353
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SEQ ID NO:355
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SEQ ID NO:356
Reverse complement of SEQ ID NO:355
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SEQ ID NO:357
>NM_001321571.2 Homo sapiens calcium/calmodulin dependent protein kinase II delta (CAMK2D), transcript variant 12, mRNA

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SEQ ID NO:359
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Reverse complement of SEQ ID NO:363
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We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a target gene, comprising

an antisense strand which is complementary to the target gene;

a sense strand which is complementary to the antisense strand and forms a double stranded region with the antisense strand; and

one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand.

2. The dsRNA agent of claim 1, wherein the C_{22} hydrocarbon chain is saturated or unsaturated.

3. The dsRNA agent of claim 1 or 2, wherein the C_{22} hydrocarbon chain is linear or branched

4. The dsRNA agent of any one of claims 1-3, wherein the internal positions include all positions except two or three terminal positions from each end of the at least one strand.

5. The dsRNA agent of claim 4, wherein the internal positions exclude a cleavage site region of the sense strand.

6. The dsRNA agent of claim 5, wherein the internal positions exclude positions 9-12 or positions 11-13, counting from the 5'-end of the sense strand.

7. The dsRNA agent of claim 4, wherein the internal positions exclude a cleavage site region of the antisense strand.

8. The dsRNA agent of claim 7, wherein the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand.

9. The dsRNA agent of any one of claims 1-8, wherein the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 4-8 and 13-18 on the sense strand, and positions 6-10 and 15-18 on the antisense strand, counting from the 5'end of each strand.

10. The dsRNA agent of claim 9, wherein the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17

on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

11. The dsRNA agent of claim 10, wherein the one or more C_{22} hydrocarbon chains are conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand.

12. The dsRNA agent of any one of claims 1-11, wherein the sense the antisense strands are each independently 15-30 nucleotides in length; 19 to 25 nucleotides in length; or 21 to 23 nucleotides in length.

13. The dsRNA agent of any one of claims 1-12, wherein the one or more C_{22} hydrocarbon chains is an aliphatic, alicyclic, or polyalicyclic compound.

14. ThedsRNA agent of claim 13, wherein the one or more C_{22} hydrocarbon chains contains a functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

15. The dsRNA agent of any one of claims 1-14, wherein the one or more C_{22} hydrocarbon chains is a C_{22} acid.

16. The dsRNA agent of claim 15, wherein the C_{22} acid is selected from the group consisting of docosanoic acid, 6-octyltetradecanoic acid, 10-hexylhexadecanoic acid, all-cis-7,10,13,16,19-docosapentaenoic acid, all-cis-4,7,10,13,16,19-docosahexaenoic acid, all-cis-13,16-docosadienoic acid, all-cis-7,10,13,16-docosatetraenoic acid, all-cis-4,7,10,13,16-docosapentaenoic acid, and cis-13-docosenoic acid.

17. The dsRNA agent of any one of claims 1-14, wherein the one or more C_{22} hydrocarbon chains is a C_{22} alcohol.

18. The dsRNA agent of claim 17, wherein the C_{22} alcohol is selected from the group consisting of 1-docosanol, 6-octyltetradecan-1-ol, 10-hexylhexadecan-1-ol, cis-13-docosen-1-ol, docosan-9-ol, docosan-2-ol, docosan-10-ol, docosan-11-ol, andcis-4,7,10,13,16,19-docosahexanol.

19. The dsRNA agent of any one of claims 1-14, wherein the one or more C_{22} hydrocarbon chains is a C_{22} amide.

20. The dsRNA agent of claim 19, wherein the C_{22} amide is selected from the group consisting of (E)-Docos-4-enamide, (E)-Docos-5-enamide, (Z)-Docos-9-enamide, (E)-Docos-11-enamide,12-Docosenamide, (Z)-Docos-13-enamide, (Z)-N-Hydroxy-13-docoseneamide, (E)-Docos-14-enamide, 6-cis-Docosenamide, 14-Docosenamide Docos-11-enamide, (4E,13E)-Docosa-4,13-dienamide, and (5E,13E)-Docosa-5,13-dienamide.

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21. The dsRNA agent of any one of claims 1-20, wherein the one or more C_{22} hydrocarbon chains is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s).

22. The dsRNA agent of claim 21, wherein the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

23. The dsRNA agent of any one of claims 1-22, wherein the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a linker containing an ether, thioether, urea, carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

24. The dsRNA agent of any one of claims 1-22, wherein the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a linker or a carrier or via internucleotide phosphate linkage.

25. The dsRNA agent of any one of claims 1-24, wherein the dsRNA agent comprises at least one single-stranded overhang.

26. The dsRNA agent of claim 25, wherein the single-stranded overhang is 1, 2 or 3 nucleotides in length.

27. The dsRNA agent of any one of claims 1-26, wherein at least one end of the dsRNA agent is blunt-ended.

28. The dsRNA agent of any one of claims 1-27, wherein the one or more C_{22} hydrocarbon chains is conjugated to a nucleobase, sugar moiety, or internucleosidic phosphate linkage.

29. The dsRNA agent of any one of claims 1-28, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

30. The dsRNA agent of claim 29, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

31. The dsRNA agent of any one of claims 1-28, further comprising a modified phosphate at the 5'-end of the antisense strand.

32. The dsRNA agent of any one of claims 1-31, wherein the one or more C_{22} hydrocarbon chains or targeting ligand is conjugated via a biocleavable linker selected from the group consisting of a DNA linker, an RNA linker, a disulfide linker, an amide linker, a protease cleavable peptide linker, a funtionalized monosaccharide linker, a galactosamine oligosaccharide linker, a glucosamine linker, a glucose linker, a galactose linker, a mannose linker, and combinations thereof.

33. The dsRNA agent of any one of claims 1-32, wherein the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

34. The dsRNA agent of any one of claims 1-33, wherein the target gene is selected from the group consisting of adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4 (KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); and Phosphodiesterase 1 (PDE1).

35. The dsRNA agent of any one of claims 1-33, wherein the target gene is selected from the group consisting of Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin (LEP); folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); and Activin A Receptor Type 1C (ACVR1C).

36. The dsRNA agent of any one of claims 1-33, wherein the target gene is selected from the group consisting of myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

37. A cell containing the dsRNA agent of any one of claims 1-36.

38. A pharmaceutical composition for inhibiting expression of the target gene, comprising the dsRNA agent of any one of claims 1-36.

39. A method of inhibiting expression of a target gene in a skeletal muscle cell, a cardiac muscle cell, or an adipocyte, comprising contacting the cell with a dsRNA agent that inhibits expression of a target gene, wherein the dsRNA agent comprises

an antisense strand which is complementary to the target gene;

a sense strand which is complementary to the antisense strand and forms a double stranded region with the antisense strand; and

one or more C_{22} hydrocarbon chains conjugated to one or more internal positions on at least one strand.

40. The method of claim 39, wherein the C_{22} hydrocarbon chain is saturated or unsaturated.

41. The method of claim 39 or 40, wherein the C_{22} hydrocarbon chain is linear or branched

42. The method of any one of claims 39-41, wherein the internal positions include all positions except two or three terminal positions from each end of the at least one strand.

43. The method of claim 42, wherein the internal positions exclude a cleavage site region of the sense strand.

44. The method of claim 43, wherein the internal positions exclude positions 9-12 or positions 11-13, counting from the 5'-end of the sense strand.

45. The method of claim 42, wherein the internal positions exclude a cleavage site region of the antisense strand.

46. The method of claim 45, wherein the internal positions exclude positions 12-14, counting from the 5'-end of the antisense strand.

47. The method of any one of claims 39-46, wherein the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 4-8 on the sense strand, positions 13-18 on the sense strand, positions 6-10 on the antisense strand and positions 15-18 on the antisense strand, counting from the 5'end of each strand.

48. The method of claim 47, wherein the one or more C_{22} hydrocarbon chains are conjugated to one or more of the following internal positions: positions 5, 6, 7, 15, and 17 on the sense strand, and positions 15 and 17 on the antisense strand, counting from the 5'-end of each strand.

49. The method of claim 48, wherein the one or more C_{22} hydrocarbon chains are conjugated to position 6 on the sense strand, counting from the 5'-end of the sense strand.

50. The method of any one of claims 39-49, wherein the sense strand and the antisense strand are each independently 15-30 nucleotides in length; 19 to 25 nucleotides in length; or 21 to 23 nucleotides in length.

51. The method of any one of claims 39-50, wherein the one or more C_{22} hydrocarbon chains is an aliphatic, alicyclic, or polyalicyclic compound.

52. The method of claim 51, wherein the one or more C_{22} hydrocarbon chains contains a functional group selected from the group consisting of hydroxyl, amine, carboxylic acid, sulfonate, phosphate, thiol, azide, and alkyne.

53. The method of any one of claims 39-52, wherein the one or more C_{22} hydrocarbon chains is a C_{22} acid.

54. The method of claim 53, wherein the C_{22} acid is selected from the group consisting of docosanoic acid, 6-octyltetradecanoic acid, 10-hexylhexadecanoic acid, all-cis-7,10,13,16,19-docosapentaenoic acid, all-cis-4,7,10,13,16,19-docosahexaenoic acid, all-cis-13,16-docosadienoic acid, all-cis-7,10,13,16-docosatetraenoic acid, all-cis-4,7,10,13,16-docosapentaenoic acid, and cis-13-docosenoic acid.

55. The method of any one of claims 39-52, wherein the one or more C_{22} hydrocarbon chains is a C_{22} alcohol.

56. The method of claim 55, wherein the C_{22} alcohol is selected from the group consisting of 1-docosanol, 6-octyltetradecan-1-ol, 10-hexylhexadecan-1-ol, cis-13-docosen-1-ol, docosan-9-ol, docosan-2-ol, docosan-10-ol, docosan-11-ol, and cis-4,7,10,13,16,19-docosahexanol.

57. The method of any one of claims 39-52, wherein the one or more C_{22} hydrocarbon chains is a C_{22} amide.

58. The method of claim 57, wherein the C_{22} amide is selected from the group consisting of (E)-Docos-4-enamide, (E)-Docos-5-enamide, (Z)-Docos-9-enamide, (E)-Docos-11-enamide,12-Docosenamide, (Z)-Docos-13-enamide, (Z)-N-Hydroxy-13-docoseneamide, (E)-Docos-14-enamide, 6-cis-Docosenamide, 14-Docosenamide Docos-11-enamide, (4E,13E)-Docosa-4,13-dienamide, and (5E,13E)-Docosa-5,13-dienamide.

59. The method of any one of claims 39-58, wherein the one or more C_{22} hydrocarbon chains is conjugated via a carrier that replaces one or more nucleotide(s) in the internal position(s).

60. The method of claim 59, wherein the carrier is a cyclic group selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl; or is an acyclic moiety based on a serinol backbone or a diethanolamine backbone.

61. The method of any one of claims 39-60, wherein the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a linker containing an ether, thioether, urea,

carbonate, amine, amide, maleimide-thioether, disulfide, phosphodiester, sulfonamide linkage, a product of a click reaction, or carbamate.

62. The method of any one of claims 39-60, wherein the one or more C_{22} hydrocarbon chains is conjugated to the dsRNA agent via a linker or via a carrier or via an internucleotide phosphate linkage.

63. The method of any one of claims 39-62, wherein the dsRNA agent comprises at least one single-stranded overhang.

64. The method of claim 63, wherein the single-stranded overhang is 1, 2 or 3 nucleotides in length.

65. The method of any one of claims 39-64, wherein at least one end of the dsRNA agent is blunt-ended.

66. The method of any one of claims 39-65, wherein the one or more C_{22} hydrocarbon chains is conjugated to a nucleobase, sugar moiety, or internucleosidic phosphate linkage.

67. The method of any one of claims 39-66, further comprising a phosphate or phosphate mimic at the 5'-end of the antisense strand.

68. The method of claim 67, wherein the phosphate mimic is a 5'-vinyl phosphonate (VP).

69. The method of any one of claims 39-68, wherein the one or more C_{22} hydrocarbon chains or targeting ligand is conjugated via a bio-cleavable linker selected from the group consisting of a DNA linker, an RNA linker, a disulfide linker, an amide linker, a protease cleavable peptide linker, a funtionalized monosaccharide linker, a galactosamine oligosaccharide linker, a glucosamine linker, a glucose linker, a galactose linker, a mannose linker, and combinations thereof.

70. The method of any one of claims 39-69, wherein the 3' end of the sense strand is protected via an end cap which is a cyclic group having an amine, said cyclic group being selected from the group consisting of pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolanyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuranyl, and decalinyl.

71. The method of any one of claims 39-70, wherein the target gene is selected from the group consisting of adrenoceptor beta 1 (ADRB1); calcium voltage-gated channel subunit alpha1 C (CACNA1C); calcium voltage-gated channel subunit alpha1 G (CACNA1G) (T type calcium cchannel); angiotensin II receptor type 1(AGTR1); Sodium Voltage-Gated Channel Alpha Subunit 2 (SCN2A); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 1 (HCN1); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4 (HCN4); Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 3 (HCN3); Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5); Potassium Inwardly Rectifying Channel Subfamily J Member 3 (KCNJ3); Potassium Inwardly Rectifying Channel Subfamily J Member 4 (KCNJ4); phospholamban (PLN); calcium/calmodulin dependent protein kinase II delta (CAMK2D); or Phosphodiesterase 1 (PDE1).

72. The method of any one of claims 39-70, wherein the target gene is selected from the group consisting of Delta 4-Desaturase, Sphingolipid 1 (DEGS1); leptin (LEP); folliculin (FLCN); Zinc Finger Protein 423 (ZFP423); Cyclin Dependent Kinase 6 (CDK6); Regulatory Associated Protein Of MTOR Complex 1 (RPTOR); Mechanistic Target Of Rapamycin Kinase, (mTOR); Forkhead Box P1 (FOXP1); Phosphodiesterase 3B (PDE3B); annnd Activin A Receptor Type 1C (ACVR1C).

73. The method of any one of claims 39-70, wherein the target gene is selected from the group consisting of myostatin (MSTN); Cholinergic Receptor Nicotinic Alpha 1 Subunit (CHRNA1); Cholinergic Receptor Nicotinic Beta 1 Subunit (CHRNB1); Cholinergic Receptor Nicotinic Delta Subunit (CHRND); Cholinergic Receptor Nicotinic Epsilon Subunit (CHRNE); Cholinergic Receptor Nicotinic Gamma Subunit (CHRNG); Collagen Type XIII Alpha 1 Chain (COL13A1); Docking Protein 7 (DOK7); LDL Receptor Related Protein 4 (LRP4); Muscle Associated Receptor Tyrosine Kinase (MUSK); Receptor Associated Protein Of The Synapse (RAPSN); Sodium Voltage-Gated Channel Alpha Subunit 4 (SCN4A); and Double Homeobox 4 (DUX4).

74. The method of any one of claims 39-73, wherein the cell is within a subject.

75. The method of claim 74, wherein the subject is a human.

76. A method of treating a subject having a skeletal muscle disorder, a cardiac muscle disorder, or an adipose tissue disorder, comprising:

administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-36, thereby treating the subject.

77. The method of claim 76, wherein the cardiac disorder is selected from the group consisting of obstructive hypertrophic cardiomyopathy (HOCM); familial hypertrophic cardiomyopathy (FHC); heart failure with preserved ejection fraction (HFPEF); atrial fibrillation (AFIB); ventricular fibrillation (VFIB); angina; myocardial infarction (MI); heart failure or heart failure with reduced ejection fraction (HFREF); supraventricular tachycardia (SVT); hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmia, and congestive heart failure (CHF).

78. The method of claim 76, wherein the adipose disorder is a metabolic disorder.

79. The method of claim 78, wherein the metabolic disorder is selected from the group consisting of a disorder of carbohydrates, a lipid metabolism disorder, hypertension, a cardiovascular disease, and a disorder of body weight.

80. The method of claim 76, wherein the skeletal muscle disorder is selected from the group consisting of Myostatin-related muscle hypertrophy, congenital myasthenic syndrome, and facioscapulohumeral muscular dystrophy (FSHD).

81. The method of any one of claims 76-80, wherein the dsRNA agent is administered to the subject subcutaneously.

82. The method of any one of claims 76-80, wherein the dsRNA agent is administered to the subject intramusclularly.

83. The method of any one of claims 76-80, wherein the dsRNA agent is administered to the subject intavenously.

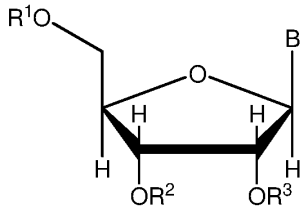
84. The method of any one of claims 76-80, wherein the dsRNA agent is administered to the subject intraperitonealy.

85. The method of any one of claims 76-84, further comprising administering to the subject an additional agent or a therapy suitable for treatment or prevention of a a cardiac muscle disorder, or an adipose tissue disorder.

86. The method of claim 78, wherein the metabolic disorder is a disorder of body weight and wherein administration of the dsRNA agent inhibits expression of a target gene by no more than 40%, no more than 50%, no more than 60% or no more than 70%.

87. The method of claim 86, wherein the target gene is leptin.

88. A method of synthesizing a nucleoside monomer having the structure of Formula (I):



FORMULA (I)

wherein:

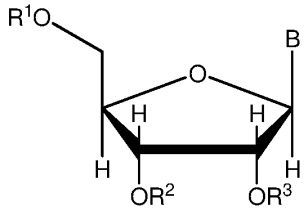
B is a modified or unmodified nucleobase;

R^1 is a hydroxyl protecting group;

R^2 is H or phosphoramidite;

R^3 is $C_{22}H_{45}$

wherein the compound of formula (I) is free or substantially free of a compound of Formula (II)



FORMULA (II)

wherein:

B is a modified or unmodified nucleobase;

R^1 is a hydroxyl protecting group;

R^2 is $C_{22}H_{45}$

R^3 is H

89. The method of claim 88, wherein the hydroxyl protecting group is selected from the group consisting of 4,4'-dimethoxytrityl (DMT), monomethoxytrityl (MMT), 9-fluorenylmethylcarbonate (Fmoc), o-nitrophenylcarbonyl, p-phenylazophenylcarbonyl, phenylcarbonyl, p-chlorophenylcarbonyl, and 5'-(α-methyl-2-nitropiperonyl)oxycarbonyl (MeNPOC).