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(54) Title: 3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE (HMGCR) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: The present invention relates to RNAi agents, e.g., double stranded RNA (dsRNA) agents, targeting the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene. The invention also relates to methods of using such RNAi agents to inhibit expression of an HMGCR gene and to methods of preventing and treating an HMGCR-associated disorder, e.g., disorder of lipid metabolism, e.g., hyperlipidemia.



WO 2023/009687 A1

3-HYDROXY-3-METHYLGLUTARYL-COA REDUCTASE (HMGCR) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

5 This application claims the benefit of priority to U.S. Provisional Application No. 63/226,873, filed on July 29, 2021, and U.S. Provisional Application No. 63/284,720, filed on December 1, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

10 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) catalyzes the conversion of 3-hydroxy-3-methyl-glutaryl-CoA into mevalonic acid, the rate limiting step in cholesterol biosynthesis. The enzymatic activity of HMGCR in the cholesterol biosynthesis pathway is regulated *via* a negative feedback mechanism mediated by sterols and non-sterol metabolites derived from mevalonate, which is the product of the reaction catalyzed by HMGCR. Normally in mammalian cells, HMGCR is
15 suppressed by cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) *via* the LDL receptor. Competitive inhibitors of HMGCR induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol.

Disorders of lipid metabolism can lead to elevated levels of serum lipids, such as triglycerides
20 and/or cholesterol. High cholesterol levels have been associated with an increased risk of atherosclerosis and cardiovascular disease (Lewington S, *et al.* (2007), *Lancet* 370 (9602): 1829–39). Elevated cholesterol levels contribute to formation of atheromatous plaque in the arteries which may lead to progressive narrowing or blockage of the involved arteries. Occlusion of a coronary artery can result in myocardial infarction or heart attack, while occlusion of an artery supplying the brain may
25 lead to stroke.

Statins are a widely known drug class of cholesterol lowering drugs. Statins act by competitively inhibiting HMGCR enzymatic activity, and reduce the rate by which HMGCR is able to produce mevalonate, thus, reducing the overall synthesis of cholesterol. Although statins have been found to reduce cardiovascular disease and mortality in those who are at high risk, some patients
30 experience side effects of statins including muscle pain, increased risk of hyperlipidemia mellitus, and abnormalities in liver enzyme tests (Naci H, *et al.* (2013). *Circ Cardiovasc Qual Outcomes* 6 (4): 390–9). Additionally, patients may experience rare but severe adverse effects, particularly statin-induced myopathy (Abd TT, *et al.* (2011) *Expert opinion on drug safety* 10 (3): 373–87). Accordingly, there is a need in the art for alternative treatments for subjects having disorders of lipid
35 metabolism.

SUMMARY OF THE INVENTION

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). The HMGCR gene may be within a cell, *e.g.*, a cell within a subject, such as a human subject. The present invention also provides methods of using the iRNA compositions of the invention for inhibiting the expression of an HMGCR gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of an HMGCR gene, *e.g.*, a subject suffering or prone to suffering from an HMGCR-associated disorder, *e.g.* a disorder of lipid metabolism, *e.g.*, hyperlipidemia.

Accordingly, in an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of any one of SEQ ID NOs:1-8 and 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 1, 2, or 3 nucleotides from the corresponding portion of the nucleotide sequence of any one of SEQ ID NOs:9-16.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in a cell, wherein said dsRNA comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HMGCR, and wherein the region of complementarity comprises at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2-5.

In one embodiment, the dsRNA agent comprises a sense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising a contiguous nucleotide sequence which has at least 85%, *e.g.*, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, nucleotide sequence identity over its entire length to any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.

In one embodiment, the dsRNA agent comprises a sense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising at least 15, *e.g.*, 15, 16, 17, 18, 19, 20, 21, 22, or 23 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.

In one embodiment, the dsRNA agent comprises a sense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the sense strands in any one of Tables 2-3 and an antisense strand comprising or consisting of a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2-3.

In one embodiment, the dsRNA agent comprises at least one modified nucleotide.

In one embodiment, substantially all of the nucleotides of the sense strand are modified nucleotides; substantially all of the nucleotides of the antisense strand are modified nucleotides; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, all of the nucleotides of the sense strand are modified nucleotides; all of the nucleotides of the antisense strand are modified nucleotides; or all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythymidine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.

In one embodiment, at least one of the modified nucleotides is selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

5 In one embodiment, at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a glycol modified nucleotide (GNA), *e.g.*, Ggn, Cgn, Tgn, or Agn, a nucleotide with a 2' phosphate, *e.g.*, G2p, C2p, A2p or U2p, a nucleotide comprising a phosphorothioate group, and a vinyl-phosphonate nucleotide; and combinations thereof.

10 In another embodiment, at least one of the modified nucleotides is a nucleotide with a thermally destabilizing nucleotide modification.

In one embodiment, the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; a destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).

15 In some embodiments, the modified nucleotide comprises a short sequence of 3'-terminal deoxythymidine nucleotides (dT).

In some embodiments, the dsRNA agent further comprises at least one phosphorothioate internucleotide linkage. In some embodiments, the dsRNA agent comprises 6-8 phosphorothioate internucleotide linkages. In one embodiment, the phosphorothioate or methylphosphonate
20 internucleotide linkage is at the 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In a related embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand. In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the
25 both the 5'- and 3'-terminus of one strand. Optionally, the strand is the antisense strand. In another embodiment, the strand is the sense strand.

The double stranded region may be 19-30 nucleotide pairs in length; 19-25 nucleotide pairs in length; 19-23 nucleotide pairs in length; 23-27 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

30 In one embodiment, each strand is independently no more than 30 nucleotides in length.

In one embodiment, the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

The region of complementarity may be at least 17 nucleotides in length; between 19 and 23 nucleotides in length; or 19 nucleotides in length.

35 In one embodiment, 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.

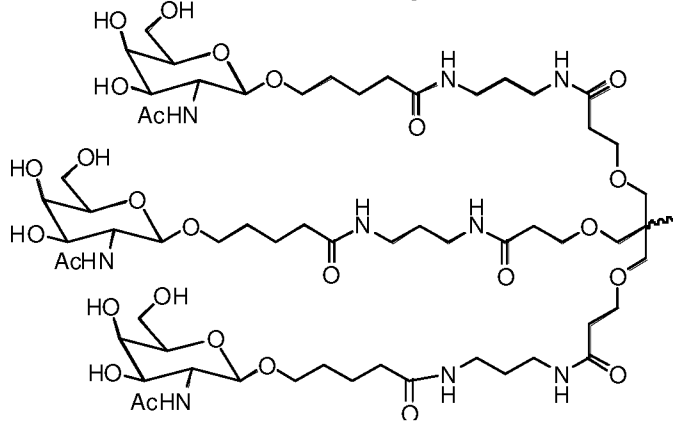
In one embodiment, the dsRNA agent further comprises a ligand.

In one embodiment, the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

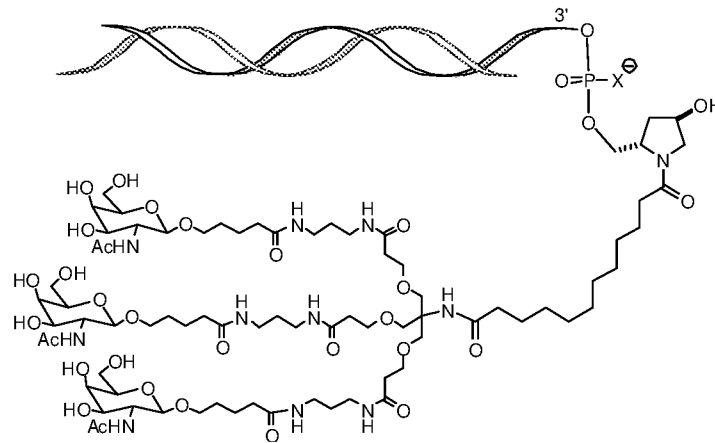
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

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

In one embodiment, the ligand is



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



and, wherein X is O or S.

In one embodiment, the X is O.

In one embodiment, the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In another embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand, *e.g.*, the antisense strand or the sense strand.

In one embodiment, the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand. In one embodiment, the strand is the antisense strand.

In one embodiment, the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

In one 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 phosphodiester 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 phosphodiester, 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 agent having 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).

The present invention also provides cells containing any of the dsRNA agents of the invention and pharmaceutical compositions comprising any of the dsRNA agents of the invention.

The pharmaceutical composition of the invention may include dsRNA agent in an unbuffered solution, *e.g.*, saline or water, or the pharmaceutical composition of the invention may include the dsRNA agent is in a buffer solution, *e.g.*, a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a method of inhibiting expression of a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene in a cell. The method includes contacting the cell with any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby inhibiting expression of the HMGCR gene in the cell.

In one embodiment, the cell is within a subject, *e.g.*, a human subject, *e.g.*, a subject having a disorder of lipid metabolism, such as hyperlipidemia.

In one embodiment, the hyperlipidemia is mixed hyperlipidemia, hypertriglyceridemia, or hypercholesterolemia

In certain embodiments, the HMGCR expression is inhibited by at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In one embodiment, inhibiting expression of HMGCR decreases HMGCR protein level in serum of the subject by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%.

In one aspect, the present invention provides a method of treating a subject having a disorder that would benefit from reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) expression. The method includes administering to the subject a therapeutically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby treating the subject having the disorder that would benefit from reduction in HMGCR expression.

In another aspect, the present invention provides a method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) expression. The method includes administering to the subject a prophylactically effective amount of any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in HMGCR expression.

In certain embodiments, the disorder is a disorder of lipid metabolism, *e.g.* a hyperlipidemia.

In some embodiments, the HMGCR-associated disorder is mixed hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia.

In certain embodiments, administration of the dsRNA to the subject causes a decrease HMGCR protein accumulation in the subject.

In a further aspect, the present invention also provides methods of inhibiting the expression of HMGCR in a subject. The methods include administering to the subject a therapeutically effective amount of any of the dsRNAs provided herein, thereby inhibiting the expression of HMGCR in the subject.

In one embodiment, the subject is human.

In one embodiment, the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

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

In one embodiment, the methods of the invention include further determining the level of HMGCR in a sample(s) from the subject.

In one embodiment, the level of HMGCR in the subject sample(s) is an HMGCR protein level in a blood or serum or liver tissue sample(s).

In certain embodiments, the methods of the invention further comprise administering to the subject an additional therapeutic agent.

In certain embodiments, the additional therapeutic agent is selected from the group consisting of a statin, a bile sequestering agent, a VLDL secretion inhibitor, a lipophilic antioxidant, an acyl-CoA cholesterol acyl transferase inhibitor, a farnesoid X receptor antagonist, a sterol regulatory binding protein cleavage activating protein (SCAP) activator, a microsomal triglyceride transfer protein (MTP) inhibitor, an ApoE-related peptide, a cholesteryl ester transfer protein (CETP) inhibitor, a therapeutic antibody against HMGCR, and a combination of any of the foregoing.

The present invention also provides kits comprising any of the dsRNAs of the invention or any of the pharmaceutical compositions of the invention, and optionally, instructions for use. In one embodiment, the invention provides a kit for performing a method of inhibiting expression of HMGCR gene in a cell by contacting a cell with a double stranded RNAi agent of the invention in an amount effective to inhibit expression of the HMGCR in the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to a subject.

The present invention further provides an RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of the invention.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene. The gene may be within a cell, *e.g.*, a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (HMGCR) in mammals.

10 The iRNAs of the invention have been designed to target the human 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene, including portions of the gene that are conserved in the HMGCR orthologs of other mammalian species. Without intending to be limited by theory, it is believed that a combination or sub-combination of the foregoing properties and the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved
15 efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an HMGCR gene.

20 The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is up to about 30 nucleotides or less in length, *e.g.*, 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, which region is substantially complementary to at least part of an mRNA transcript of an HMGCR gene.

25 In certain embodiments, one or both of the strands of the double stranded RNAi agents of the invention is up to 66 nucleotides in length, *e.g.*, 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length, with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of an HMGCR gene. In some embodiments, such iRNA agents having longer length antisense strands may, for example, include a second RNA strand (the sense
30 strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of iRNAs of the invention enables the targeted degradation of mRNAs of the corresponding gene (HMGCR gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting an HMGCR gene can potently mediate RNAi, resulting in
35 significant inhibition of expression of an HMGCR gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia.

Accordingly, the present invention provides methods and combination therapies for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an HMGCR gene, *e.g.*, a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR)-associated disease, such as a disorder of lipid metabolism, *e.g.*, hyperlipidemia, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an HMGCR gene.

The present invention also provides methods for preventing at least one symptom in a subject having a disorder that would benefit from inhibiting or reducing the expression of an HMGCR gene, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an HMGCR gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of an HMGCR gene, *e.g.*, subjects susceptible to or diagnosed with an HMGCR-associated disorder.

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 $\pm 10\%$. In certain embodiments, about means $\pm 5\%$. 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, “3-hydroxy-3-methylglutaryl-CoA reductase,” used interchangeably with the term “HMGCR,” refers to an amino acid sequence from any vertebrate or mammalian source, including, but not limited to, human, bovine, chicken, rodent, mouse, rat, porcine, ovine, primate, monkey, and guinea pig, unless specified otherwise. The term also refers to fragments and variants of native HMGCR that maintain at least one *in vivo* or *in vitro* activity of a native HMGCR. The term encompasses full-length unprocessed precursor forms of HMGCR as well as mature forms resulting from post-translational cleavage of the signal peptide.

The sequence of a human HMGCR mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519311560 (NM_000859.3; SEQ ID NO:1; reverse complement, SEQ ID NO:9) and GenBank Asscension No. GI: 196049379 (NM_001130996.2; SEQ ID NO:2; reverse complement, SEQ ID NO:10). The predicted nucleotide and amino acid sequence of a human HMGCR can also be found at, for example, GenBank Accession No. GI: 767935799 (XM_011543357.1 ; SEQ ID NO:3; reverse complement, SEQ ID NO:11); GenBank Accession No. GI: 767935801 (XM_011543358.1; SEQ ID NO:4; reverse complement, SEQ ID NO:12) and GenBank Accession No. GI: 767935803 (XM_011543359.1SEQ ID NO:5; reverse complement, SEQ ID NO:13) The predicted nucleotide and amino acid sequence of a *Cynomolgus* monkey HMGCR can be found at, for example, GenBank Accession No. GI: 544437803 (XM_005557178.1; SEQ ID NO:6; reverse complement, SEQ ID NO:14). The nucleotide and amino acid sequence of a mouse HMGCR can be found at, for example, GenBank Accession No. GI: 160358777 (NM_008255.2; SEQ ID NO:7; reverse complement, SEQ ID NO:15). The nucleotide and amino acid sequence of a rat HMGCR can be found at, for example, GenBank Accession No. GI: 40538851 (NM_013134.2; SEQ ID NO:8; reverse complement, SEQ ID NO:16).

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

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

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.

5 The term HMGCR, as used herein, also refers to variations of the HMGCR gene including variants provided in the SNP database. Numerous sequence variations within the HMGCR 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=HMGCR, the entire contents of which is incorporated herein by reference as of the date of filing this application.

10 As used herein, “target sequence” refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HMGCR gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodiment, the target portion of the sequence will be at least long enough to serve as a substrate for siRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an HMGCR gene.

15 The target sequence may be from about 19-36 nucleotides in length, *e.g.*, about 19-30 nucleotides in length. For example, the target sequence can be about 19-30 nucleotides, 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 certain embodiments, the target sequence is 19-23 nucleotides in length, optionally 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.

25 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.

30 “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. 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 35 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.

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. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, *e.g.*, inhibits, the expression of an HMGCR gene in a cell, *e.g.*, a liver cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNA that interacts with a target RNA sequence, *e.g.*, an HMGCR 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 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). Thus, in one aspect the invention relates to a single stranded RNA (siRNA) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, *i.e.*, an HMGCR gene. Accordingly, the term “siRNA” is also used herein to refer to an iRNA as described above.

In certain embodiments, the RNAi agent may be a single-stranded siRNA (ssRNAi) 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 siRNAs 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 certain embodiments, an “iRNA” for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a “double stranded RNA 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.*, an HMGCR gene. In some embodiments of the invention, 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, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an “iRNA” may include ribonucleotides with chemical modifications; an iRNA 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 modified nucleobase, or any combination thereof. 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 invention 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 “iRNA” or “RNAi agent” for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an 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 19 to 36 base pairs in length, *e.g.*, about 19-30 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 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 region is 19-21 base pairs in length, *e.g.*, 21 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the disclosure.

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 least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. 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.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not be, 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 of the RNAi agent, at least one strand comprises a 3' overhang of at least 1 nucleotide. In another
5 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
10 comprise an overhang of at least 1 nucleotide.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, *e.g.*, an HMGCR gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that
15 interacts with a target RNA sequence, *e.g.*, an HMGCR 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 double stranded iRNA. 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
20 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
25 present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, the antisense 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 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
30 replaced with a nucleoside thiophosphate.

In certain embodiments, 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
35 the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotides, *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 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, 10-25 nucleotides, 10-20 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 extended 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.

“Blunt” or “blunt end” means that there are no unpaired nucleotides at that end of the double stranded RNA agent, *i.e.*, no nucleotide overhang. A “blunt ended” double stranded RNA agent is double stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (*i.e.*, agents with one overhang and one blunt end) or with no nucleotide overhangs at either end. 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.*, an HMGCRC mRNA.

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.*, an HMGCRC 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, or 3 nucleotides of the 5'- or 3'-end of the iRNA. 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, 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 an HMGCR 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 an HMGCR gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of an HMGCR gene is important, especially if the particular region of complementarity in an HMGCR gene is known to have polymorphic sequence variation within the population.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of an iRNA 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, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C 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 iRNA, *e.g.*, within a dsRNA as described herein, include
5 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
10 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
15 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,
20 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”
25 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 double stranded RNA 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) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding an HMGCR gene). For
30 example, a polynucleotide is complementary to at least a part of an HMGCR mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an HMGCR gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target HMGCR sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target HMGCR sequence and comprise a
35 contiguous nucleotide sequence which is at least 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of SEQ ID NOs:1-8, or a fragment of any one of SEQ ID NOs:1-8, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target HMGCR 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 any one of Tables 2-5, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2-5, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% 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 HMGCR 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: 9-16, or a fragment of any one of SEQ ID NOs:9-16, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% 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 HMGCR 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, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2-5, such as about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100% complementary.

In general, an "iRNA" includes ribonucleotides with chemical modifications. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a dsRNA molecule, are encompassed by "iRNA" for the purposes of this specification and claims.

In certain embodiments of the instant disclosure, inclusion of a deoxy-nucleotide if present within an RNAi agent can be considered to constitute a modified nucleotide.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA *via* an antisense inhibition mechanism. The single-stranded antisense oligonucleotide 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 oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16,

17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase “contacting a cell with an iRNA,” such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell *in vitro* with the iRNA or contacting a cell *in vivo* with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the individual performing the method, or alternatively, the iRNA 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 iRNA.

Contacting a cell *in vivo* may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the iRNA may contain or be coupled to a ligand, *e.g.*, GalNAc, that directs the iRNA to a site of interest, *e.g.*, the liver. 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 iRNA and subsequently transplanted into a subject.

In certain embodiments, contacting a cell with an iRNA includes “introducing” or “delivering the iRNA into the cell” by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be *in vitro* or *in vivo*. For example, for *in vivo* introduction, iRNA 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.*, an iRNA or a plasmid from which an iRNA 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), a non-primate (such as 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 an embodiment, the subject is a human, such as a human being treated or assessed for a disease or disorder that would benefit from reduction in HMGCR expression; a human at risk for a disease or disorder that would benefit from reduction in HMGCR expression; a human having a disease or disorder that would benefit from reduction in HMGCR expression; or human being treated for a disease or disorder that would benefit from reduction in HMGCR 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, such as reducing at least one sign or symptom of an HMGCR-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted HMGCR expression; diminishing the extent of unwanted HMGCR activation or stabilization; amelioration or palliation of unwanted HMGCR 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 HMGCR 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%, 35%, 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 HMGCR 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, “lower” is the decrease in the difference between the level of a marker or symptom for a subject suffering from a disease and a level accepted within the range of normal for an individual. 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 an HMGCR-associated disorder towards or to a level in a normal subject not suffering from an HMGCR-associated disorder. 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, may be treated or ameliorated by a reduction in expression of an HMGCR gene, 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 an HMGCR-associated disorder, *e.g.*, a disorder of lipid metabolism, *e.g.*, hyperlipidemia. 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 “3-hydroxy-3-methylglutaryl-CoA reductase-associated disorder” or “HMGCR-associated disorder,” is a disease or disorder that is caused by, or associated with, HMGCR gene expression or HMGCR protein production. The term “HMGCR-associated disorder” includes a disease, disorder or condition that would benefit from a decrease in HMGCR gene expression, replication, or protein activity. Exemplary HMGCR-associated diseases include acquired or inherited “disorders of lipid metabolism” which include any disorder associated with or caused by a disturbance in lipid metabolism. For example, this term includes any disorder, disease or condition characterized

by abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood or a condition that can lead to abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood, such as a hyperlipidemia, and other forms of lipid imbalance such as hypercholesterolemia, hypertriglyceridemia, mixed hyperlipidemia, as well as the pathological conditions associated with these disorders, e.g., congestive heart disease (CHD) and atherosclerosis.

Exemplary 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, and 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), familial partial lipodystrophy type 1 (FPLD1), hypercholesterolemia, mixed hyperlipidemia (or mixed hyperlipoproteinemia familial, gout associated with hypercholesterolemia, xanthomatosis (subcutaneous cholesterol deposits), hyperlipidemia with heterogeneous LPL deficiency, and hyperlipidemia with high LDL, heterogeneous LPL deficiency, and an induced or acquired disorder, such as a disorder induced or acquired as a result of a disease.

As used herein, the term “serum lipid” refers to any major lipid present in the blood. Serum lipids may be present in the blood either in free form or as a part of a protein complex, e.g., a lipoprotein complex. Non-limiting examples of serum lipids include triglycerides, cholesterol, such as total cholesterol, low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and intermediate-density lipoprotein cholesterol (IDL-C).

In some embodiments, the disorder of lipid metabolism is a hyperlipidemia. As used herein, the term “hyperlipidemia” refers to any any disorder, disease or condition characterized by abnormal elevation of levels of any or all lipids, such as cholesterol and triglycerides, and/or lipoproteins in the blood or a condition that can lead to abnormal elevation of levels of any or all lipids and/or lipoproteins in the blood.

In one embodiment, the hyperlipidemia is hypertriglyceridemia.

As used herein, the term “hypertriglyceridemia” refers to a condition in which triglyceride levels are elevated, often caused or exacerbated by uncontrolled hyperlipidemia mellitus, obesity, and sedentary habits. This condition is a risk factor for coronary artery disease. Hypertriglyceridemia is usually asymptomatic until triglycerides are greater than 1000-2000 mg/dL. Signs and symptoms may include the following: pain in the mid-epigastric, chest, or back regions; nausea, vomiting, dyspnea, xanthomas, corneal arcus, and/or xanthelasmas

In some embodiments, the hyperlipidemia is hypercholesterolemia.

As used herein the term “hypercholesterolemia” refers to a form of hyperlipidemia (elevated levels of lipids in the blood) in which there are high levels of cholesterol in the serum of a subject, e.g., at least about 240 mg/dL of total cholesterol.

In other embodiments, the hyperlipidemia is mixed hyperlipidemia.

5 As used herein the term “mixed hyperlipidemia” also referred to as type 5 hyperlipidemia refers to a form of hyperlipidemia in which there are elevated levels of VLDL and chylomicrons found in plasma in the serum of a subject, e.g., at least about 240 mg/dL of total cholesterol.

Cardiovascular diseases associated with disorders of lipid metabolism are also considered “disorders of lipid metabolism”, as defined herein. These diseases may include coronary artery
10 disease (also called ischemic heart disease), atherosclerosis, inflammation associated with coronary artery disease, restenosis, peripheral vascular diseases, and stroke.

Disorders related to body weight are also considered “disorders of lipid metabolism”, as defined herein. Such disorders may include obesity, metabolic syndrome including independent components of metabolic syndrome (e.g., central obesity, FBG/pre-hyperlipidemia/hyperlipidemia,
15 hypercholesterolemia, hypertriglyceridemia, and hypertension), 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 “disorders of lipid metabolism”, as defined herein. Such disorders may include hyperlipidemia, hypertension, and polycystic ovarian syndrome related to insulin resistance. Other exemplary disorders of lipid metabolism may also include renal
20 transplantation, nephrotic syndrome, Cushing's syndrome, acromegaly, systemic lupus erythematosus, dysglobulinemia, lipodystrophy, glycogenosis type I, and Addison's disease.

“Therapeutically effective amount,” as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having an HMGCR-associated disorder, is sufficient
25 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 an RNAi agent that, when administered to a subject having an HMGCR-associated disorder, is sufficient
30 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
35 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 "prophylactically effective amount" also includes an amount of an RNAi agent that produces some desired effect at a reasonable benefit/risk ratio applicable to any treatment. The iRNA employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such
5 treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds (including salts), materials, 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,
10 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
15 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. Such carriers are known in the art. Pharmaceutically acceptable carriers include carriers for administration by injection.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues
20 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, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, 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. In certain embodiments, samples may be derived from the liver
25 (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes). In some embodiments, a "sample derived from a subject" refers to urine obtained from the subject. A "sample derived from a subject" can refer to blood or blood derived serum or plasma from the subject.

30 II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of an HMGCR gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of an HMGCR gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing an HMGCR-associated disorder, *e.g.*, disorder of lipid
35 metabolism, *e.g.*, hyperlipidemia. The dsRNAi agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an HMGCR gene. The region of complementarity is about 19-30 nucleotides in length (*e.g.*, about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, or 19 nucleotides in length).

Upon contact with a cell expressing the HMGCRC gene, the iRNA inhibits the expression of the HMGCRC gene (*e.g.*, a human, a primate, a non-primate, or a rat HMGCRC gene) by at least about 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 or flow cytometric techniques. In certain embodiments, inhibition of expression is determined by the qPCR method provided in the examples herein with the siRNA at, *e.g.*, a 10 nM concentration, in an appropriate organism cell line provided therein. In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, a mouse or an AAV-infected mouse expressing the human target gene, *e.g.*, when administered as single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression.

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. The target sequence can be derived from the sequence of an mRNA formed during the expression of an HMGCRC 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 duplex structure is 19 to 30 base pairs in length. Similarly, the region of complementarity to the target sequence is 19 to 30 nucleotides in length.

In some embodiments, the dsRNA is about 19 to about 23 nucleotides in length, or about 25 to about 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 in length may 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 19 to about 30 base pairs, *e.g.*, about 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. 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, an iRNA agent useful to target HMGCR 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.*, 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have superior inhibitory properties relative to their blunt-ended counterparts. 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 an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art. Double stranded RNAi 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. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an anti-sense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 2-5, and the corresponding antisense strand of the sense strand is

selected from the group of sequences of any one of Tables 2-5. 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 an HMGCRCR gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 2-5, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2-5.

In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although the sequences in, for example, Table 3, are not described as modified or conjugated sequences, the RNA of the invention *e.g.*, a dsRNA of the invention, may comprise any one of the sequences set forth in any one of Tables 2-5 that is unmodified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2-5 which are un-modified, un-conjugated, modified, or conjugated, as described herein.

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.*, *EMBO* 2001, 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 in any one of Tables 2-5, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having any one of the sequences in any one of Tables 2-5 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 19, 20, or more contiguous nucleotides derived from any one of the sequences of any one of Tables 2-5, and differing in their ability to inhibit the expression of an HMGCRCR gene by not more than about 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 2-5 identify a site(s) in an HMGCRCR transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 19 contiguous nucleotides from any one of the sequences provided in any one of Tables 2-5 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an HMGCRCR gene.

III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is unmodified, and does not comprise, *e.g.*, chemical modifications or conjugations known in the art and described herein. In other embodiments, the RNA of an iRNA of the invention, *e.g.*, a dsRNA, is
5 chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, *i.e.*, not more than 5, 4, 3, 2, or 1 unmodified nucleotides are present in a strand of the iRNA.

10 The nucleic acids featured in the invention 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. Modifications include, for example, end modifications, *e.g.*, 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation,
15 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 iRNA compounds useful in the
20 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
25 modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates,
30 thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. In some embodiments of the invention, the dsRNA agents of the invention are in a free acid form. In other embodiments of the
35 invention, the dsRNA agents of the invention are in a salt form. In one embodiment, the dsRNA agents of the invention are in a sodium salt form. In certain embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for substantially all of the phosphodiester and/or phosphorothioate groups present in the agent. Agents

in which substantially all of the phosphodiester and/or phosphorothioate linkages have a sodium counterion include not more than 5, 4, 3, 2, or 1 phosphodiester and/or phosphorothioate linkages without a sodium counterion. In some embodiments, when the dsRNA agents of the invention are in the sodium salt form, sodium ions are present in the agent as counterions for all of the phosphodiester and/or phosphorothioate groups present in the agent.

Representative U.S. Patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Patent Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH₂ component parts.

Representative U.S. Patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Patent Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, in which both the sugar and the internucleoside linkage, *i.e.*, the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound in which an RNA mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative US patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Patent Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference.

Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen *et al.*, *Science*, 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH₂--NH--CH₂--, --CH₂--N(CH₃)--O--CH₂-- [known as a methylene (methylimino) or MMI backbone], --CH₂--O--N(CH₃)--CH₂--, --CH₂--N(CH₃)--N(CH₃)--CH₂-- and --N(CH₃)--CH₂--CH₂-- of the above-referenced U.S. Patent No. 5,489,677, and the amide backbones of the above-referenced U.S. Patent No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Patent No. 5,034,506. The native phosphodiester backbone can be represented as O-P(O)(OH)-OCH₂-.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, *e.g.*, dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Exemplary suitable modifications include O[(CH₂)_nO]_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON[(CH₂)_nCH₃]₂, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin *et al.*, *Helv. Chim. Acta*, 1995, 78:486-504) *i.e.*, an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminoethoxy, *i.e.*, a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethyl (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--CH₂--O--CH₂--N(CH₃)₂. Further exemplary modifications include : 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH₃), 2'-aminopropoxy (2'-OCH₂CH₂CH₂NH₂) and 2'-fluoro (2'-F). Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Patent 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; and 5,700,920, certain of which are commonly owned with the instant application,. The entire contents of each of the foregoing are hereby incorporated herein by reference.

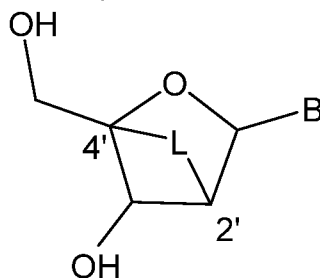
An iRNA can also include nucleobase (often referred to in the art simply as “base”) modifications or substitutions. As used herein, “unmodified” or “natural” nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythymidine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch *et al.*, Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. Patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Patent Nos. 3,687,808, 4,845,205; 5,130,300; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an RNAi agent of the disclosure can also be modified to include one or more bicyclic sugar moieties. A “bicyclic sugar” is a furanosyl ring modified by a ring formed by the bridging of two carbons, whether adjacent or non-adjacent. A “bicyclic nucleoside” (“BNA”) is a

nucleoside having a sugar moiety comprising a ring formed by bridging two carbons, whether adjacent or non-adjacent, of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring, optionally, via the 2'-acyclic oxygen atom. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH₂-O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. *et al.*, (2005) *Nucleic Acids Research* 33(1):439-447; Mook, OR. *et al.*, (2007) *Mol Canc Ther* 6(3):833-843; Grunweller, A. *et al.*, (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge.

A locked nucleoside can be represented by the structure (omitting stereochemistry),



wherein B is a nucleobase or modified nucleobase and L is the linking group that joins the 2'-carbon to the 4'-carbon of the ribose ring. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH₂)—O-2' (LNA); 4'-(CH₂)—S-2'; 4'-(CH₂)₂—O-2' (ENA); 4'-CH(CH₃)—O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH₂OCH₃)—O-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4'-C(CH₃)(CH₃)—O-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4'-CH₂—N(OCH₃)-2' (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4'-CH₂—O—N(CH₃)-2' (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4'-CH₂—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a nitrogen protecting group (see, *e.g.*, U.S. Patent No. 7,427,672); 4'-CH₂—C(H)(CH₃)-2' (see, *e.g.*, Chattopadhyaya *et al.*, *J. Org. Chem.*, 2009, 74, 118-134); and 4'-CH₂—C(=CH₂)-2' (and analogs thereof; see, *e.g.*, U.S. Patent No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative U.S. Patents and U.S. Patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Patent Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283;

US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α -L-ribofuranose and β -D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH₃)-O-2' bridge (*i.e.*, L in the preceding structure). In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and -C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, U.S. Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, an iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is 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 monomer with bonds between C1'-C4' have been 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 has been removed (see *Nuc. Acids Symp. Series*, 52, 133-134 (2008) and Fluiters *et al.*, *Mol. Biosyst.*, 2009, 10, 1039 hereby incorporated by reference).

Representative U.S. publications that teach the preparation of UNA include, but are not limited to, U.S. Patent No. 8,314,227; and U.S. Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-O-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3'-phosphate, inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), and inverted abasic 2'-deoxyribonucleotide (iAb) and others. Disclosure of this modification can be found in WO 2011/005861.

In one example, the 3' or 5' terminal end of an oligonucleotide is linked to an inverted 2'-deoxy-modified ribonucleotide, such as inverted dT(idT), inverted dA (idA), or an inverted abasic 2'-deoxyribonucleotide (iAb). In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'-end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted abasic ribonucleotide (iAb). In another example, the 3'-end of a sense strand is linked via a 3'-3'-phosphorothioate linkage to an inverted dA (idA).

In one particular example, the inverted 2'-deoxy-modified ribonucleotide is linked to the 3'-end of an oligonucleotide, such as the 3'-end of a sense strand described herein, where the linking is via a 3'-3' phosphodiester linkage or a 3'-3'-phosphorothioate linkage.

In another example, the 3'-terminal nucleotides of a sense strand is an inverted dA (idA) and is linked to the preceding nucleotide via a 3'-3'- linkage (e.g., 3'-3'-phosphorothioate linkage).

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example U.S. Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNA agents of the invention include agents with chemical modifications as disclosed, for example, in WO2013/075035, the entire contents of each of which are incorporated herein by reference. As shown herein and in WO2013/075035, one or more motifs of three identical modifications on three consecutive nucleotides may be introduced into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNA agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNA agents capable of inhibiting the expression of a target gene (i.e., HMGCR gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, for example, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA (“dsRNA”), also referred to herein as “dsRNAi agent.” The duplex region of a dsRNAi agent may be, for example, the duplex region can be 27-30 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 19, 20, 21, 22, 23, 24, 25, 26, and 27 nucleotides in length.

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but not limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof.

For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand, or both strands of the dsRNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In some embodiments, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some embodiments, this 3'-overhang is present in the antisense strand. In some embodiments, this 3'-overhang is present in the sense strand.

The dsRNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'- end of the sense strand or, alternatively, at the 3'-end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (*i.e.*, the 3'-end of the sense strand) or *vice versa*. Generally, the antisense strand of the dsRNAi agent has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by

theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In certain embodiments, the dsRNAi agent is a double blunt-ended of 19 nucleotides 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, and 13 from the 5' end.

In other embodiments, the dsRNAi agent is a double blunt-ended of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, and 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, and 13 from the 5' end.

In yet other embodiments, the dsRNAi agent is a double blunt-ended of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, and 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, and 13 from the 5' end.

In certain embodiments, the dsRNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, and 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, and 13 from the 5' end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. In one embodiment, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, *e.g.*, in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (such as, GalNAc₃).

In certain embodiments, the dsRNAi agent comprises a sense and an antisense strand, 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 the first strand comprise at least 8 ribonucleotides; the 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
5 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
10 at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the 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.

15 In certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi 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 the 3' end of the first strand and the 5' end of the second strand form a blunt end and the
20 second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein Dicer cleavage of the dsRNAi agent results in an siRNA comprising the 3'-end of the second strand, thereby reducing expression of
25 the target gene in the mammal. Optionally, the dsRNAi agent further comprises a ligand.

In certain embodiments, the sense strand of the dsRNAi 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.

30 In certain embodiments, the antisense strand of the dsRNAi 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 a dsRNAi agent having a duplex region of 19-23 nucleotides 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; the 10, 11, 12 positions;
35 the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first 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 dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In some embodiments, the sense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistries of the motifs are distinct from each other, and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

In other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end, or both ends of the strand.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two, or three nucleotides.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications

each from one strand fall on one end of the duplex region, having an overlap of one, two, or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

5 In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2'-hydroxyl on the
10 ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

 As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a
15 non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3'- or 5' terminal position, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the
20 double strand region of an RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5'-end or ends can be phosphorylated.

25 It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5'- or 3'-overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3'- or 5'-overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2'
30 position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

 In some embodiments, each residue of the sense strand and antisense strand is independently
35 modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-O-methyl or 2'-fluoro modifications, or others.

In certain embodiments, the N_a or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB...", "AABBAABBAABB...", "AABAABAABAAB...", "AAABAAABAAB...", "AAABBBAAABBB..." or "ABCABCABCABC...", etc.

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB...", "ACACAC..." "BDBDBD..." or "CDCDCD...", etc.

In some embodiments, the dsRNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and *vice versa*. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' to 3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In another particular example, the alternating motif in the antisense strand is "BABABA" from 3'-5' of the strand, where each A is a 2'-deoxy-2'-fluoro modified nucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is "ABABAB" sfrom 5' 3' of the strand and the alternating motif in the antisense strand is "BABABA" from 3'-5' of the strand, where each A is an unmodified ribonucleotide and each B is a 2'-Omethyl modified nucleotide.

In one particular example, the alternating motif in the sense strand is “ABABAB” sfrom 5’ 3’ of the strand and the alternating motif in the antisense strand is “BABABA” from 3’ -5’ of the strand, where each A is a 2’ -deoxy-2’ -fluoro modified nucleotide and each B is a 2’ -O-methyl modified nucleotide.

5 In some embodiments, the dsRNAi agent comprises the pattern of the alternating motif of 2’-O-methyl modification and 2’-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2’-O-methyl modification and 2’-F modification on the antisense strand initially, *i.e.*, the 2’-O-methyl modified nucleotide on the sense strand base pairs with a 2’-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may
10 start with the 2’-F modification, and the 1 position of the antisense strand may start with the 2’-O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or
15 antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

In some embodiments, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a
20 different modification than the modification of the motif. For example, the portion of the sequence containing the motif is “...N_aYYYN_b...,” where “Y” represents the modification of the motif of three identical modifications on three consecutive nucleotide, and “N_a” and “N_b” represent a modification to the nucleotide next to the motif “YYY” that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a or N_b may be present or absent when
25 there is a wing modification present.

The iRNA 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, antisense strand, or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every
30 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
35 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, a double-stranded RNAi agent comprises 6-8 phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5’-end and two

phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent comprises a 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 the 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 paired nucleotide next to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, or the 5'-end of the antisense strand.

In some embodiments, the 2-nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the dsRNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may 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.

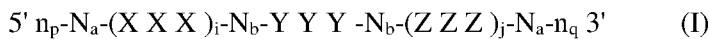
In certain embodiments, the dsRNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from 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 other embodiments, the nucleotide at the 3'-end of the sense strand is deoxythymidine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxythymidine (dT). For example, there is a short sequence of deoxythymidine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

In certain embodiments, the sense strand sequence may be represented by formula (I):



wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

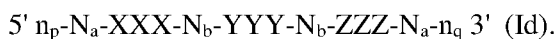
wherein N_b and Y do not have the same modification; and

XXX , YYY , and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. In one embodiment, YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8; 7, 8, 9; 8, 9, 10; 9, 10, 11; 10, 11, 12; or 11, 12, 13) of the sense strand, the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:



When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a can

independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. In one embodiment, N_b is 0, 1, 2, 3, 4, 5, or 6. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

10 $5' n_p-N_a-YYY-N_a-n_q 3'$ (Ia).

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

15 $5' n_q'-N_a'-(Z'Z'Z')_k-N_b'-Y'Y'Y'-N_b'-(X'X'X')_l-N_a'-n_p' 3'$ (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

20 each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

25 $X'X'X'$, $Y'Y'Y'$, and $Z'Z'Z'$ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

The $Y'Y'Y'$ motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the $Y'Y'Y'$ motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. In one embodiment, the $Y'Y'Y'$ motif occurs at positions 11, 12, 13.

In certain embodiments, $Y'Y'Y'$ motif is all 2'-OMe modified nucleotides.

35 In certain embodiments, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

$5' n_q'-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_a'-n_p' 3'$ (IIb);

$5' n_q'-N_a'-Y'Y'Y'-N_b'-X'X'X'-n_p' 3'$ (IIc); or

5' n_q'-N_a'-Z'Z'Z'-N_b'-Y'Y'Y'-N_b'-X'X'X'-N_a'-n_p' 3' (IIId).

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. In one embodiment, N_b is 0, 1, 2, 3, 4, 5, or 6.

In other embodiments, k is 0 and l is 0 and the antisense strand may be represented by the formula:

5' n_p'-N_a'-Y'Y'Y'-N_a'-n_q' 3' (Ia).

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of X', Y' and Z' may be the same or different from each other.

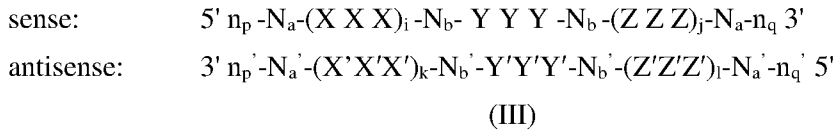
Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X', Y', and Z', in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In some embodiments, the sense strand of the dsRNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nt, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In some embodiments the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain X'X'X' motif or Z'Z'Z' motifs as wing modifications at the opposite end of the duplex region; and X'X'X' and Z'Z'Z' each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with an antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the dsRNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the iRNA duplex represented by formula (III):



10 wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

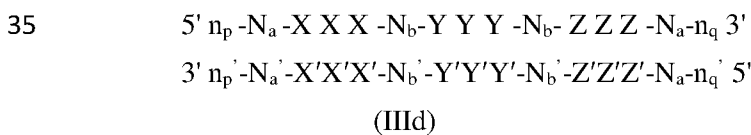
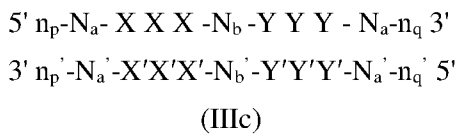
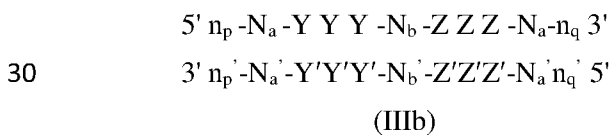
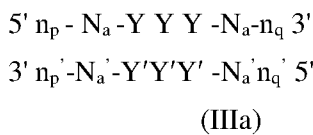
15 each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

20 XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and l is 0; or k is 1 and l is 0; k is 0 and l is 1; or both k and l are 0; or both k and l are 1.

25 Exemplary combinations of the sense strand and antisense strand forming an iRNA duplex include the formulas below:



When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleotides. Each N_a

5 independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIIc), each N_b , N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or

10 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (III d), each N_b , N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a , N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a , N_a' , N_b , and N_b' independently comprises modifications

15 of alternating pattern.

Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (III d) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (III d), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least

20 two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the dsRNAi agent is represented by formula (IIIb) or (III d), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all

25 form base pairs with the corresponding Z' nucleotides.

When the dsRNAi agent is represented as formula (IIIc) or (III d), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all

form base pairs with the corresponding X' nucleotides.

In certain embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

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In certain embodiments, when the dsRNAi agent is represented by formula (III d), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (III d), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications and $n_p' > 0$ and at least one n_p' is linked to a neighboring nucleotide *via* phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (III d), the N_a modifications

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are 2'-O-methyl or 2'-fluoro modifications, $n_{p'} > 0$ and at least one $n_{p'}$ is linked to a neighboring nucleotide *via* phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other
5 embodiments, when the RNAi agent is represented by formula (III_d), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, $n_{p'} > 0$ and at least one $n_{p'}$ is linked to a neighboring nucleotide *via* phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, when the dsRNAi agent is represented by formula (III_a), the N_a
10 modifications are 2'-O-methyl or 2'-fluoro modifications, $n_{p'} > 0$ and at least one $n_{p'}$ is linked to a neighboring nucleotide *via* phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, the dsRNAi agent is a multimer containing at least two duplexes
15 represented by formula (III), (III_a), (III_b), (III_c), and (III_d), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In some embodiments, the dsRNAi agent is a multimer containing three, four, five, six, or
20 more duplexes represented by formula (III), (III_a), (III_b), (III_c), and (III_d), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (III_a),
25 (III_b), (III_c), and (III_d) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

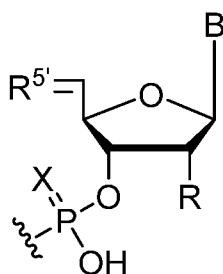
In certain embodiments, an RNAi agent of the invention may contain a low number of
nucleotides containing a 2'-fluoro modification, *e.g.*, 10 or fewer nucleotides with 2'-fluoro
30 modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent of the invention contains 10 nucleotides with a 2'-fluoro modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 6 nucleotides with a 2'-fluoro modification in the antisense strand. In another specific embodiment, the RNAi agent of the invention contains 6 nucleotides with a 2'-fluoro
35 modification, *e.g.*, 4 nucleotides with a 2'-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

In other embodiments, an RNAi agent of the invention may contain an ultra low number of
nucleotides containing a 2'-fluoro modification, *e.g.*, 2 or fewer nucleotides containing a 2'-fluoro

modification. For example, the RNAi agent may contain 2, 1 or 0 nucleotides with a 2'-fluoro modification. In a specific embodiment, the RNAi agent may contain 2 nucleotides with a 2'-fluoro modification, *e.g.*, 0 nucleotides with a 2-fluoro modification in the sense strand and 2 nucleotides with a 2'-fluoro modification in the antisense strand.

5 Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Patent No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

In certain embodiments, the compositions and methods of the disclosure include a vinyl
10 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 C₁₋₂₀alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

15 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.

In one embodiment, R^{5'} is =C(H)-P(O)(OH)₂ and the double bond between the C5' carbon
20 and R^{5'} is in the *E* orientation. In another embodiment, R is methoxy and R^{5'} is =C(H)-P(O)(OH)₂ and the double bond between the C5' carbon and R^{5'} is in the *E* orientation. In another embodiment, X is S, R is methoxy, and R^{5'} is =C(H)-P(O)(OH)₂ and the double bond between the C5' carbon and R^{5'} is in the *E* orientation.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the
25 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 phosphonate modifications are also contemplated for the compositions and methods of
the instant disclosure. An exemplary vinyl phosphonate structure includes the preceding structure,
30 where R^{5'} is =C(H)-OP(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).

As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases,

the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA can be replaced with another moiety, *e.g.*, a non-carbohydrate (such as, cyclic) carrier to which is attached a carbohydrate ligand. 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). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide *via* a carrier. The carriers include (i) at least one “backbone attachment point,” such as, 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 into the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing backbone, of a ribonucleic acid. A “tethering attachment point” (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic 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 ring.

The iRNA may be conjugated to a ligand *via* a carrier, wherein the carrier can be cyclic group or acyclic group. In one embodiment, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazoliny, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxaliny, pyridazinonyl, tetrahydrofuryl, and decalin. In one embodiment, the acyclic group is a serinol backbone or diethanolamine backbone.

i. Thermally Destabilizing Modifications

In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein “seed region” means at positions 2-9 of the 5'-end of the referenced strand, or at positions 2-8 of the 5'-end of the referenced strand. For example, thermally destabilizing modifications can be incorporated in the seed region of the antisense strand to reduce or inhibit off-target gene silencing.

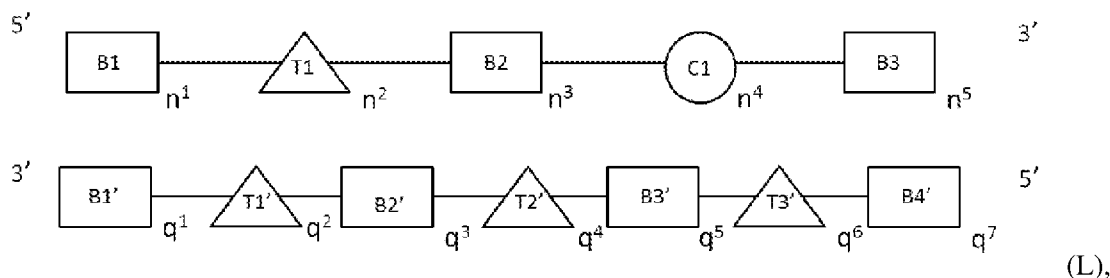
The term “thermally destabilizing modification(s)” includes modification(s) that would result with a dsRNA with a lower overall melting temperature (T_m) than the T_m of the dsRNA without having such modification(s). For example, the thermally destabilizing modification(s) can decrease

the T_m of the dsRNA by 1 – 4 °C, such as one, two, three or four degrees Celcius. And, the term “thermally destabilizing nucleotide” refers to a nucleotide containing one or more thermally destabilizing modifications.

It has been discovered that dsRNAs with an antisense strand comprising at least one thermally destabilizing modification of the duplex within the first 9 nucleotide positions, counting from the 5' end, of the antisense strand have reduced off-target gene silencing activity. Accordingly, in some embodiments, the antisense strand comprises at least one (*e.g.*, one, two, three, four, five or more) thermally destabilizing modification of the duplex within the first 9 nucleotide positions of the 5' region of the antisense strand. In some embodiments, one or more thermally destabilizing modification(s) of the duplex is/are located in positions 2-9, such as, positions 4-8, from the 5'-end of the antisense strand. In some further embodiments, the thermally destabilizing modification(s) of the duplex is/are located at position 6, 7 or 8 from the 5'-end of the antisense strand. In still some further embodiments, the thermally destabilizing modification of the duplex is located at position 7 from the 5'-end of the antisense strand. In some embodiments, the thermally destabilizing modification of the duplex is located at position 2, 3, 4, 5 or 9 from the 5'-end of the antisense strand.

The thermally destabilizing modifications, described below, can include, but are not limited to, abasic modification; mismatch with the opposing nucleotide in the opposing strand; and sugar modification such as 2'-deoxy modification, acyclic nucleotide, *e.g.*, unlocked nucleic acids (UNA) or glycol nucleic acid (GNA); and 2'-5'-linked ribonucleotides (“3'-RNA”).

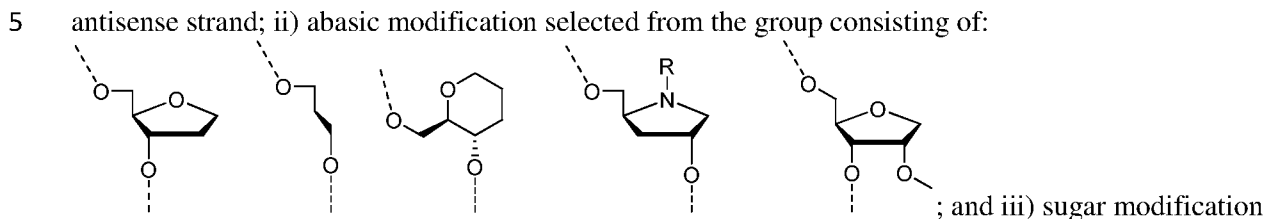
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



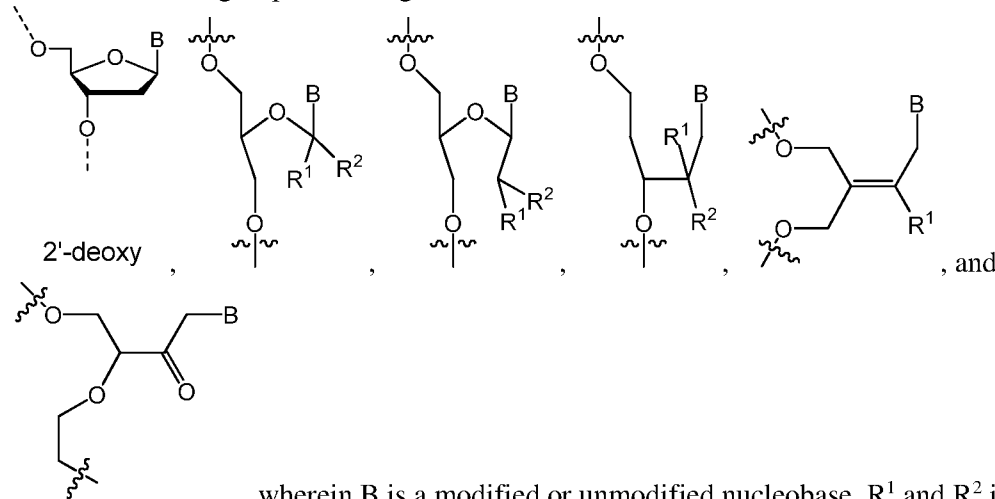
In formula (L), B1, B2, B3, B1', B2', B3', and B4' each are independently a nucleotide containing a modification selected from the group consisting of 2'-O-alkyl, 2'-substituted alkoxy, 2'-substituted alkyl, 2'-halo, ENA, and BNA/LNA. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe modifications. In one embodiment, B1, B2, B3, B1', B2', B3', and B4' each contain 2'-OMe or 2'-F modifications. In one embodiment, at least one of B1, B2, B3, B1', B2', B3', and B4' contain 2'-O-N-methylacetamido (2'-O-NMA, 2'-O-CH₂C(O)N(Me)H) modification.

C1 is a thermally destabilizing nucleotide placed 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, or at positions 2-9 of the 5'-end of the referenced strand). For example, C1 is at a position of the sense strand that pairs with a nucleotide at positions 2-8 of the 5'-end of the antisense strand. In one example, C1 is at position 15 from the 5'-end of the sense strand. C1 nucleotide bears the thermally destabilizing modification

which can include abasic modification; mismatch with the opposing nucleotide in the duplex; and sugar modification such as 2'-deoxy modification or acyclic nucleotide *e.g.*, unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA). In one embodiment, C1 has thermally destabilizing modification selected from the group consisting of: i) mismatch with the opposing nucleotide in the antisense strand; ii) abasic modification selected from the group consisting of:

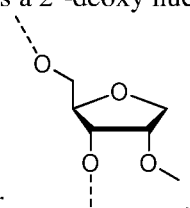


selected from the group consisting of:



, wherein B is a modified or unmodified nucleobase, R¹ and R² independently are

10 H, halogen, OR₃, or alkyl; and R₃ is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In one embodiment, the thermally destabilizing modification in C1 is a mismatch selected from the group consisting of G:G, G:A, G:U, G:T, A:A, A:C, C:C, C:U, C:T, U:U, T:T, and U:T; and optionally, at least one nucleobase in the mismatch pair is a 2'-deoxy nucleobase. In one example, the thermally



destabilizing modification in C1 is GNA or

15 T1, T1', T2', and T3' each independently represent a nucleotide comprising a modification providing the nucleotide a steric bulk that is less or equal to the steric bulk of a 2'-OMe modification. A steric bulk refers to the sum of steric effects of a modification. Methods for determining steric effects of a modification of a nucleotide are known to one skilled in the art. The modification can be at the 2' position of a ribose sugar of the nucleotide, or a modification to a non-ribose nucleotide, acyclic nucleotide, or the backbone of the nucleotide that is similar or equivalent to the 2' position of the ribose sugar, and provides the nucleotide a steric bulk that is less than or equal to the steric bulk of a 2'-OMe modification. For example, T1, T1', T2', and T3' are each independently selected from 20 DNA, RNA, LNA, 2'-F, and 2'-F-5'-methyl. In one embodiment, T1 is DNA. In one embodiment,

T1' is DNA, RNA or LNA. In one embodiment, T2' is DNA or RNA. In one embodiment, T3' is DNA or RNA.

n^1 , n^3 , and q^1 are independently 4 to 15 nucleotides in length.

n^5 , q^3 , and q^7 are independently 1-6 nucleotide(s) in length.

5 n^4 , q^2 , and q^6 are independently 1-3 nucleotide(s) in length; alternatively, n^4 is 0.

q^5 is independently 0-10 nucleotide(s) in length.

n^2 and q^4 are independently 0-3 nucleotide(s) in length.

Alternatively, n^4 is 0-3 nucleotide(s) in length.

10 In one embodiment, n^4 can be 0. In one example, n^4 is 0, and q^2 and q^6 are 1. In another example, n^4 is 0, and q^2 and q^6 are 1, 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).

15 In one embodiment, n^4 , q^2 , and q^6 are each 1.

In one embodiment, n^2 , n^4 , q^2 , q^4 , and q^6 are each 1.

In one embodiment, C1 is at position 14-17 of the 5'-end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^4 is 1. In one embodiment, C1 is at position 15 of the 5'-end of the sense strand

20 In one embodiment, T3' starts at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1.

In one embodiment, T1' starts at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

25 In an exemplary embodiment, T3' starts from position 2 from the 5' end of the antisense strand and T1' starts from position 14 from the 5' end of the antisense strand. In one example, T3' starts from position 2 from the 5' end of the antisense strand and q^6 is equal to 1 and T1' starts from position 14 from the 5' end of the antisense strand and q^2 is equal to 1.

In one embodiment, T1' and T3' are separated by 11 nucleotides in length (*i.e.* not counting the T1' and T3' nucleotides).

30 In one embodiment, T1' is at position 14 from the 5' end of the antisense strand. In one example, T1' is at position 14 from the 5' end of the antisense strand and q^2 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose.

35 In one embodiment, T3' is at position 2 from the 5' end of the antisense strand. In one example, T3' is at position 2 from the 5' end of the antisense strand and q^6 is equal to 1, and the modification at the 2' position or positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

In one embodiment, T1 is at the cleavage site of the sense strand. In one example, T1 is at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1. In an exemplary embodiment, T1 is at the cleavage site of the sense strand at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1,

5 In one embodiment, T2' starts at position 6 from the 5' end of the antisense strand. In one example, T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1.

In an exemplary embodiment, T1 is at the cleavage site of the sense strand, for instance, at position 11 from the 5' end of the sense strand, when the sense strand is 19-22 nucleotides in length, and n^2 is 1; T1' is at position 14 from the 5' end of the antisense strand, and q^2 is equal to 1, and the
10 modification to T1' is at the 2' position of a ribose sugar or at positions in a non-ribose, acyclic or backbone that provide less steric bulk than a 2'-OMe ribose; T2' is at positions 6-10 from the 5' end of the antisense strand, and q^4 is 1; and T3' is at position 2 from the 5' end of the antisense strand, and q^6 is equal to 1, and the modification to T3' is at the 2' position or at positions in a non-ribose, acyclic or backbone that provide less than or equal to steric bulk than a 2'-OMe ribose.

15 In one embodiment, T2' starts at position 8 from the 5' end of the antisense strand. In one example, T2' starts at position 8 from the 5' end of the antisense strand, and q^4 is 2.

In one embodiment, T2' starts at position 9 from the 5' end of the antisense strand. In one example, T2' is at position 9 from the 5' end of the antisense strand, and q^4 is 1.

In one embodiment, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 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
25 strand).

In one embodiment, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 1, B3' is 2'-OMe or 2'-F, q^5 is 6, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two
30 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).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.
35

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4,

T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 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).

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

10 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 6, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 7, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 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).

15 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1.

20 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 6, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within positions 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).

25 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

30 In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 5, T2' is 2'-F, q⁴ is 1, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; optionally with at least 2 additional TT at the 3'-end of the antisense strand; with two phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand (counting from the 5'-end of the sense strand), and two phosphorothioate internucleotide linkage modifications within positions 18-23 of the antisense strand (counting from the 5'-end of the antisense strand).

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).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-OMe, and q^7 is 1; with two

phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand

(counting from the 5'-end), 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).

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, T2' is 2'-F, q^4 is 2, B3' is 2'-OMe or 2'-F, q^5 is 5, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with

two phosphorothioate internucleotide linkage modifications within positions 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).

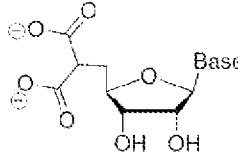
In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1.

In one embodiment, B1 is 2'-OMe or 2'-F, n^1 is 8, T1 is 2'F, n^2 is 3, B2 is 2'-OMe, n^3 is 7, n^4 is 0, B3 is 2'-OMe, n^5 is 3, B1' is 2'-OMe or 2'-F, q^1 is 9, T1' is 2'-F, q^2 is 1, B2' is 2'-OMe or 2'-F, q^3 is 4, q^4 is 0, B3' is 2'-OMe or 2'-F, q^5 is 7, T3' is 2'-F, q^6 is 1, B4' is 2'-F, and q^7 is 1; with two

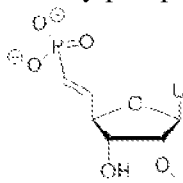
phosphorothioate internucleotide linkage modifications within positions 1-5 of the sense strand


(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).

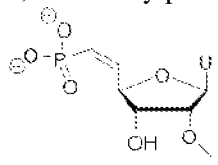
The RNAi agent can comprise a phosphorus-containing group at the 5'-end of the sense strand or antisense strand. The 5'-end phosphorus-containing group can be 5'-end phosphate (5'-P), 5'-end phosphorothioate (5'-PS), 5'-end phosphorodithioate (5'-PS₂), 5'-end vinylphosphonate (5'-

VP), 5'-end methylphosphonate (MePhos), or 5'-deoxy-5'-C-malonyl (). When

the 5'-end phosphorus-containing group is 5'-end vinylphosphonate (5'-VP), the 5'-VP can be either



5'-*E*-VP isomer (*i.e.*, *trans*-vinylphosphonate, , 5'-*Z*-VP isomer (*i.e.*, *cis*-



vinylphosphonate, or mixtures thereof.

In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the sense strand. In one embodiment, the RNAi agent comprises a phosphorus-containing group at the 5'-end of the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-P. In one embodiment, the RNAi agent comprises a 5'-P in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS. In one embodiment, the RNAi agent comprises a 5'-PS in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-VP. In one embodiment, the RNAi agent comprises a 5'-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*E*-VP in the antisense strand. In one embodiment, the RNAi agent comprises a 5'-*Z*-VP in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS₂. In one embodiment, the RNAi agent comprises a 5'-PS₂ in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-PS₂. In one embodiment, the RNAi agent comprises a 5'-deoxy-5'-*C*-malonyl in the antisense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-*E*-VP, 5'-*Z*-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The dsRNA agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The dsRNAi RNA agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1. The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5' - P.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5' - PS.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5' - VP. The 5'-VP may be 5'-E-VP, 5'-Z-VP, or combination thereof.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5' - PS₂.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-VP (e.g., a 5'-E-VP, 5'-Z-VP, or combination thereof), and a targeting ligand.

In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-OMe, and q⁷ is 1; with two phosphorothioate internucleotide linkage modifications within position 1-5 of the sense strand (counting from the 5'-end), 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, T2' is 2'-F, q⁴ is 2, B3' is 2'-OMe or 2'-F, q⁵ is 5, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-P and a targeting ligand. In one embodiment, the 5'-P is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F,

q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS and a targeting ligand. In one embodiment, the 5'-PS is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-VP (*e.g.*, a 5'-E-VP, 5'-Z-VP, or combination thereof) and a targeting ligand. In one embodiment, the 5'-VP is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-PS₂ and a targeting ligand. In one embodiment, the 5'-PS₂ is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In one embodiment, B1 is 2'-OMe or 2'-F, n¹ is 8, T1 is 2'F, n² is 3, B2 is 2'-OMe, n³ is 7, n⁴ is 0, B3 is 2'-OMe, n⁵ is 3, B1' is 2'-OMe or 2'-F, q¹ is 9, T1' is 2'-F, q² is 1, B2' is 2'-OMe or 2'-F, q³ is 4, q⁴ is 0, B3' is 2'-OMe or 2'-F, q⁵ is 7, T3' is 2'-F, q⁶ is 1, B4' is 2'-F, and q⁷ is 1; 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). The RNAi agent also comprises a 5'-deoxy-5'-C-malonyl and a targeting ligand. In one embodiment, the 5'-deoxy-5'-C-malonyl is at the 5'-end of the antisense strand, and the targeting ligand is at the 3'-end of the sense strand.

In a particular embodiment, an RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker; and
 - (iii) 2'-F modifications at positions 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, an RNAi agent of the present invention comprises:

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

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, 10, and 12 to 21, 2'-F modifications at positions 7, and 9, and a deoxy-nucleotide (*e.g.* dT) at position 11 (counting from the 5' end); and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-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
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

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

(iii) 2'-OMe modifications at positions 1 to 9, and 12 to 21, and 2'-F modifications at positions 10, and 11; and

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

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

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

(iii) 2'-F modifications at positions 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

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

and

(b) an antisense strand having:

(i) a length of 23 nucleotides;

(ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

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

(iii) 2'-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

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

and

(b) an antisense strand having:

(i) a length of 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 deoxy-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 RNAi 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, a RNAi agent of the present invention comprises:

(a) a sense strand having:

(i) a length of 21 nucleotides;

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

(iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and

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

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 21 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 6, 8, and 12 to 21, and 2'-F modifications at positions 7, and 9 to 11; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

- (b) an antisense strand having:
- (i) a length of 23 nucleotides;
 - (ii) 2'-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 RNAi 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, a RNAi agent of the present invention comprises:

- (a) a sense strand having:
- (i) a length of 19 nucleotides;
 - (ii) an ASGPR ligand attached to the 3'-end, wherein said ASGPR ligand comprises three GalNAc derivatives attached through a trivalent branched linker;
 - (iii) 2'-OMe modifications at positions 1 to 4, 6, and 10 to 19, and 2'-F modifications at positions 5, and 7 to 9; and
 - (iv) phosphorothioate internucleotide linkages between nucleotide positions 1 and 2, and between nucleotide positions 2 and 3 (counting from the 5' end);

and

(b) an antisense strand having:

(i) a length of 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 RNAi 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 certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in any one of Tables 2-5. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA *e.g.*, into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1994, 4:1053-1060), a thioether, *e.g.*, beryl-S-tritylthiol (Manoharan *et al.*, *Ann. N.Y. Acad. Sci.*, 1992, 660:306-309; Manoharan *et al.*, *Biorg. Med. Chem. Lett.*, 1993, 3:2765-2770), a thiocholesterol (Oberhauser *et al.*, *Nucl. Acids Res.*, 1992, 20:533-538), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J*, 1991, 10:1111-1118; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327-330; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49-54), a phospholipid, *e.g.*, dihexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969-973), or adamantane acetic acid (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651-3654), a palmityl moiety (Mishra *et al.*, *Biochim. Biophys. Acta*, 1995, 1264:229-237), or an octadecylamine or hexylamino-carboxyloxycholesterol moiety (Crooke *et al.*, *J. Pharmacol. Exp. Ther.*, 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting, or lifetime of an iRNA agent into which it is incorporated. In some embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. In some embodiments, ligands do not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylglucosamine, N-acetylgalactosamine, or hyaluronic

acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic. In certain embodiments, the ligand is a multivalent galactose, *e.g.*, an N-acetyl-galactosamine.

Other examples of ligands include dyes, intercalating agents (*e.g.* acridines), cross-linkers (*e.g.* psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases (*e.g.* EDTA), lipophilic molecules, *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)cholonic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, [MPEG]₂, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.* biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu³⁺ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF- κ B.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxol, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide
5 A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins, *etc.* Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid,
10 dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (*e.g.* as PK modulating ligands). In addition, aptamers
15 that bind serum components (*e.g.* serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be
20 reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems® (Foster City,
25 Calif.). Any other methods for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on
30 a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis
35 of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the

standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. In one embodiment, such a lipid or lipid-based molecule binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to inhibit, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In certain embodiments, the lipid based ligand binds HSA. In one embodiment, it binds HSA with a sufficient affinity such that the conjugate will be distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In other embodiments, the lipid based ligand binds HSA weakly or not at all. In one embodiment, the conjugate will be distributed to the kidney. Other moieties that target to kidney cells can also be used in place of, or in addition to, the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents

In another aspect, the ligand is a cell-permeation agent, such as, a helical cell-permeation agent. In one embodiment, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopodia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. In one embodiment, the helical agent is an alpha-helical agent, which has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. 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 attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and

absorption. The peptide or peptidomimetic moiety 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.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO: xx). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:xx) containing a hydrophobic MTS can also be a targeting moiety.

The peptide moiety can be a “delivery” peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:xx) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:xx) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent *via* an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, *e.g.*, glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidomimetics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand, *e.g.*, PECAM-1 or VEGF.

A “cell permeation peptide” is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α -helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β -defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni *et al.*, Nucl. Acids Res. 31:2717-2724, 2003).

35

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is advantageous for the *in vivo*

delivery of nucleic acids, as well as compositions suitable for *in vivo* therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate *per se* made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a
5 compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific
10 monosaccharides include C5 and above (*e.g.*, C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (*e.g.*, C5, C6, C7, or C8).

In certain embodiments, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide.

In certain embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). GalNAc
15 conjugates, which comprise one or more N-acetylgalactosamine (GalNAc) derivatives, are described, for example, in US 8,106,022, the entire content of which is hereby incorporated herein by reference. In some embodiments, the GalNAc conjugate serves as a ligand that targets the iRNA to particular cells. In some embodiments, the GalNAc conjugate targets the iRNA to liver cells, *e.g.*, by serving as a ligand for the asialoglycoprotein receptor of liver cells (*e.g.*, hepatocytes).

In some embodiments, the carbohydrate conjugate comprises one or more GalNAc
20 derivatives. The GalNAc derivatives may be attached *via* a linker, *e.g.*, a bivalent or trivalent branched linker. In some embodiments the GalNAc conjugate is conjugated to the 3' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 3' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein. In some embodiments the
25 GalNAc conjugate is conjugated to the 5' end of the sense strand. In some embodiments, the GalNAc conjugate is conjugated to the iRNA agent (*e.g.*, to the 5' end of the sense strand) *via* a linker, *e.g.*, a linker as described herein.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc
30 derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a trivalent linker. In other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a tetravalent linker.

In certain embodiments, the double stranded RNAi agents of the invention comprise one
35 GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

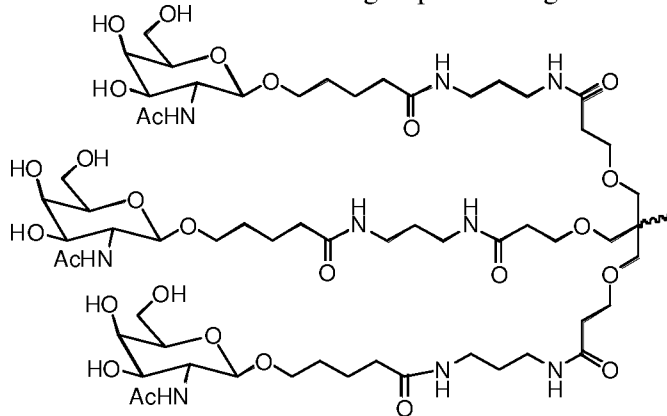
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

5

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

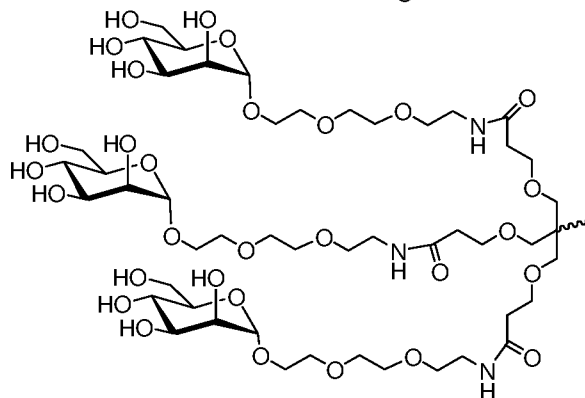
10

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:

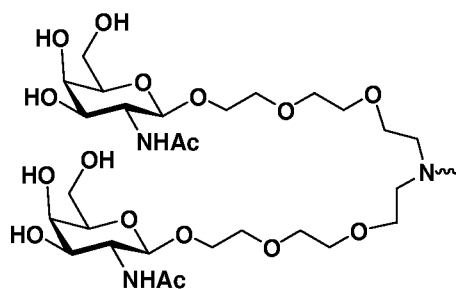


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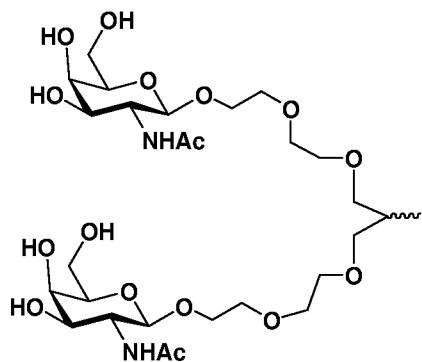
Formula II,



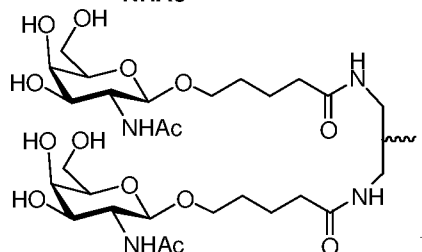
Formula III,



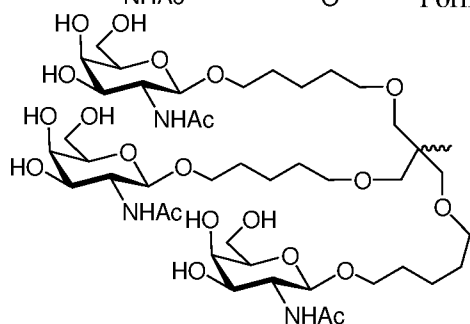
Formula IV,



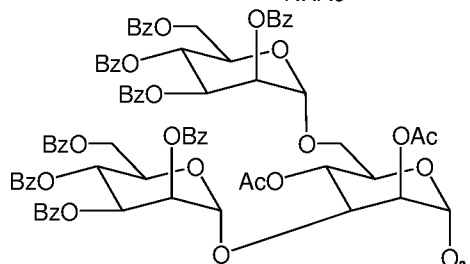
Formula V,



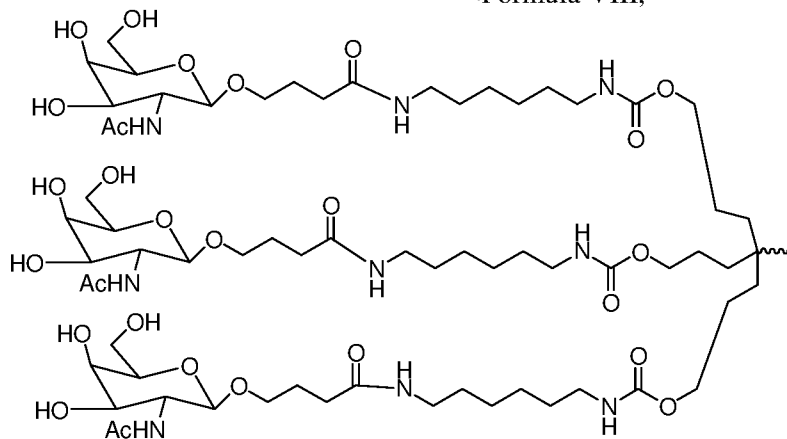
Formula VI,



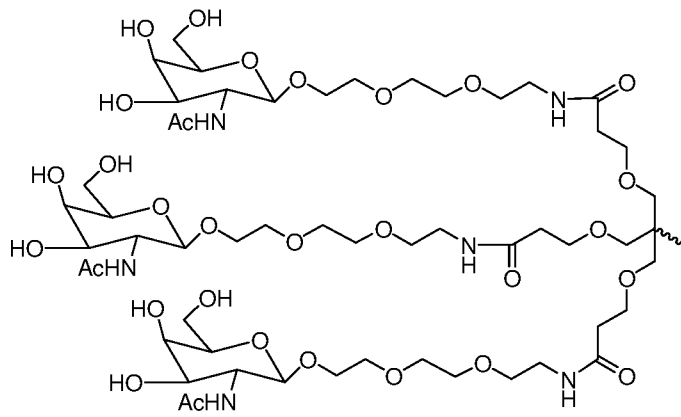
Formula VII,



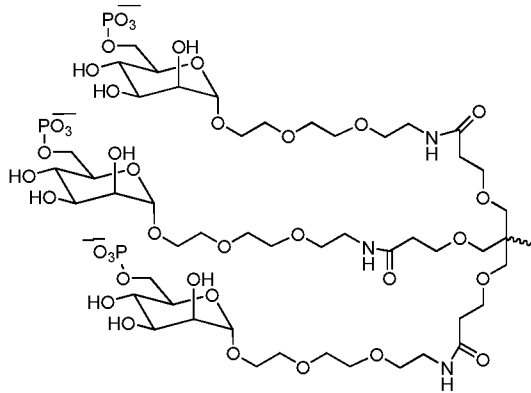
Formula VIII,



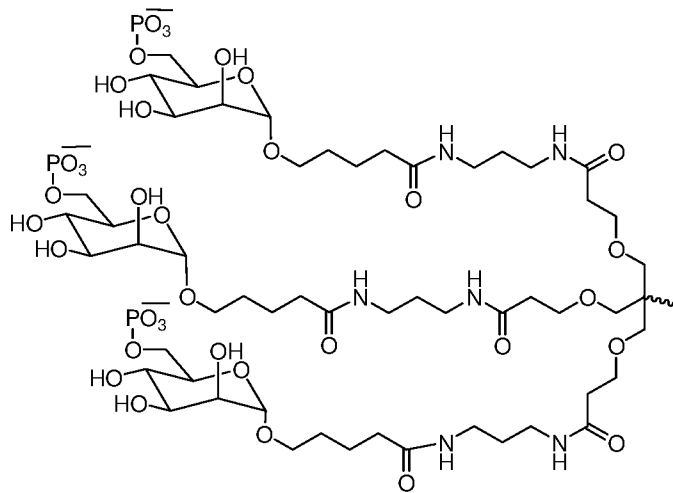
Formula IX,



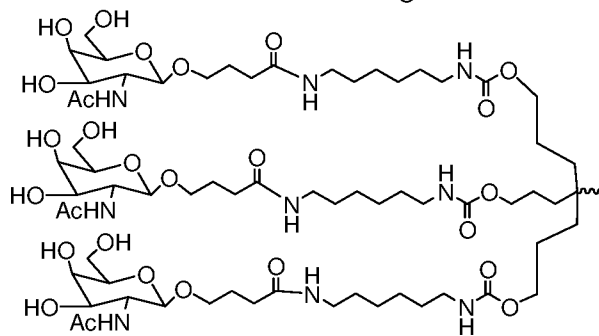
Formula X,



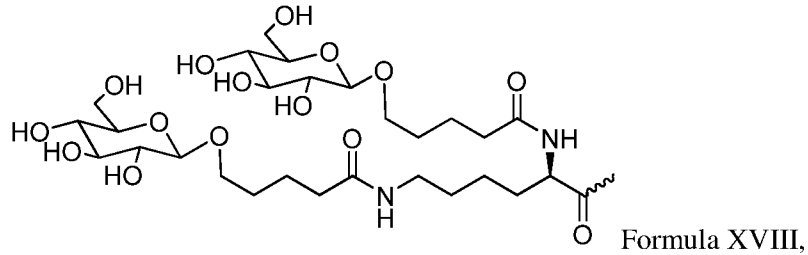
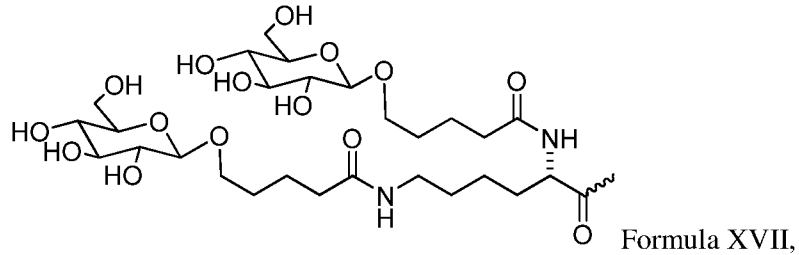
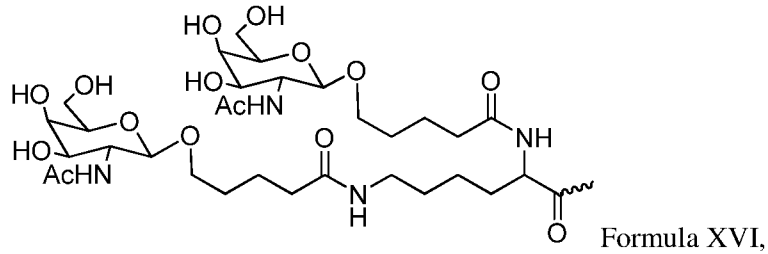
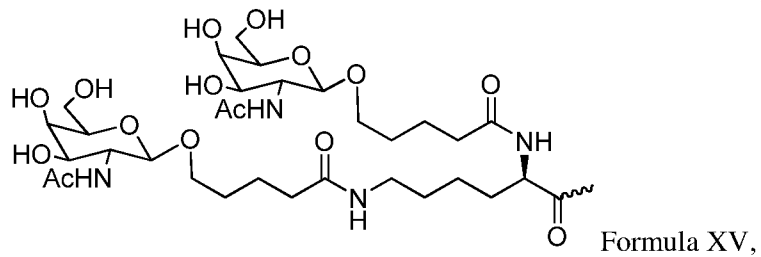
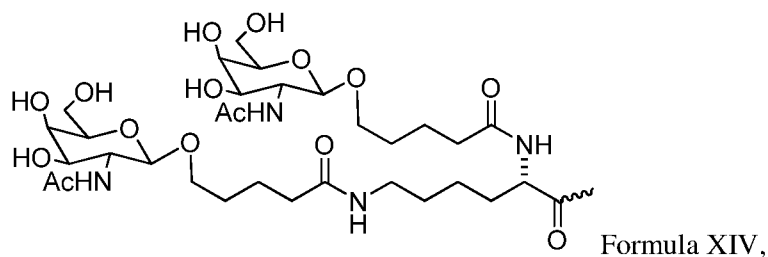
Formula XI,



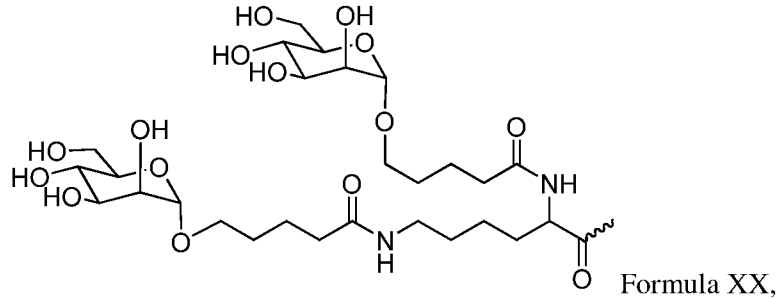
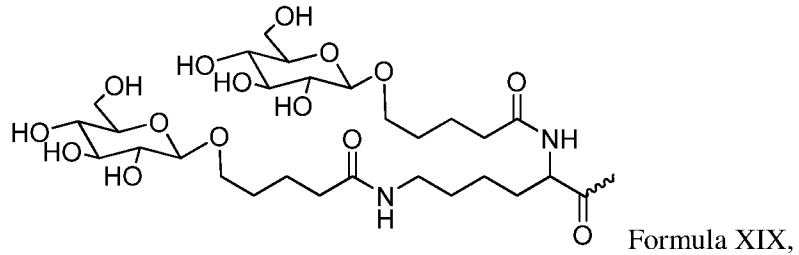
Formula XII,

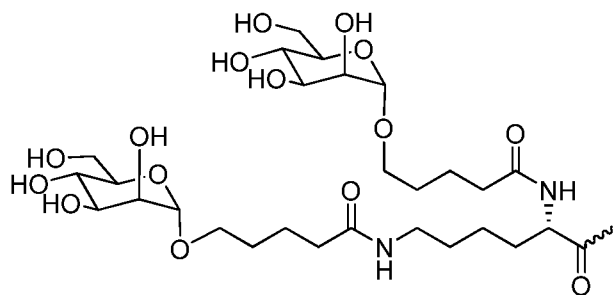


Formula XIII,

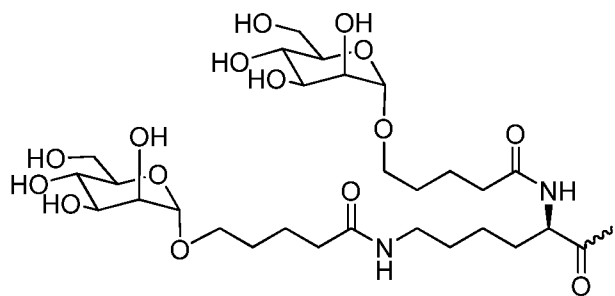


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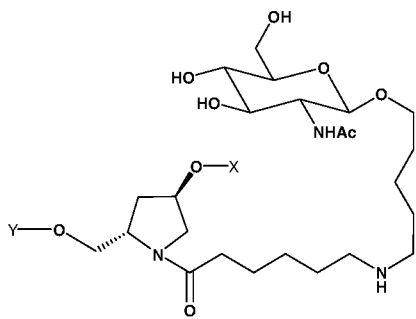




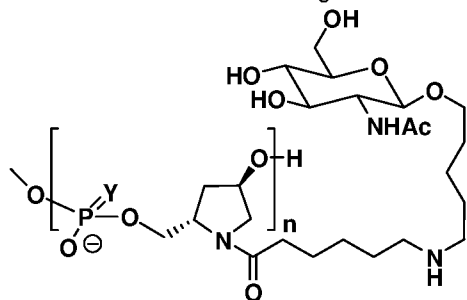
Formula XXI,



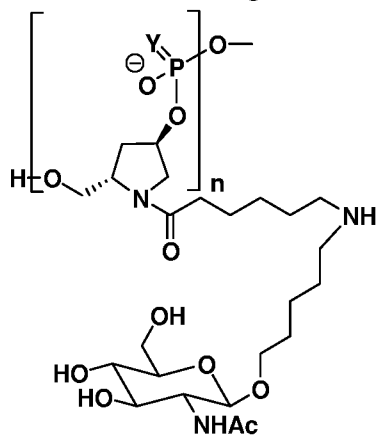
Formula XXII,



Formula XXIII;

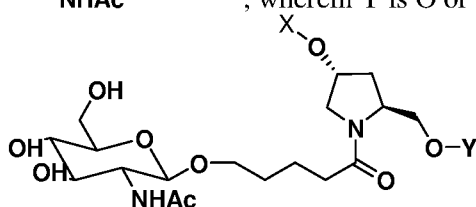


, wherein Y is O or S and n is 3 -6 (Formula XXIV);

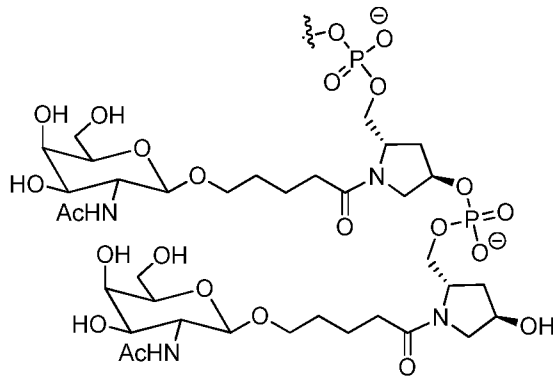
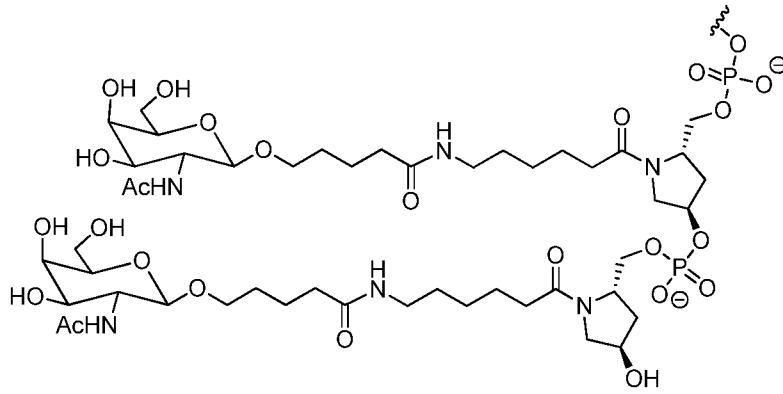


, wherein Y is O or S and n is 3-6 (Formula XXV);

5

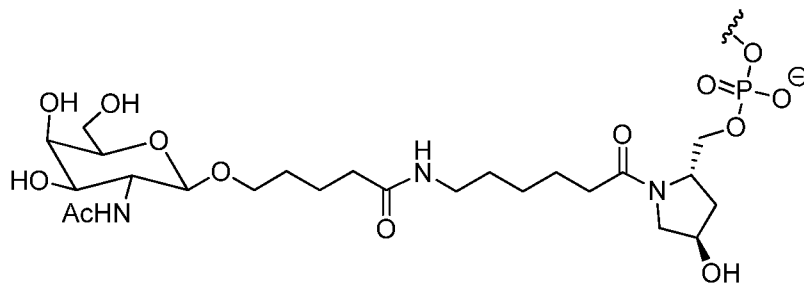


Formula XXVI;

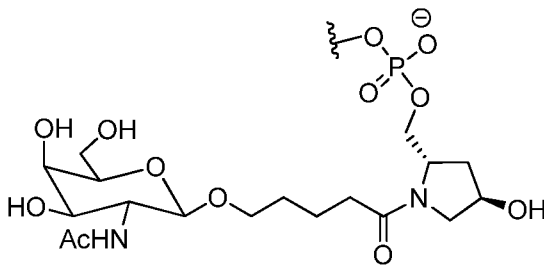


Formula XXX;

Formula XXXI;

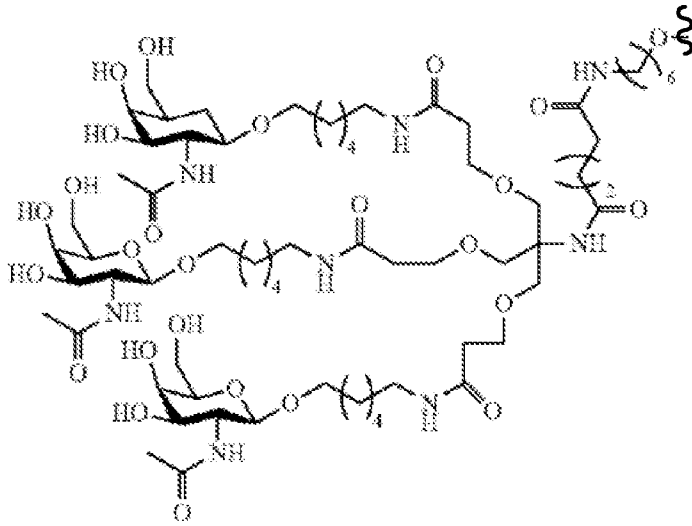


, and



Formula XXXII;

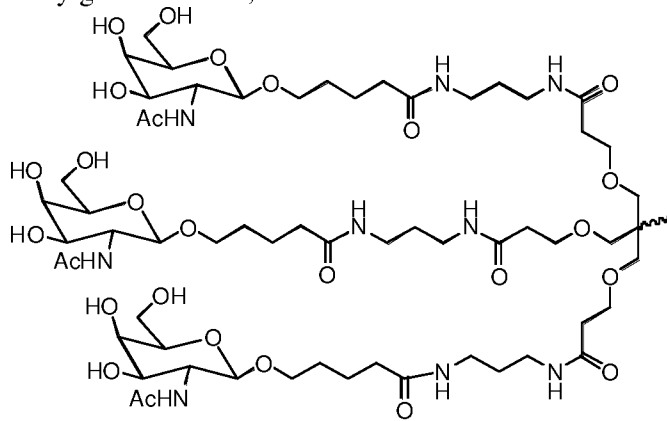
5 Formula XXXIII.



Formula XXXIV.

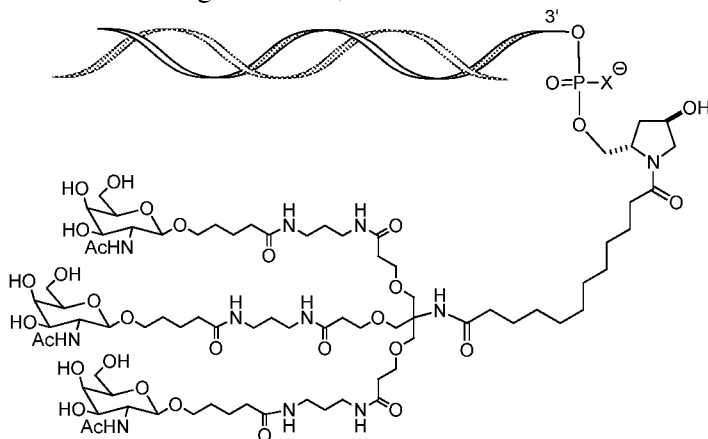
In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In one embodiment, the monosaccharide is an N-acetylgalactosamine, such as

5

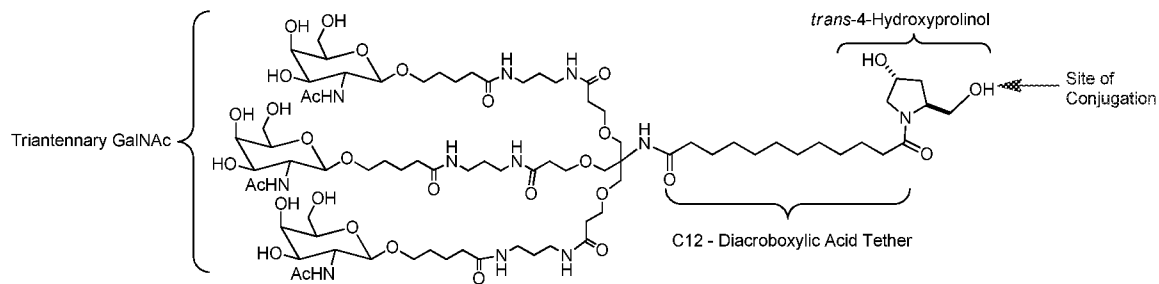


Formula II.

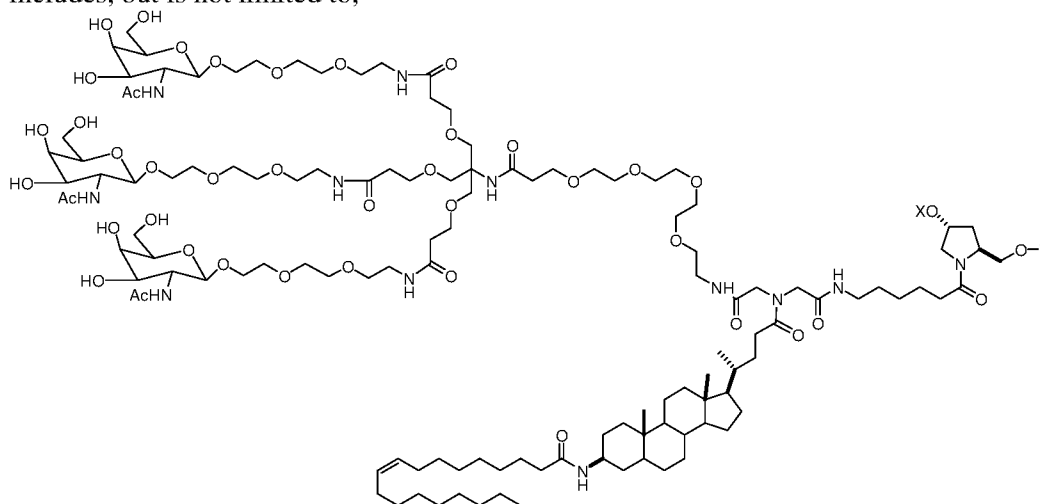
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S.



In some embodiments, the RNAi agent is conjugated to L96 as defined in Table 1 and shown below:



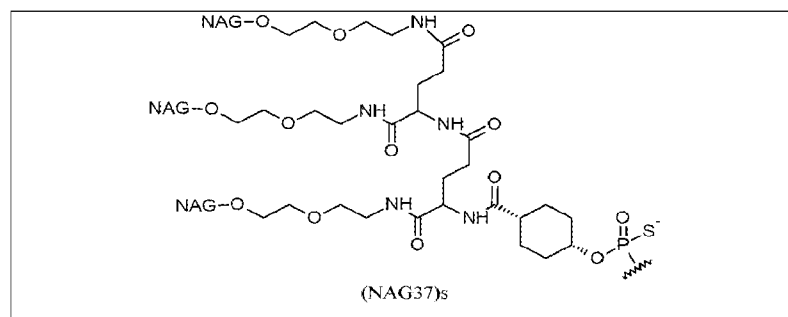
Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXXVI), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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:

15



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In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention *via* a bivalent linker. In yet other
5 invention *via* a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one or more GalNAc or GalNAc derivative attached to the iRNA agent. The GalNAc may be attached to any nucleotide *via* a linker on the sense strand or antisense strand. The GalNAc may be attached to the 5'-end of the sense strand, the 3' end of the sense strand, the 5'-end of the antisense strand, or the 3' –
10 end of the antisense strand. In one embodiment, the GalNAc is attached to the 3' end of the sense strand, *e.g.*, *via* a trivalent linker.

In other embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of linkers, *e.g.*, monovalent linkers.
15

In some embodiments, for example, when the two strands of an iRNA agent of the invention is part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached *via* a monovalent linker.
20

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates and linkers suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.
25

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, *e.g.*, covalently attaches two parts of a compound. Linkers typically comprise a direct
30 bond or an atom such as oxygen or sulfur, a unit such as NR₂, C(O), C(O)NH, SO, SO₂, SO₂NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclalkyl, heterocyclalkenyl,
35 heterocyclalkynyl, aryl, heteroaryl, heterocycl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl,

alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclalkyl, alkylheterocyclalkenyl, alkylheterocyclalkynyl, alkenylheterocyclalkyl, alkenylheterocyclalkenyl, alkenylheterocyclalkynyl, alkynylheterocyclalkyl, alkynylheterocyclalkenyl, alkynylheterocyclalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylheteroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO₂, N(R₈), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R₈ is hydrogen, acyl, aliphatic, or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-17, or 8-16 atoms.

10 A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In an exemplary embodiment, the cleavable linking group is cleaved at least about 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or more, or at least 100 times faster in a target cell or under a first reference condition (which can, *e.g.*, be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, *e.g.*, be selected to mimic or represent conditions found in the blood or serum).

15 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.

20 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 linkers will have a cleavable linking group that is cleaved at a selected pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

25 A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

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. 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 can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In certain embodiments, useful candidate compounds are cleaved at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, 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).

i. Redox cleavable linking groups

In certain embodiments, a cleavable linking group is a redox cleavable linking group that is 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 known 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 one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 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.

ii. Phosphate-based cleavable linking groups

In other embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is 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-, wherein Rk at each occurrence can be, independently, C1-C20 alkyl, C1-C20 haloalkyl, C6-C10 aryl, or C7-C12 aralkyl. Exemplary embodiments include -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-, and -O-P(S)(H)-S-. In certain embodiments a phosphate-based linking group is -O-P(O)(OH)-O-. These candidates can be evaluated using methods analogous to those described above.

10 *iii. Acid cleavable linking groups*

In other embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In certain 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, esters, and esters of amino acids. Acid cleavable groups can have the general formula -C=NN-, C(O)O, or -OC(O). An exemplary 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.

25 *iv. Ester-based linking groups*

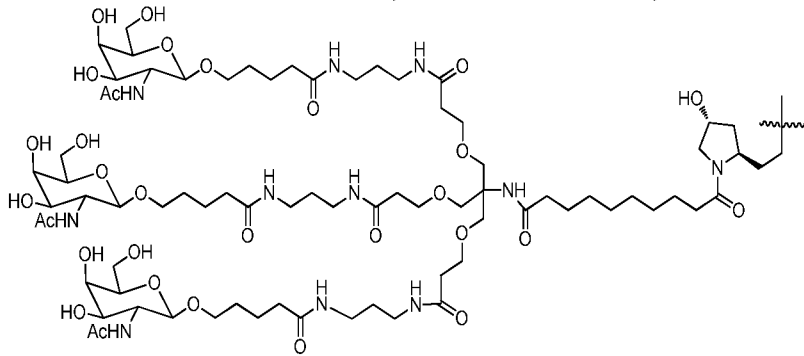
In other embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include, but are not limited to, esters of alkylene, alkenylene 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.

30 *v. Peptide-based cleaving groups*

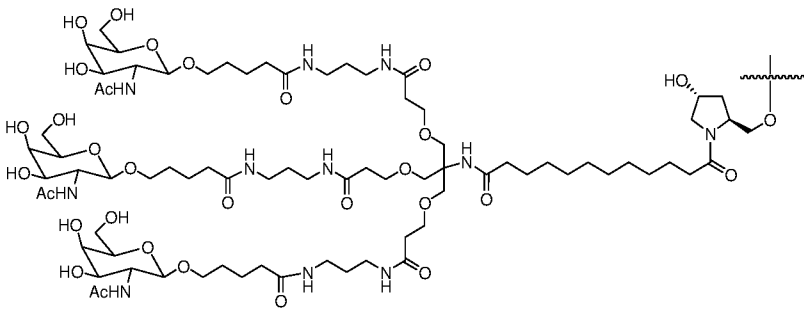
In yet other embodiments, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (*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 alkynylene. 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-based cleavable linking groups have the general formula –NHCHRAC(O)NHCHRBC(O)-, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

5 In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,

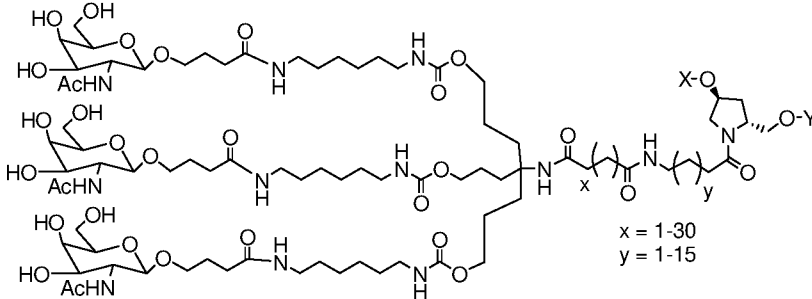


(Formula XXXVII),

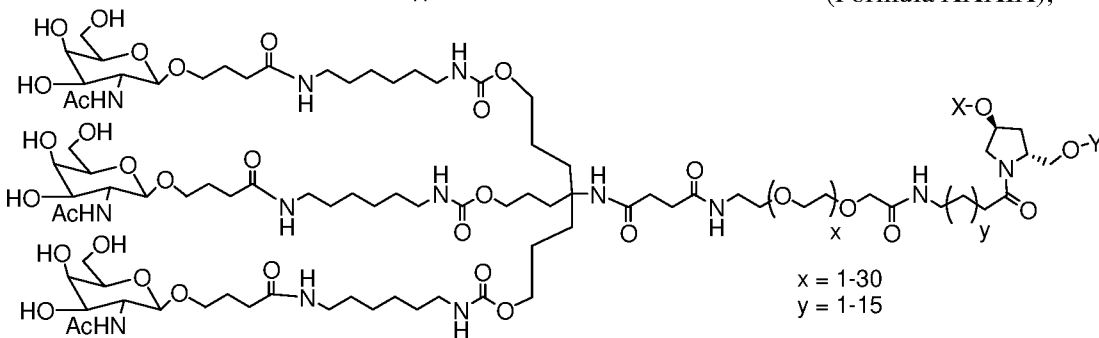


(Formula XXXVIII),

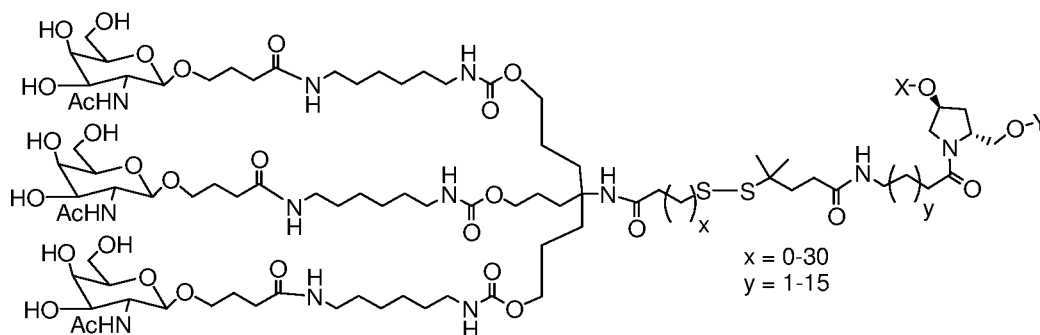
10



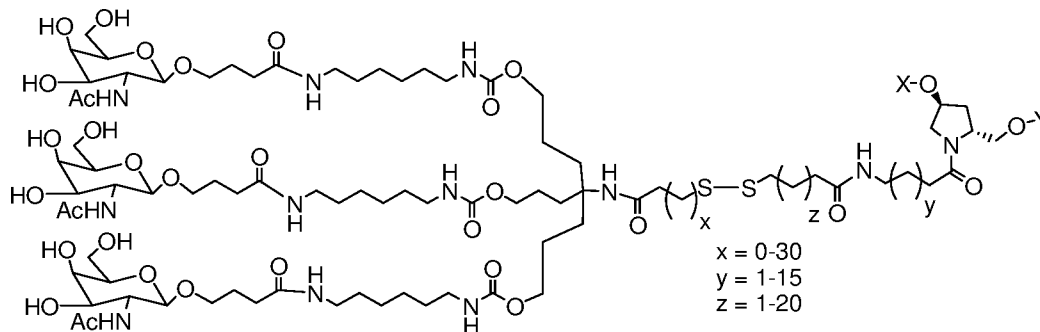
(Formula XXXIX),



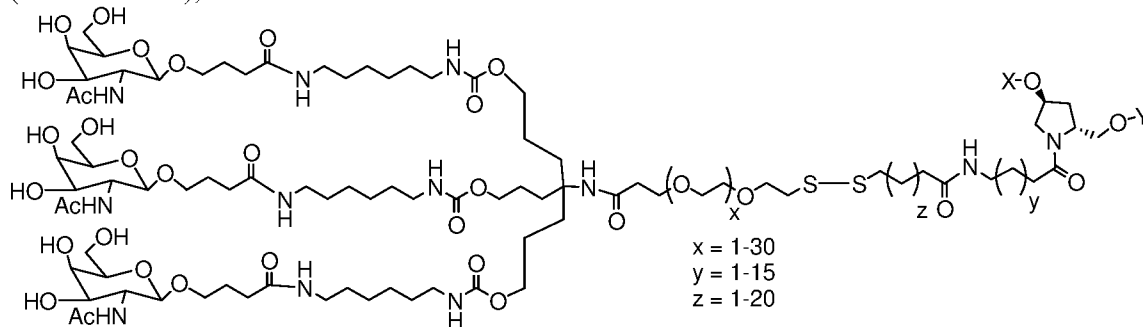
(Formula XL),



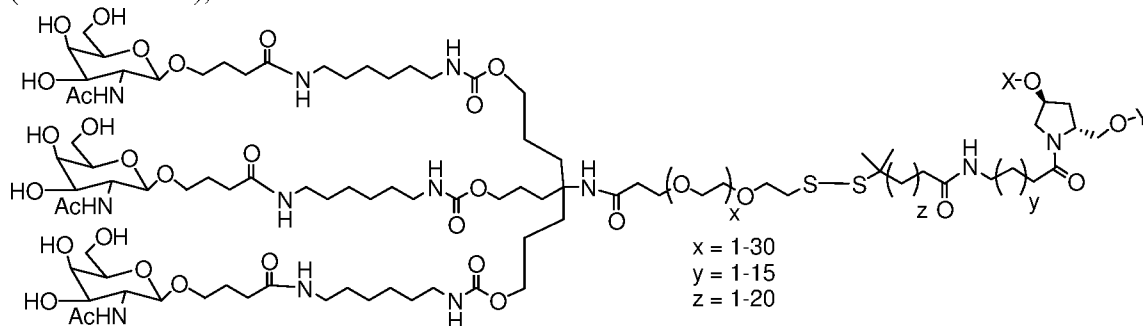
(Formula XLI),



5 (Formula XLII),



(Formula XLIII), and

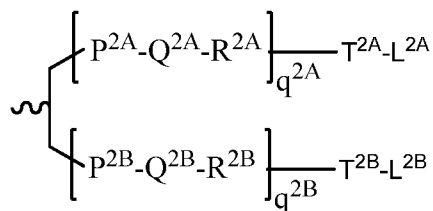


(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

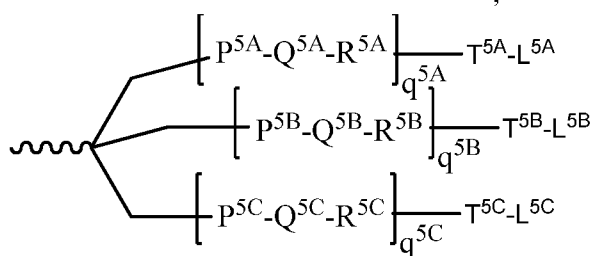
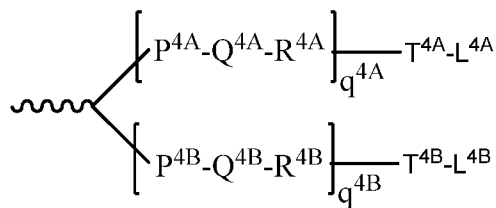
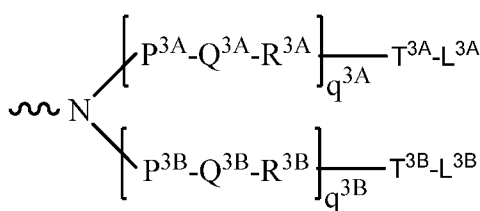
10 In certain embodiments of the compositions and methods of the invention, a ligand is one or more “GalNAc” (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

Formula XXXV



Formula XLVI



, or

;

Formula XL'

Formula XLVIII

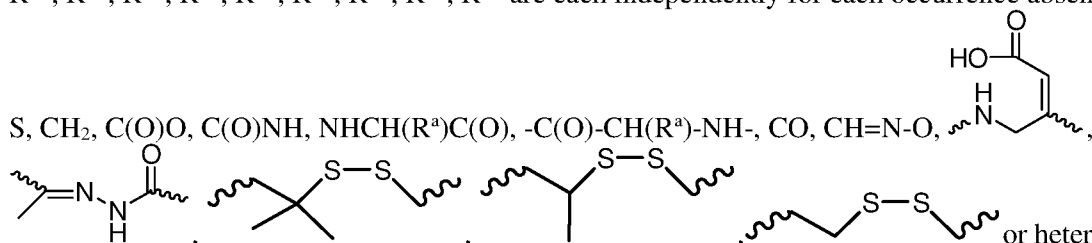
5 wherein:

q^{2A}, q^{2B}, q^{3A}, q^{3B}, q^{4A}, 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;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each

10 Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, N(R^N), C(R')=C(R''), C≡C or C(O);

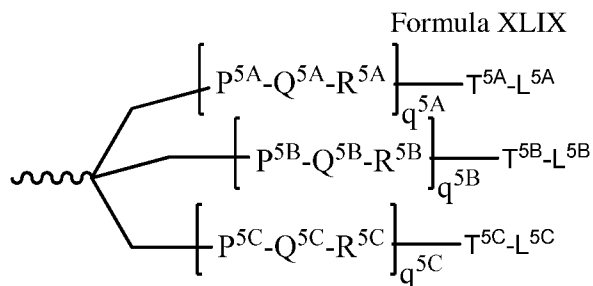
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O,



L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide,

oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a

20 target gene, such as those of formula (XLIX):



wherein L^{5A} , L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

5 Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative U.S. Patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Patent 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; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 10 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; 5,214,136; 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; 5,688,941; 6,294,664; 6,320,017; 15 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are 20 chimeric compounds.

“Chimeric” iRNA compounds or “chimeras,” in the context of this invention, are iRNA compounds, such as, dsRNAi agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, *i.e.*, a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA 25 increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA 30 inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. *et al.*, *Biochem. Biophys. Res. Comm.*, 2007, 365(1):54-61; 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. Lett.*, 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), or 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). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

25 IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention 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 susceptible to or diagnosed with an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either *in vitro* or *in vivo*. *In vivo* delivery may also be performed directly by administering a composition comprising an iRNA, *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 iRNA. 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 an iRNA of the invention (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 iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of

the delivered molecule in the target tissue. RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., *et al.* (2004) *Nucleic Acids* 32:e49; Tan, PH., *et al.* (2005) *Gene Ther.* 12:59-66; Makimura, H., *et al.* (2002) *BMC Neurosci.* 3:18; Shishkina, GT., *et al.* (2004) *Neuroscience* 129:521-528; Thakker, ER., *et al.* (2004) *Proc. Natl. Acad. Sci. U.S.A.*

5 101:17270-17275; Akaneya, Y., *et al.* (2005) *J. Neurophysiol.* 93:594-602). Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into
10 mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., *et al.* (2004) *Nature* 432:173-178).

In an alternative embodiment, the iRNA 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 an iRNA molecule (negatively charged) and also
15 enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, 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 iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic- iRNA
20 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 iRNAs include DOTAP (Sorensen, DR., *et al.* (2003), *supra*; Verma, UN, *et al.* (2003), *supra*), "solid nucleic acid
25 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, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical
30 compositions of iRNAs 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 an HMGCR gene in a cell, comprising contacting said cell with the double-stranded RNAi agent of the disclosure. In one embodiment, the cell is a hepatic cell, optionally a
35 hepatocyte. In one embodiment, the cell is an extrahepatic cell.

A. Vector encoded iRNAs of the Invention

iRNA targeting the HMGCR 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; Skillern, A, *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 5 00/22114, and Conrad, U.S. Patent No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to 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.*, 10 *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

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 15 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 20 and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, *e.g.*, promoters, enhancers, *etc.*, to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

V. Pharmaceutical Compositions of the Invention

25 The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for preventing or treating an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia.

30 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 subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an HMGCR gene.

35 In some embodiments, the pharmaceutical compositions of the invention are sterile. In another embodiment, the pharmaceutical compositions of the invention are pyrogen free.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an HMGCR gene. In general, a suitable dose of an iRNA of the invention will

be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, such as, about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimen may include administration of
5 a therapeutic amount of iRNA on a regular basis, such as every month, once every 3-6 months, or once a year. In certain embodiments, the iRNA is administered about once per month to about once per six months.

After an initial treatment regimen, the treatments can be administered on a less frequent basis. Duration of treatment can be determined based on the severity of disease.

10 In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that doses are administered at not more than 1, 2, 3, or 4 month intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered about once per month. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered quarterly (*i.e.*, about every three months). In other
15 embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered twice per year (*i.e.*, about once every six months).

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to mutations present in the subject, previous treatments, the general health or age of the subject, and other diseases present. Moreover,
20 treatment of a subject with a prophylactically or therapeutically effective amount, as appropriate, of a composition can include a single treatment or a series of treatments.

The pharmaceutical compositions of the present disclosure can 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 topical (including ophthalmic, vaginal, rectal, intranasal, transdermal),
25 oral, or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal, *e.g.*, via an implanted device; or intracranial, *e.g.*, by intraparenchymal, intrathecal or intraventricular, administration.

The iRNA can be delivered in a manner to target a particular tissue, such as the liver.

Pharmaceutical compositions and formulations for topical administration can include
30 transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the RNAi agents featured in the disclosure are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating
35 agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (*e.g.*, dimyristoylphosphatidyl glycerol DMPG) and cationic (*e.g.*, dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). RNAi

agents featured in the disclosure can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, RNAi agents can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-20 alkyl ester (*e.g.*, isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof. Topical formulations are described in detail in US 6,747,014, which is incorporated herein by reference.

10 In one embodiment, the siRNAs, double stranded RNA agents of the invention, are administered to a cell in a pharmaceutical composition by a topical route of administration. In one embodiment, the pharmaceutical composition may include an siRNA compound mixed with a topical delivery agent. The topical delivery agent can be a plurality of microscopic vesicles. The microscopic vesicles can be liposomes. In some embodiments the liposomes are cationic liposomes.

15 In another embodiment, the dsRNA agent is admixed with a topical penetration enhancer. In one embodiment, the topical penetration enhancer is a fatty acid. The fatty acid can be arachidonic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C1-10 alkyl ester, monoglyceride, diglyceride or pharmaceutically acceptable salt thereof.

20 In another embodiment, the topical penetration enhancer is a bile salt. The bile salt can be cholic acid, dehydrocholic acid, deoxycholic acid, glucolic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate, sodium glycodihydrofusidate, polyoxyethylene-9-lauryl ether or a pharmaceutically acceptable salt thereof.

25 In another embodiment, the penetration enhancer is a chelating agent. The chelating agent can be EDTA, citric acid, a salicylate, a N-acyl derivative of collagen, laureth-9, an N-amino acyl derivative of a beta-diketone or a mixture thereof.

30 In another embodiment, the penetration enhancer is a surfactant, *e.g.*, an ionic or nonionic surfactant. The surfactant can be sodium lauryl sulfate, polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether, a perfluorchemical emulsion or mixture thereof.

35 In another embodiment, the penetration enhancer can be selected from a group consisting of unsaturated cyclic ureas, 1-alkyl-alkones, 1-alkenylazacyclo-alkanones, steroidal anti-inflammatory agents and mixtures thereof. In yet another embodiment the penetration enhancer can be a glycol, a pyrrol, an azone, or a terpenes.

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, *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.

The iRNA molecules of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include one or more species of iRNA 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 to a cell, *e.g.*, a liver cell. 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.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids, and self-emulsifying semisolids. Formulations include those that target the liver.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers.

A. Additional Formulations

i. Emulsions

The compositions of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi *et al.*, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed

with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution either in the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed.

Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic, and amphoteric (see *e.g.*, Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New

York, NY Rieger, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and antioxidants (Block, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

The application of emulsion formulations *via* dermatological, oral, and parenteral routes, and methods for their manufacture have been reviewed in the literature (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Idson, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

ii. Microemulsions

In one embodiment of the present invention, the compositions of iRNAs and nucleic acids are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see *e.g.*, Ansel's *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Allen, LV., Popovich NG., and Ansel HC., 2004, Lippincott Williams & Wilkins (8th ed.), New York, NY; Rosoff, in *Pharmaceutical Dosage Forms*, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: *Controlled Release of Drugs: Polymers and Aggregate Systems*, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215).

iii. Microparticles

An iRNA of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present

in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also
5 enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, *i.e.*, surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see *e.g.*, Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, NY, 2002; Lee *et al.*, Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above
10 mentioned classes of penetration enhancers and their use in manufacture of pharmaceutical compositions and delivery of pharmaceutical agents are well known in the art.

v. Excipients

In contrast to a carrier compound, a “pharmaceutical carrier” or “excipient” is a
15 pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Such agent are well known in the art.

vi. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such
25 as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations
30 can be sterilized and, if desired, mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings, or aromatic substances, and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension
35 including, for example, sodium carboxymethylcellulose, sorbitol, or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating an HMGCR-associated disorder, *e.g.*, a disorder of lipid metabolism.

Toxicity and prophylactic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose prophylactically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50, such as, an ED80 or ED90, with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the prophylactically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (*e.g.*, achieving a decreased concentration of the polypeptide) that includes the IC50 (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) or higher levels of inhibition as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents used for the prevention or treatment of an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Methods For Inhibiting HMGCR Expression

The present invention also provides methods of inhibiting expression of an HMGCR gene in a cell. The methods include contacting a cell with an RNAi agent, *e.g.*, double stranded RNA agent, in an amount effective to inhibit expression of HMGCR in the cell, thereby inhibiting expression of HMGCR in the cell. In some embodiments of the disclosure, expression of an HMGCR gene is inhibited preferentially in the liver (*e.g.*, hepatocytes).

Contacting of a cell with an iRNA, *e.g.*, a double stranded RNA agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the iRNA includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the iRNA. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. 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 GalNAc₃ ligand, or any other ligand that directs the RNAi agent to a site of interest.

5 The term “inhibiting,” as used herein, is used interchangeably with “reducing,” “silencing,” “downregulating”, “suppressing”, and other similar terms, and includes any level of inhibition.

The phrase “inhibiting expression of an HMGCR gene” is intended to refer to inhibition of expression of any HMGCR gene (such as, *e.g.*, a mouse HMGCR gene, a rat HMGCR gene, a monkey HMGCR gene, or a human HMGCR gene) as well as variants or mutants of an HMGCR gene. Thus, the HMGCR gene may be a wild-type HMGCR gene, a mutant HMGCR gene, or a
10 transgenic HMGCR gene in the context of a genetically manipulated cell, group of cells, or organism.

“Inhibiting expression of an HMGCR gene” includes any level of inhibition of an HMGCR gene, *e.g.*, at least partial suppression of the expression of an HMGCR gene. The expression of the HMGCR gene may be assessed based on the level, or the change in the level, of any variable associated with HMGCR gene expression, *e.g.*, HMGCR mRNA level or HMGCR protein level. It is
15 understood that HMGCR is expressed in various tissues, including the liver.

The expression of an HMGCR may also be assessed indirectly based on other variables associated with HMGCR gene expression, *e.g.*, level of 3-hydroxy-3-methylglutaryl-CoA reductase expression in the cytoplasm, nuclear localization of 3-hydroxy-3-methylglutaryl-CoA reductase, or expression of certain target genes such as Jun, c-Myc and CyclinD-1 or other oncogenes under
20 transcription control of 3-hydroxy-3-methylglutaryl-CoA reductase.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with HMGCR expression 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
25 as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of an HMGCR gene is inhibited by at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some embodiments, expression of an HMGCR gene is inhibited by at least 70%. It is further understood that inhibition of HMGCR expression in certain tissues, *e.g.*, in
30 liver, without a significant inhibition of expression in other tissues, *e.g.*, brain, may be desirable. In some embodiments, expression level is determined using the assay method provided in Example 2 with a 10 nM siRNA concentration in the appropriate species matched cell line.

In certain embodiments, inhibition of expression *in vivo* is determined by knockdown of the human gene in a rodent expressing the human gene, *e.g.*, an AAV-infected mouse expressing the
35 human target gene (*i.e.*, HMGCR), *e.g.*, when administered as a single dose, *e.g.*, at 3 mg/kg at the nadir of RNA expression. Knockdown of expression of an endogenous gene in a model animal system can also be determined, *e.g.*, after administration of a single dose at, *e.g.*, 3 mg/kg at the nadir of RNA expression. Such systems are useful when the nucleic acid sequence of the human gene and

the model animal gene are sufficiently close such that the human iRNA provides effective knockdown of the model animal gene. RNA expression in liver is determined using the PCR methods provided in Example 2.

Inhibition of the expression of an HMGCR 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 an HMGCR gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of an HMGCR 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 an iRNA or not treated with an iRNA targeted to the gene of interest). In some embodiments, the inhibition is assessed by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

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

In other embodiments, inhibition of the expression of an HMGCR gene may be assessed in terms of a reduction of a parameter that is functionally linked to HMGCR gene expression, *e.g.*, HMGCR protein level in blood or serum from a subject. HMGCR gene silencing may be determined in any cell expressing HMGCR, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of an HMGCR protein may be manifested by a reduction in the level of the HMGCR protein that is expressed by a cell or group of cells or in a subject sample (*e.g.*, the level of protein in a blood sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition 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, or the change in the level of protein in a subject sample, *e.g.*, blood or serum derived therefrom.

A control cell, a group of cells, or subject sample that may be used to assess the inhibition of the expression of an HMGCR gene includes a cell, group of cells, or subject sample that has not yet been contacted with an RNAi agent of the invention. For example, the control cell, group of cells, or subject sample may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent or an appropriately matched population control.

The level of HMGCR mRNA that is expressed by a cell or group of cells may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of HMGCR in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the HMGCR 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, and microarray analysis.

In some embodiments, the level of expression of HMGCR 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 HMGCR. 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 mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to HMGCR mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix® gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of HMGCR mRNA.

An alternative method for determining the level of expression of HMGCR 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, U.S. 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.*, U.S. 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 invention, the level of expression of HMGCR is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System). In some embodiments, expression level is determined by the method provided in Example 2 using, *e.g.*, a 10 nM siRNA concentration, in the species matched cell line.

The expression levels of HMGCR 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 U.S. 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 HMGCR expression level may also comprise using nucleic acid probes in solution.

In some embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein. In some embodiments, expression level is determined by the method provided in Example 2 using a 10nM siRNA concentration in the species matched cell line.

The level of HMGCR 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.

In some embodiments, the efficacy of the methods of the invention are assessed by a decrease in HMGCR mRNA or protein level (*e.g.*, in a liver biopsy).

In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in tumor formation. Reducing tumor, as used herein, includes any decrease in the size, number, or severity of tumor, or to a prevention or reduction in the formation of tumor, within a tissue of a subject, as may be assessed *in vitro* or *in vivo* using any method known in the art.

In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of HMGCR may be assessed using measurements of the level or change in the level of HMGCR mRNA or HMGCR protein in a sample derived from fluid or tissue from the specific site within the subject (*e.g.*, liver or blood).

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.

VII. Prophylactic and Treatment Methods of the Invention

The present invention also provides methods of using an iRNA of the invention or a composition containing an iRNA of the invention to inhibit expression of HMGCR, thereby preventing or treating an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia. In the methods of the invention the cell may be contacted with the siRNA *in vitro* or *in vivo*, *i.e.*, the cell may be within a subject.

A cell suitable for treatment using the methods of the invention may be any cell that expresses an HMGCR gene, *e.g.*, a liver cell. A cell suitable for use in the methods of the invention may be a mammalian cell, *e.g.*, a primate cell (such as a human cell, including human cell in a chimeric non-human animal, or a non-human primate cell, *e.g.*, a monkey cell or a chimpanzee cell), or a non-
5 primate cell. In certain embodiments, the cell is a human cell, *e.g.*, a human liver cell. In the methods of the invention, HMGCR expression is inhibited in the cell by at least 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay.

The *in vivo* methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least
10 a part of an RNA transcript of the HMGCR gene of the mammal to which the RNAi agent is to be administered. The composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, intraocular (*e.g.*, periocular, conjunctival, subtenon, intracameral, intravitreal,
15 intraocular, anterior or posterior juxtasceral, subretinal, subconjunctival, retrobulbar, or intracanalicular injection), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), 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
20 certain embodiments, the compositions are administered by intramuscular injection.

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 invention also provides methods for inhibiting the expression of an
25 HMGCR gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets an HMGCR gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the HMGCR gene, thereby inhibiting expression of the HMGCR gene in the cell. Reduction in gene expression can be assessed by any methods known in the art and by methods, *e.g.* qRT-PCR, described herein, *e.g.*, in
30 Example 2. Reduction in protein production can be assessed by any methods known in the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the HMGCR gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the HMGCR protein expression. A reduction in the expression of HMGCR may also be assessed indirectly by measuring a decrease in
35 biological activity of HMGCR, *e.g.*, a decrease in the level of serum lipid, triglycerides, cholesterol and/or free fatty acids

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with an HMGCR-associated disorder, such as a disorder of lipid metabolism, *e.g.*, hyperlipidemia.

The present invention further provides methods of prophylaxis in a subject in need thereof.

5 The treatment methods of the invention include administering an iRNA of the invention to a subject, *e.g.*, a subject that would benefit from a reduction of HMGCR expression, in a prophylactically effective amount of a dsRNA targeting an HMGCR gene or a pharmaceutical composition comprising a dsRNA targeting an HMGCR gene.

10 In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in HMGCR expression, *e.g.*, an HMGCR-associated disorder, such as a disorder of lipid metabolism, *e.g.*, hyperlipidemia.

Treatment of a subject that would benefit from a reduction and/or inhibition of HMGCR gene expression includes therapeutic treatment (*e.g.*, a subject is having a disorder of lipid metabolism) and prophylactic treatment (*e.g.*, the subject is not having a disorder of lipid metabolism or a subject may be at
15 risk of developing a disorder of lipid metabolism).

In some embodiments, the HMGCR-associated disorder is a disorder of lipid metabolism. Examples of disorder of lipid metabolism include but are not limited to,

In some embodiments, the HMGCR-associated disorder is hyperlipidemia.

20 In some embodiments, the RNAi agent is administered to a subject in an amount effective to inhibit HMGCR expression in a cell within the subject. The amount effective to inhibit HMGCR expression in a cell within a subject may be assessed using methods discussed above, including methods that involve assessment of the inhibition of HMGCR mRNA, HMGCR protein, or related variables, such as tumor formation.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is
25 administered in the absence of a pharmaceutical composition. The naked iRNA 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 iRNA can be adjusted such that it is suitable for administering to a subject.

30 Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from an inhibition of HMGCR gene expression are subjects susceptible to or diagnosed with an HMGCR-associated disorder, such as disorder of lipid metabolism, *e.g.*, hyperlipidemia. In an embodiment, the method includes administering a
35 composition featured herein such that expression of the target an HMGCR gene is decreased, such as for about 1, 2, 3, 4, 5, 6, 1-6, 1-3, or 3-6 months per dose. In certain embodiments, the composition is administered once every 3-6 months.

In one embodiment, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target HMGCR gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

5 Administration of the iRNA according to the methods of the invention may result prevention or treatment of an HMGCR-associated disorder, *e.g.*, disorder of lipid metabolism, *e.g.*, hyperlipidemia. Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

10 In one embodiment, the iRNA is administered subcutaneously, *i.e.*, by subcutaneous injection. One or more injections may be used to deliver the desired dose of iRNA 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 regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as once 15 per month to once a year. In certain embodiments, the iRNA is administered about once per month to about once every three months, or about once every three months to about once every six months.

The invention further provides methods and uses of an iRNA agent or a pharmaceutical composition thereof for treating a subject that would benefit from reduction and/or inhibition of HMGCR gene expression, *e.g.*, a subject having an HMGCR-associated disorder, in combination with 20 other pharmaceuticals and/or other therapeutic methods, *e.g.*, with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating these disorders.

25 Accordingly, in some aspects of the invention, the methods which include administration of an iRNA agent of the invention, further include administering to the subject one or more additional therapeutic agents.

For example, in certain embodiments, an iRNA targeting HMGCR is administered in combination with, *e.g.*, an agent useful in treating an HMGCR-associated disorder as described herein or otherwise known in the art. For example, additional agents and treatments suitable for treating a subject that would benefit from reduction in HMGCR expression, *e.g.*, a subject having an HMGCR- 30 associated disorder, may include agents currently used to treat symptoms of HMGCR-associated disorder

In some embodiments, additional agents suitable for treating a subject that would benefit from reduction in HMGCR expression, *e.g.*, a subject having a disorder of lipid metabolism, may include agents that lower one or more serum lipids. Non-limiting examples of such agents may include 35 cholesterol synthesis inhibitors, such as existing HMGCR inhibitors, *e.g.*, statins. Statins may include atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor), lovastatin extended-release (Altoprev), pitavastatin (Livalo), pravastatin (Pravachol), rosuvastatin (Crestor), and simvastatin (Zocor). Other agents useful in treating a disorder of lipid metabolism may include bile sequestering

agents, such as cholestyramine and other resins; VLDL secretion inhibitors, such as niacin; lipophilic antioxidants, such as Probucol; acyl-CoA cholesterol acyl transferase inhibitors; farnesoid X receptor antagonists; sterol regulatory binding protein cleavage activating protein (SCAP) activators; microsomal triglyceride transfer protein (MTP) inhibitors; ApoE-related peptide; and therapeutic antibodies against HMGCR. The additional therapeutic agents may also include agents that raise high density lipoprotein (HDL), such as cholesteryl ester transfer protein (CETP) inhibitors. Furthermore, the additional therapeutic agents may also include dietary supplements, *e.g.*, fish oil. The iRNA and additional therapeutic agents may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

In some embodiments, second agents suitable for administration as a combination therapy in conjunction with the RNAi agents described herein are 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, or corticosteroids, and a combination of any of the foregoing.

The iRNA agent and an additional therapeutic agent and/or treatment may be administered at the same time and/or in the same combination, *e.g.*, parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times and/or by another method known in the art or described herein.

VIII. Kits

In certain aspects, the instant disclosure provides kits that include a suitable container containing a pharmaceutical formulation of a siRNA compound, *e.g.*, a double-stranded siRNA compound, or siRNA compound, (*e.g.*, a precursor, *e.g.*, a larger siRNA compound which can be processed into a siRNA compound, or a DNA which encodes an siRNA compound, *e.g.*, a double-stranded siRNA compound, or ssiRNA compound, or precursor thereof).

Such kits include one or more dsRNA agent(s) and instructions for use, *e.g.*, instructions for administering a prophylactically or therapeutically effective amount of a dsRNA agent(s). The dsRNA agent may be in a vial or a pre-filled syringe. The kits may optionally further comprise means for administering the dsRNA agent (*e.g.*, an injection device, such as a pre-filled syringe), or means for measuring the inhibition of HMGCR (*e.g.*, means for measuring the inhibition of HMGCR mRNA, HMGCR protein, and/or HMGCR activity). Such means for measuring the inhibition of HMGCR may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for determining the therapeutically effective or prophylactically effective amount.

In certain embodiments the individual components of the pharmaceutical formulation may be provided in one container, *e.g.*, a vial or a pre-filled syringe. 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 siRNA compound preparation, and at least another for a carrier compound. 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.

- 5 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.

This invention is further illustrated by the following examples which should not be construed
10 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.

15 EXAMPLES

Example 1. siRNA 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
20 molecular biology.

siRNA Design

siRNAs targeting the 3-hydroxy-3-methylglutaryl-CoA reductase gene (HMGCR, human: NCBI refseqID NM_000859.3, NCBI Gene ID: 3156) were designed using custom R and Python
25 scripts. The human NM_000859.3 mRNA has a length of 4530 bases.

Detailed lists of the unmodified HMGCR sense and antisense strand nucleotide sequences are shown in Tables 2 and 4. Detailed lists of the modified HMGCR sense and antisense strand nucleotide sequences are shown in Tables 3 and 5.

It is to be understood that, throughout the application, a duplex name without a decimal is
30 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 were designed, synthesized, and prepared using methods known in the art.

35 Briefly, siRNA sequences were synthesized on a 1 μ mol 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.

5 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
10 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 μ L 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
15 (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 μ L of dimethyl sulfoxide (DMSO) and 300 μ L 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
20 1 mL of 9:1 acetonitrile: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
25 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 μ M in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed
30 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 2. *In vitro* screening methods of Duplexes in Tables 2 and 3

Cell culture and 96-well transfections

35 Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ 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 μ l of Opti-MEM plus 0.3 μ l of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat #

13778-150) to 2.5 μ l of each siRNA duplex to an individual well in a 96-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty μ l of complete growth media without antibiotic containing $\sim 1.5 \times 10^4$ 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 final duplex concentration.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen™, part #: 610-12)

Cells were lysed in 75 μ l of Lysis/Binding Buffer containing 3 μ L 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 90 μ L) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10 μ L 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 1 μ l 10X Buffer, 0.4 μ l 25X dNTPs, 1 μ l Random primers, 0.5 μ l Reverse Transcriptase, 0.5 μ l RNase inhibitor and 6.6 μ l of H₂O 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 (μ l) of cDNA were added to a master mix containing 0.5 μ l of human GAPDH TaqMan Probe (4326317E), 0.5 μ l human HMGCR, 2 μ l nuclease-free water and 5 μ l 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 $\Delta\Delta$ Ct method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC₅₀s are 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: cuuAcGcuGAGuAcuucGAdTsdT and antisense UCGAAGuACUcAGCGuAAGdTsdT.

Table 8 shows the results of a single dose screen in Hep3b cells transfected with the indicated agents from Tables 2 and 3.

Example 3. *In vitro* screening methods of Duplexes in Tables 4 and 5*Cell culture and transfections*

Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% CO₂ in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization.

Primary mouse hepatocytes (PMH) were freshly isolated from a C57BL/6 female mouse (Charles River Laboratories International, Inc. Willmington, MA) less than 1 hour prior to transfections and grown in primary hepatocyte media. Cells were resuspended at 0.11x10⁶ cells/ml in InVitroGRO CP Rat (plating) medium (Celsis In Vitro Technologies, catalog number S01494).

During transfections, cells were plated onto a BD BioCoat 96 well collagen plate (BD, 356407) at 10,000 cells per well and incubated at 37°C in an atmosphere of 5% CO₂.

Transfection was carried out by adding 4.9µl of Opti-MEM plus 0.1µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5µl 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 William's E Medium (Life Tech) containing about 5,000 Hep3b 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 and 0.1nM final duplex concentration in Hep3b cells and single dose experiments were performed at 20 nM final duplex concentration in PMH.

Total RNA isolation using DYNABEADS mRNA Isolation Kit (Invitrogen, part #: 610-12)

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADS (Invitrogen, cat # 61012). Fifty µl of Lysis/Binding Buffer and 25 µl of Lysis Buffer containing 3µL of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 µl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 µl Elution Buffer, re-captured and supernatant was removed.

cDNA synthesis using ABI High capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, Cat #4368813)

Ten µl of a master mix containing 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of H₂O per reaction were added to RNA isolated per well. Plates were sealed, mixed and incubated on an electromagnetic shaker for 10 minutes at room temperature, and then incubated at 37°C for 2 hours. Plates were then incubated at 81°C for 8 minutes.

Real time PCR

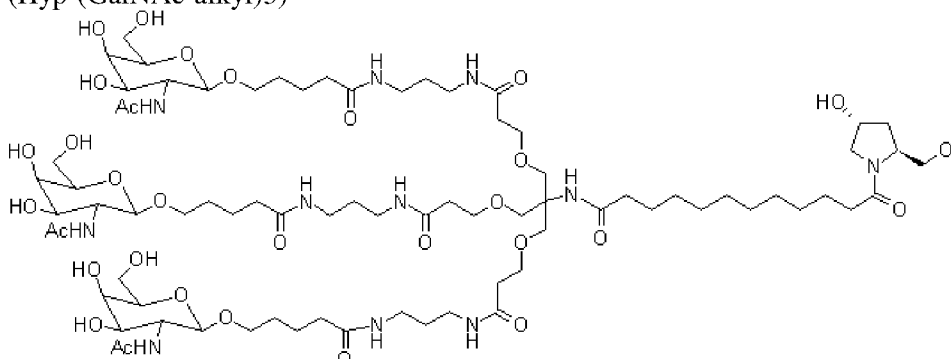
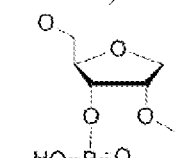
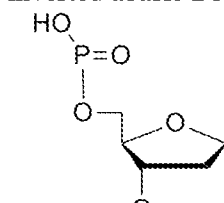
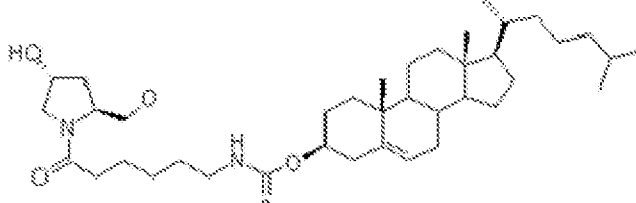
Two μl of cDNA were added to a master mix containing 0.5 μl of human GAPDH TaqMan Probe (4326317E), 0.5 μl human HMGCR (Hs00168352_m1), and 5 μl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche) using the $\Delta\Delta\text{Ct}$ (RQ) assay. Each duplex was tested in at least two times and data were normalized to cells transfected with a non-targeting control siRNA.

To calculate relative fold change, real time data were analyzed using the $\Delta\Delta\text{Ct}$ method and normalized to assays performed with cells transfected with a non-targeting control siRNA. The results from the assays with Hep3b cells are shown in Table 6 and the results from the assays performed in PMH cells are shown in Table 7.

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).

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

Abbreviation	Nucleotide(s)
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
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) 
Y34	2-hydroxymethyl-tetrahydrofuran-4-methoxy-3-phosphate (abasic 2'-OMe furanose) 
Y44	inverted abasic DNA (2-hydroxymethyl-tetrahydrofuran-5-phosphate) 
L10	N-(cholesterylcarboxamidocaproyl)-4-hydroxyprolinol (Hyp-C6-Chol) 
(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

Abbreviation	Nucleotide(s)
dA	2'-deoxyadenosine-3'-phosphate
dAs	2'-deoxyadenosine-3'-phosphorothioate
dC	2'-deoxycytidine-3'-phosphate
dCs	2'-deoxycytidine-3'-phosphorothioate
dG	2'-deoxyguanosine-3'-phosphate
dGs	2'-deoxyguanosine-3'-phosphorothioate
dT	2'-deoxythymidine-3'-phosphate
dTs	2'-deoxythymidine-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

Table 2. Unmodified Sense and Antisense Strand Sequences of HMGCR dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Range in HMGCR mRNA NM_000859.3	Antisense Sequence 5' to 3'	SEQ ID NO:	Range in HMGCR mRNA NM_000859.3
AD-1659648	GCCUCUAGUGAGAUUCUGGAGU		62-82	ACUCCAGAUCUCACUAGAGGCCA		60-82
AD-1659675	GGAUUCUGUAGCUACAAUGUU		89-109	AACAUUGUAGCUACAGAAUCCUU		87-109
AD-1659693	GUUGUCAAGACUUUUUCGAAU		107-127	AUUCGAAAAGUCUUGACAAACAU		105-127
AD-1659762	GAUGUCCAUGAACAUUUUAU		197-217	AUAAACAUGUUCUUGGACAUCAU		195-217
AD-1659780	UACUGGUACAACAAUAGAUCUU		215-235	AAGAUCUUUUUUUACCAGUAAA		213-235
AD-1659812	AUGAAUGUCCAAAGUUUGAAU		247-267	AUUCAAAACUUUUGGACAUUCAUAA		245-267
AD-1659843	AGCAGUGACAUUAUAAUUCUU		279-299	AAGAAUUUAUAAUGUCACUGCUCA		277-299
AD-1659859	CUGACAAUAACACGAUGCAUU		297-317	AAUGCAUCGUGUUUUUUGUCAGAA		295-317
AD-1659887	UGUAUUUUACUUCAGUUCU		325-345	AGAACUGGAAGUAAAUAUACAGG		323-345
AD-1659914	UACGUCAAACUUGGAUCAAAAU		352-372	AUUUUGAUCCAAGUUGACGUAAA		350-372
AD-1659950	CCUUUUCACAAUUUUUCUCAAU		392-412	AUUGAGAAAUUUGUGAAAAGGCC		390-412
AD-1659973	UUGUAUUCAGUACAGUUGUCU		415-435	AGACAACUGUACUGAAUACAAAA		413-435
AD-1659991	UCAUUCACUUCUUAGACAAAU		433-453	AUUUGUCUAAAGAAUGUGAAUGACA		431-453
AD-1660027	AUGAAGCUUUUGCCUUUUUCU		469-489	AGAAAAAGGGCAAAGCUUCAUUC		467-489
AD-1660047	CUACUUUUGAUUGACCUUUUCU		489-509	AGAAAGGUCAAUCAAAGUAGGA		487-509
AD-1660073	AAGCACAUUAGCAAAGUUUUGU		515-535	ACAAACUUUUGCUAAUUGUCUUGC		513-535
AD-1660102	CCAAUCACAGGAUGAAGUAU		544-564	AUACUUCUCCUGUGAGUUUGGAA		542-564
AD-1660136	UCGUGGAUUGGCAAUUUUAGU		578-598	ACUAAAAUUGCCAUUCCACGAGC		576-598
AD-1660176	GCUCUUUGUUGAUUGUCUUUGUU		618-638	AACAAGACAUUCAACAAGAGCAU		616-638
AD-1660211	UUGAAAUUUUGUGCUGCUUUUU		673-693	AAAAGCAGCACAUAAUUUCAAGC		671-693
AD-1660238	UGUCAGUUUCUUGCCAACUACU		700-720	AGUAGUUGGCAAAGAUCUGACAUG		698-720
AD-1660260	CGUGUUC AUGACUUUCUUCU		722-742	AGGAAGAAGUCAUGAACACGAA		720-742
AD-1660293	CUUUGUAUUAGAGCUUUUCUCU		755-775	AGAGAAAAGCUCUAAUACCAAGGA		753-775
AD-1660349	GCCAUUUUGCCCGAGUUUUAU		811-831	AUAAAACUCGGGCAAAAUGGCUG		809-831

AD-1660376	AUAGCCGAUCCUGUAACUU	844-864	AAGUACAGGAUUCGGCUUAUUU	842-864
AD-1660402	GUCAGAUUAUGUCUCUU	870-890	AAGAGACAUAAUCAUCUUGACCC	868-890
AD-1660430	UUCUUGUUC AUGCUCACAGUU	898-918	AACUGAGCAUGAACAAAGAACCC	896-918
AD-1660462	AUCCUUCUCCUCAAACAGUU	931-951	AACUGUUUUGAGGAGAAAGGAUCA	929-951
AD-1660484	AGCAGUAUCUUCUAAGGUUUU	953-973	AAAACCUUAGAAAGUAUCUCGUGU	951-973
AD-1660523	UGUGUCCAAGAGAAUUGAACU	992-1012	AGUUCAAUUCUCUUGGCACACAUU	990-1012
AD-1660558	GGCAGUUUUAUCUCUCUAAAU	1027-1047	AUUUAGAGAGAUAAAACUCGCCAG	1025-1047
AD-1660580	GAUCAGCAUGGAUAUUGAACU	1049-1069	AGUUCAAUAUCCAUJGCUGAUCAU	1047-1069
AD-1660597	ACAAGUUAUACCCUAAGUUU	1067-1087	AAACUUAAGGUAAUAACUUGUUC	1065-1087
AD-1660639	GUACAUCUUCUUUGAACAAU	1109-1129	AUUUGUUCAAAGAAAGAUUGUACUU	1107-1129
AD-1660663	ACAGAAUCUACACUCUCAUUU	1134-1154	AAUGAGAGUGUAGAUUCUGUCU	1132-1154
AD-1660693	AUCACAUCUCCUGUAGUGACU	1164-1184	AGUCACUACAGGAGAUUGUGAUAG	1162-1184
AD-1660735	UGUUGUAGACGUGAACCUAUU	1206-1226	AAUAGGUUCACGUCUACAACAAU	1204-1226
AD-1660765	AAUAACCAGAAUUGUGAUUCU	1236-1256	AGAAUCACAUUUCUGGUUAUUUC	1234-1256
AD-1660805	UAAACCGAGAAAGAAAAGUUU	1276-1296	AAACUUUCUUUCUGGGUUUAUC	1274-1296
AD-1660822	UUGAGGUUAUAAAACCCUUAU	1294-1314	AUAAGGGUUUUUAUAAACCUCAACU	1292-1314
AD-1660840	UAGUGGCUGAAACAGAUACCU	1312-1332	AGGUUUCUGUUUCAGCCACUAAAG	1310-1332
AD-1660844	ACAGAGCUACAUIUUGUGGUUU	1336-1356	AAACCACAAUUGUAGCUCUGUUU	1334-1356
AD-1660874	CCUUAUCUGAUACUUAUCAU	1366-1386	AUGAUGAAGUAUCGAGUAAGGAG	1364-1386
AD-1660909	GAAACCUGAAAUUGAACUUCU	1401-1421	AGGAAGUUCAAUUUCAGGUUCCU	1399-1421
AD-1660949	AAGAAUGUCUACAGAUACUUU	1441-1461	AAAGUAUCUGUAGACAUUCUUCA	1439-1461
AD-1660967	UUGGGAUUGCAGAGAAAGGUU	1459-1479	AAACUUUCUCUGCAUUCCCAAGU	1457-1479
AD-1660985	GUGCAAAAUUCCUUAGUGAUU	1477-1497	AAUCACUAAGGAAUUUUGCACC	1475-1497
AD-1661014	AUCCAGUUAGUCAAUUGCUAAU	1506-1526	AUUAGCAUUGACUAAUCUGGAUGA	1504-1526
AD-1661057	AAACUCUGAUGGAAACUCAU	1549-1569	AAUGAGUUUCCAUCAGAGUUUCC	1547-1569
AD-1661092	AUUCGCCGACAGUUAUUUCU	1584-1604	AGAAAAGUAACUGUCGGCGAAUAG	1582-1604
AD-1661120	UUUCAGAACCUUCUUCUCUCU	1612-1632	AGAGAGAAAGAGGUUCUGAAAGC	1610-1632
AD-1661153	ACAGGGAUUAUAUUACUCCU	1645-1665	AGGAGUAAUUAUAAUCCUUGUAA	1643-1665
AD-1661186	CUUGUUGUGAGAAUGUUUAUUU	1678-1698	AAUAACAUUCUCACAAACAAGCU	1676-1698

AD-1661258	CAACAACAGAAAGGUUGUCUUU	1771-1791	AAAGACAACCUUCUGUUGGCC	1769-1791
AD-1661300	GCAGAGCAAUAGGUUCUUGGUU	1813-1833	AACCAAGACCUAUUUGCUCUGCAG	1811-1833
AD-1661378	UUCACGUGCUUGUGACUCUU	1891-1911	AAGAGUCACAAGCACGUGGAAGA	1889-1911
AD-1661429	AAGGUUCGCAGUGAUAAAGU	1942-1962	ACUUUAUCACUGCGAACCCUUCA	1940-1962
AD-1661459	ACAGCACUAGCAGAUUUGCAU	1972-1992	AUGCAAAUCUGCUAGUGCUGUCA	1970-1992
AD-1661481	UCUACAGAAACUUCAUACAAU	1994-2014	AUUGUAUGAAGUUUCUGUAGACG	1992-2014
AD-1661513	GCAACUUUAUUAUCCGUUUCU	2026-2046	AGAAACGGAUUAAGGUUGCGU	2024-2046
AD-1661528	GAUGAACAUGAUUUCAAAGGU	2072-2092	ACUUUGAAAUCAUGUUCAUCCC	2070-2092
AD-1661562	CUUUCAAAACUUCACGAGUAU	2106-2126	AUACUCGUGAAGUUUUGAAAGUG	2104-2126
AD-1661584	UCCCUGAAAUGCAGAUUCUAU	2128-2148	AUAGAAUCUGCAUUCAGGGAAA	2126-2148
AD-1661602	UAGCCGUUAGUGGUAACUAUU	2146-2166	AAUAGUUACACAUACCGGCUAGA	2144-2166
AD-1661620	AUUGUACUGACAAGAAACCUU	2164-2184	AAGGUUUCUUGUCAGUACAUAAG	2162-2184
AD-1661639	UGCUGCUAUAAAUUGGAUAGU	2183-2203	ACUAUCCAAUUUAJAGCAGCAGG	2181-2203
AD-1661670	UCUGUUGUUUGAAGCUGUU	2217-2237	AACAGCUUCACAAAACAACAGAUU	2215-2237
AD-1661705	UGUCAGAGAAGUAUUAAGAU	2252-2272	AUCUUUAUACUUCUCUGACAAC	2250-2272
AD-1661748	GUCAACAUUAACAAGAAUUUU	2295-2315	AAAAUUCUUGUUAAUGUUGACCU	2293-2315
AD-1661856	AUGCAGCACAGAAUGUUGGUU	2404-2424	AACCAACAUCUGUGCUGCAUCC	2402-2424
AD-1661874	GUAGUUCAAACUGUAUUACUU	2422-2442	AAGUAAUACAGUUUGAAACUACCA	2420-2442
AD-1661892	UUUAAUGGAAGCAAGUGGUCU	2441-2461	AGACCACUUGCUUCCAUAUAAAGU	2439-2461
AD-1661910	UCCCCAAAUGAAGAUUUUAUU	2459-2479	AAUAAAUCUUCAUUUGUGGGACC	2457-2479
AD-1661942	CCAUCUAUAGAGAUAGGAACU	2496-2516	AGUUCUAUCUCUAUAGAUAGGCA	2494-2516
AD-1661995	AGCCUUGUUUGCAGAUAGCUAGU	2549-2569	ACUAGCAUCUGCAAACAGGCUUG	2547-2569
AD-1662021	AAGGAGCAUJGCAAAAGAUAAUU	2575-2595	AAUUAUCUUUGCAUGCCUCCUUGA	2573-2595
AD-1662094	UGUCAAAGGUCACAUGAUUCU	2690-2710	AGAAUCAUGUGACUUUUUGACAAG	2688-2710
AD-1662115	CAACAGGUCGAAGAUCAAUUU	2711-2731	AAUUGAUUCUUCGACCUGUUGUG	2709-2731
AD-1662159	CCAAGAAGACAGCCUGAAUAU	2755-2775	AUAUUCAGGCUGUCUUCUUGGUG	2753-2775
AD-1662184	ACAGUUCUGAACUUGGAACAUU	2780-2800	AAUGUCCAGUUCAGAACUGUCG	2778-2800
AD-1662202	AUGGGCAUUGGGUUCUAAAGU	2798-2818	ACUUUAGAACCCAAUUGCCCAUGU	2796-2818
AD-1662220	AGGACUAAACAUAAAUUCUGUU	2816-2836	AACAGAUUUUAUGUUGUAGUCCUUU	2814-2836

AD-1662244	AAGCUCAAUGCAUUUGUCUUGU	2845-2865	ACAAGACA AU GCAUUGAGCUUUU	2843-2865
AD-1662275	UAGAUGUGAUCACUGAGACAU	2876-2896	AUGUCUCAGUGAUCACAUUAUU	2874-2896
AD-1662297	CUUGUUUUUGGCUCUUUCAU	2900-2920	AUGAAAAGAGCCAAAACCAAGUG	2898-2920
AD-1662320	AGGUCUCAGGUUCUUUCCAUAU	2923-2943	AAUGGAAAAGAACCUGAGACCUCU	2921-2943
AD-1662349	UCAGAUCUGAACACAGUUUAU	2952-2972	AUAAACUGUGUUCAGAUUCUGAGG	2950-2972
AD-1662368	AGUGCUUUACAUGCUGUGCUU	2971-2991	AAGCACAGCAUGUAAAGCACUAAA	2969-2991
AD-1662386	CUCUUUGAAGAGAUUUCACU	2989-3009	AGUUGAAAUCUCUUCAAAGAGCA	2987-3009
AD-1662405	AGAAUAUUUGUAUGUUAAAGCU	3010-3030	AGCUUUAACAUACAUAUUUCUUG	3008-3030
AD-1662428	AGAGAUGGUAUUCUACAGCUU	3033-3053	AAGCUGUAGAUUACCAUCUCUGA	3031-3053
AD-1662450	CCUCUGAAGGCAAAUAUAAGU	3055-3075	ACUUAUAUUUGCCUUCAGAGGUG	3053-3075
AD-1662489	UUCUUGAAGUUC AUGGUGAUU	3098-3118	AAUCACCAUGAACUUC AAGAAUU	3096-3118
AD-1662508	UCAGUGCAAUUGACCUUCUCU	3117-3137	AGAGAAGGUCAAA UGCACUGAUC	3115-3137
AD-1662537	UGCCAGUUUGAAA AUGGAUUUU	3146-3166	AAA AUCCA UUUUCAACUGGCAGG	3144-3166
AD-1662550	UAUACUGUAGCUGAUGAAAACU	3172-3192	AGUUUCAUCAGCUACAGUAUAAU	3170-3192
AD-1662568	ACUCCUGAUUUUGUAGUUAAU	3190-3210	AUUAACUACAAA AU CAGGAGUUU	3188-3210
AD-1662590	GGGAUGUAGAACUUC AAGAAU	3222-3242	AUUCUUGAAGUUCUACAUC CCG	3220-3242
AD-1662618	UAAGUUCUAAGUUC AUGUUUU	3250-3270	AAA CAUGAACUUAGAACUUAAGC	3248-3270
AD-1662649	CUGGUCUAUUUUGAUUUUUGGU	3293-3313	ACCAAAAUCAAA AUAGACCAGCA	3291-3313
AD-1662650	GUAAUCAGCAUUAUUCUUCAU	3316-3336	AUGAAGAAUAAUUGCUGAUUACCC	3314-3336
AD-1662660	UGUUUUCUUC AAGGGAAGAAU	3346-3366	AUUCUUC C UUGAAGAAAACAGG	3344-3366
AD-1662680	ACACUCUUAUUC CCAAACUUAU	3366-3386	AUAGUUUGGGAUUAAGAGUGUUU	3364-3386
AD-1662698	UACAGAAUA AUGUGUUAACU	3384-3404	AGUUUAAACAUUAUUCUGUAGU	3382-3404
AD-1662717	CAUGC AAAUAGUUCUUAUCAU	3403-3423	AUGAUAGAACUUAUUAGCAUGUU	3401-3423
AD-1662743	CAAAUCACUGUAUUUAUCUCU	3429-3449	AGAGAUAAUAACAGUGAUUUUGUU	3427-3449
AD-1662783	AGGCCUUUUUGUUAAAUAUAU	3469-3489	AUAUUAUUAAAACA AAAAGGCCUCU	3467-3489
AD-1662806	UUUGCUUAACAUGUCUUUCU	3517-3537	AGAAAAGACAUGUUUAAGCCAUC	3515-3537
AD-1662829	AUAGGCUUUUAAGAAACACU	3540-3560	AGUGUUUCUUA AAAAGCCUAAUGC	3538-3560
AD-1662847	ACAGGGUUUUGUAUUCUUUAU	3558-3578	AUAAAAGAAUACAAAACCCUGUGU	3556-3578
AD-1662874	AGAGCUCUUA AUGUUGCUUAU	3589-3609	AUAAGCAACAUAU AAGAGCUCUGA	3587-3609

AD-1662904	ACUGUCAAGUACAAGCAAGAU	3619-3639	AUCUUGCUUGUACUUGACAGUCA	3617-3639
AD-1662932	UUAGAAAUCAUUGUAGAAACU	3647-3667	AGUUUCUACAAUGAUUUUCUAAAGG	3645-3667
AD-1662973	AUGUCUCUAAGCCAAACUUUAU	3689-3709	AUAAAAGUUGGCUUAGAGACAUGG	3687-3709
AD-1663013	AAACUGUAUCAAAUCUGUAUU	3758-3778	AAUACAGAUUUUGAUACAGUUUAC	3756-3778
AD-1663030	UGUAAUAAACUUUUGCUAGU	3782-3802	ACUAGCAUAAGUUUUAUUACAAC	3780-3802
AD-1663053	AUUGGAAGUUGUUCAGAAAUU	3805-3825	AAUUUCUUGAACACUUCCAAUAA	3803-3825
AD-1663076	CUUGUGUACUGAUAAAUAUCU	3834-3854	AGUAUUUAUCAGUACACAAAGUU	3832-3854
AD-1663110	GAGAAUAUUGUUCUUUAUU	3868-3888	AUUAAAGAAACAUAUCUUUCUCUG	3866-3888
AD-1663167	GAAAGGAAAGUCAGCUUUCU	3927-3947	AGAAAGCUGACUUUCCCUUUCAU	3925-3947
AD-1663217	CUAGAACUGUGCCUAGUUCU	3977-3997	AGGAACUAGGCACAGUUCUAGGG	3975-3997
AD-1663252	GGUGACUGAAACAUCACACU	4012-4032	AGUGUGAAUGUUUCAGUCACCAA	4010-4032
AD-1663293	GAAAGCUCUUAGCUUUUCUCAU	4053-4073	AUGAGAAAGCUAAGAGGCGUUCGU	4051-4073
AD-1663301	CAGAGUUUUGAAUCUUUAUU	4082-4102	AUUAAAGAUUCAUAUACUCUCGU	4080-4102
AD-1663334	UUACAUCUAUUGCUUCAAGUU	4154-4174	AACUUGAAGCAUUAGAUGUAUA	4152-4174
AD-1663357	GCUCUUGGAAAGCUGAUGAUU	4177-4197	AAUCAUCAGCUUUCCAAGAGCAA	4175-4197
AD-1663378	UCUCUUGUAGAAGAUGGACUU	4198-4218	AAGUCCAUCUUCUACAAGAGACA	4196-4218
AD-1663439	CUUAGUGAUUGUGUCUGCAUU	4259-4279	AAUGCAGACACAAUCACUAAAGAG	4257-4279
AD-1663464	UUGUGGAAAGAUUUACCUUUUU	4284-4304	AAAAGGUAAAUCUUCCACAAUA	4282-4304
AD-1663486	GUUGUACGUAAAGCUUAAAUU	4306-4326	AAUUUAAAGCUUUACGUACAACAG	4304-4326
AD-1663505	UUGCUUUUGUUGUGACUUUUU	4325-4345	AAAAGUCCACAAACAAAAGCAAUU	4323-4345
AD-1663535	CUUUUCUGAGCUUUUCAUGU	4355-4375	ACAUGAAAAGCUCAGAAAAAGUC	4353-4375
AD-1663583	UUCAUUUUAGUGACUGUAAUU	4403-4423	AAUUACAGUCACUAAAAUGAACA	4401-4423
AD-1663612	GCUGGAUUAAUGUUUUUGUACU	4432-4452	AGUACAAAACAUAUUAUCCAGCAA	4430-4452
AD-1663641	UUCAGUAAAUAUUGAAAAGGU	4498-4518	ACCUUUUCAUUUUUACUGAAAC	4496-4518

Table 3. Modified Sense and Antisense Strand Sequences of HMGCR dsRNA Agents

Duplex Name	Sense Sequence 5' to 3'	SEQ ID NO:	Antisense Sequence 5' to 3'	SEQ ID NO:	mRNA target sequence	SEQ ID NO:
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AD-1659648	gscscuucAfgUfGfAfgaucuggaguL96		asCfsuucAfgAfuUcaCfuAfgagcscsa		UGGCCUCUAGUGAGAUUCUGGAGG
AD-1659675	gsgsauucUfgUfAfgfcauaguuL96		asAfscauUfgUfAfgcuaCfaGfaaucsusu		AAGGAUUCUGUAGCUACAAUGUU
AD-1659693	gsusugicAfaGfAfcfuuuucgaauL96		asUfsucgAfaAfaAfgucUfuGfacaacsasu		AUGUUGUCAAGACUUUUUCGAAU
AD-1659762	gsasugicCfaUfGfAfacaguuuuauL96		asUfsaaaCfaUfGfuucaUfgGfacauesasu		AUGAUGUCCAUGAACAUUUUUUAC
AD-1659780	usaseuggUfaAfcAfaaaagauuuL96		asAfsgauCfuUfaAfuuuUfaCfcaguasasa		UUUACUGGUAAACAAUAAAGAUUCUG
AD-1659812	asusgaauGfuCfcAfaaaguugaauL96		asUfsucaAfaCfuUfuuggAfcAfuucausasa		UUUUGAAUGUCCAAAAGUUUUGAAG
AD-1659843	asgsaaguGfaCfaUfuuaauuuuuL96		asAfsгааUfuUfaaauUfcAfcugcuscasa		UGAGCAGUGACAUAUAAAUUCUG
AD-1659859	csusgacaAfuAfaCfacagauuuL96		asAfsugcAfuCfGfuuuAfuUfucaggsasa		UUCUGACAAUAAACACGAUGCAUA
AD-1659887	usgsuauUfuUfAfcfuuccaguuuuL96		asGfsaacUfgGfAfguaAfaUfaucasgsg		CCUGUAUUAUUACUUCUCCAGUUUCC
AD-1659914	usasegucAfaCfuUfggaucuuuuL96		asUfsuuuGfaUfcfaagUfuGfaguasasa		UUUACGUCAAACUUGGAUCAAAA
AD-1659950	cscsuuuuUfaCfaUfuuuuuuuuuL96		asUfsugaGfaAfaaauUfgAfaaggsesc		GGCCUUUUCACAAUUUUUCUCAAG
AD-1659973	usuguaUfcAfgUfacaguuuuuuL96		asGfsacaAfcUfGfuacuGfaAfuacaasasa		UUUUGUAUUCAGUACAGUUUGUCA
AD-1659991	uscsaauAfcUfUfcfuuaagaaauL96		asUfsuuuUfcUfAfgaaGfuGfaaugasasa		UGUCAUUCACUUCUUAGACAAAG
AD-1660027	asusgaagCfuUfUfgfccuuuuuuuuL96		asGfsaaaAfaGfGfcaaaAfcGfuucaususc		GAAUGAAGCUUUUGCCCUUUUUUCC
AD-1660047	csusaauUfuGfAfuugaccuuuuuuL96		asGfsaaaGfgUfcfaucAfaAfguagsgsa		UCCUACUUUUUGAUUGACCUUUUCC
AD-1660073	asasgcacAfuUfAfgfcaaaaguuuuuuL96		asCfsaaaCfuUfUfgcuaAfuGfugcuuugsc		GCAAGCACAUUAGCAAAGUUUUGC
AD-1660102	cscsaauCfaCfaGfgaagaaguuuuL96		asUfsacuUfcAfuUfcugUfgAfguuuggsasa		UUCCAACUCACAGGAUGAAGUAAA
AD-1660136	uscsuggAfaUfGfcauuuuuuuuL96		asCfsuaaAfaUfUfgcuaUfuCfcacagsgsc		GCUCGUGGAAUUGGCAAUUUUAGG
AD-1660176	gscscuucGfuUfGfAfaugcuuuuuuuL96		asAfscaaGfaCfaAfuucaAfcAfaaggsasu		AUGCUCUUGUUGAAUGUCUUGUG

AD-1660211	ususaaaUfuAfuGfugcugcuuuuL96		asAfsaagCfaGfCfacauAfaUfuucaagsc		GCUUGAAAUAUGUGCUCUUUG
AD-1660238	usgsucagUfuCfuUfGfcaacuL96		asGfsuagUfuGfGfcaagAfaCfugacausg		CAUGUCAGUUUUGCCAAACUACU
AD-1660260	esgsuguuCfaUfGfAfeuuuuuuL96		asGfsgaaGfaAfaGfuaUfGfAfacacsasa		UUCGGUUCUAGACUUUCUUCCCC
AD-1660293	esusugguAfuUfAfgfagcuuuuL96		asGfsagaAfaGfCfucuaAfuAfeccaagsgsa		UCCUUUGUAUUAGAGCUUUUCUCG
AD-1660349	gscseuuUfuGfCfCfcgaguuuuuuL96		asUfsaaaAfcUfCfGfGgeAfaAfaugcscusg		CAGCCAUUUUGCCCCGAGUUUUUAG
AD-1660376	asusaaagCfzAfaUfCfcuguaacuL96		asAfsuuAfcAfGfgauuCfGfGfuuuuuuuu		AAAUAGCCGAAUCCUGUAACUC
AD-1660402	gsuseaagAfuGfAfuUfuaugucuuL96		asAfsagAfcAfuAfaucAfuCfuuagcscsc		GGGUCAAAGAUAUUAUGUCUCUA
AD-1660430	ususcuuUfuCfaUfGfCfaguuL96		asAfsucgUfgAfgfcaugAfaCfaagaascsc		GGUUUUUUUUAUGCUCACAGUC
AD-1660462	asuscuuCfuCfCfUfcaaaacaguuL96		asAfsucgUfuUfGfGfagAfgAfggausasa		UGAUCCUUUCUCCUCAAAAACAGUA
AD-1660484	asgsacagaUfaCfuUfCfuaaguuuuL96		asAfsaacCfuUfAfgaagUfaUfCugcsgsu		ACAGCAGUAACUUCUUAAGGUUUC
AD-1660523	usgsugucCfaAfgfAfgaauugaacuL96		asGfsmucAfaUfCfucuuUfgGfCfacacausu		AAUGUGUCCAAGAGAAUUGAACC
AD-1660558	gsgscaguUfuUfAfuUfCfucuaaaL96		asUfsmuaGfaGfAfguaAfaAfcugccsag		CUGGCAGUUUUUAUCUCUCUAAAA
AD-1660580	gsasucagCfaUfGfGfauuugaacuL96		asGfsmucAfaUfAfucaUfgCfugaucsasu		AUGAUCAGCAUGGAUAUUGAACA
AD-1660597	ascsaaguUfaUfUfAfcuuuaguuL96		asAfsacuUfaGfGfguaaUfaAfcuuususc		GAACAAGUUUAUUACCCUAAGUUU
AD-1660639	gsusacuCfuUfCfUfuuuagaaL96		asUfsmugUfuCfaaagaAfgAfcuuacsusu		AAGUACAUCUUCUUUUUGAACAAC
AD-1660663	ascsagaaUfuUfAfcfucuuuuL96		asAfsaugAfgAfgfuaGfaUfucuguscs		AGACAGAAUUCUACACUCUCAUUA
AD-1660693	asuscacaUfuUfCfCfuguaugacuL96		asGfsmucCfuAfcfaggaGfaUfugausasg		CUAUCACAUCUCCUCUGUAGUGACA
AD-1660735	usgsuuuAfgAfcfGfgaaccuuL96		asAfsuagGfuUfCfCfagcuCfaAfeaacasasu		AUUGUUUGUAGACGUGAACCUCUAUG
AD-1660765	asasuaacCfaGfAfauguuuuL96		asGfsmucCfaCfaUfuuuUfgGfuuuuususc		GAAAUAAACCAGAAAUGUGAUUCA

AD-1660805	usasaaccGfaGfAfAfaaaaaguuuL96		asAfsacuUfuUfCfuuuUfcGfguuuuasusc		GAUAAACCGAGAAAAGAAAAGUUUG
AD-1660822	ususgaggUfuAfuAfaaacccuuuL96		asUfsaagGfgUfuUfuuuuAfaCfcucaaacsu		AGUUAGGGUUUAUAAAACCCUUAG
AD-1660840	usassggCfuGfAfAfacaguuuuL96		asGfsguaUfcUfGfuuuUfcGfcacuuaasg		CUUAGUGGCUGAAAACAGAUACCC
AD-1660844	ascsgagCfuAfcAfAfuuuuggguuuL96		asAfsaccAfcAfAfauguAfgCfueigususu		AAACAGAGCUACAUUUUGUGGUUG
AD-1660874	escsuuacUfcGfAfUfacuucauuL96		asUfsgauGfaAfGfuuaUcGfaGfuuaagsasg		CUCCUUACUCGAUACUUCAUCAG
AD-1660909	gsasaaccGfaAfAfUfugaauuuL96		asGfsgaaGfuUfCfauuUfcAfgguucsesu		AGGAACCUGAAAUUUGAACUUCCC
AD-1660949	asasgaaUfUfAfcaguuuuL96		asAfsaguAfuCfUfuguagAfcAfuuuuicsa		UGAAGAAUGUCUACAGAUACUUUG
AD-1660967	ususgggaAfuGfCfAfgaagaaguuL96		asAfsccuUfuUfCfugeAfuUfcccuaagsu		ACUUUGGGAUUGCAGAGAAAAGGUG
AD-1660985	gsusgcaaAfaUfUfCfuuuaguuuL96		asAfsucaCfuAfaGfgaaUfuUfugcacsu		AGGUGCAAAAUUCCUUAGUGAUG
AD-1661014	asuscagUfuAfgUfcaauuuuuL96		asUfsuagCfaUfUfgacuAfaCfuggaugsas		UCAUCCAGUUAGUCAAUUGCUAAG
AD-1661057	asasacueUfgAfuGfgaauuuL96		asAfsugaGfuUfUfcuuCfaGfaguuuisc		GGAAACUCUGAUGGAAACUCAUG
AD-1661092	asusucgcCfGfCfAfguuuuuuL96		asGfsaaaGfuAfaCfuguCfGfcauuaasg		CUAUUCGCCGACAGUUACUUUCC
AD-1661120	ususucagAfaCfCfUfuuuuuuL96		asGfsagaGfaAfGfaaggUfuCfugaauagsc		GCUUUCAGAACCUUCUUCUCUCC
AD-1661153	ascsgaggAfuUfAfaaauuuuuL96		asGfsgagUfaAfuUfuuaAfuCfccugusasa		UUACAGGGAUUAUAAUUACUCCU
AD-1661186	csusuguuGfuGfAfGfaaunguuuuL96		asAfsauaAfcAfUfucucAfaAfaaagscu		AGCUUGUUGUGAGAAUGUUUAUUG
AD-1661258	csasacaaCfaGfAfAfgguuuuuL96		asAfsagaCfaAfcuueUfgUfuguugscsc		GGCAACAACAGAAAGGUUGUCUUG
AD-1661300	gscsagagCfaAfuAfgguuuuuL96		asAfsccaAfgAfcuuaUfgCfueugcsasg		CUGCAGAGCAAUAGGUUCUUGGUG
AD-1661378	ususccacGfuGfCfUfuguuuuL96		asAfsagUfcAfcfaagcAfcGfuggaugsas		UCUUCACGUGCUUGUGACUCUG
AD-1661429	asasggguUfcGfCfAfguuuuuuL96		asCfsuumAfuCfAfcugcGfaAfccuuicsa		UGAAGGGUUCGCAGUGAUAAAAGG

AD-1661459	ascsgacCfuAfgCfagauuu gcauL96	asUfsgcaAfaUfCfugcuAfgUfgeugusca	UGACAGCACUAGCAGAUUUUGCAC
AD-1661481	uscnuacaGfaAfaCfuucauacaauL96	asUfsuguAfuGfAfauguUfeUfguagascsg	CGUCUACAGAAACUUCUAUACAAG
AD-1661513	gscsaaccUfuUfaUfaucgguuuuL96	asGfsaaaCfGfAfuuaaAfaGfguugcsgsu	ACGCAACCUUUUAUUAUCCGUUUUCC
AD-1661528	gsasugaCfaUfGfAfuuaaagguL96	asCfscuuUfgAfaucaUfgUfuaucscsc	GGGAUGAACAUGAUUUUCAAAGGG
AD-1661562	csusuucaAfaAfcUfucacgaguauL96	asUfsacuCfGfGfaaguUfuUfgaaagsusg	CACUUUCAAACUUCACGAGUAU
AD-1661584	uscscugAfaAfuGfcagauuuuL96	asUfsagaAfuCfuUfgcauUfuCfagggasasa	UUUCCUGAAAUGCAGAUUUCUAG
AD-1661602	usasccgUfuAfgUfguuaeuuuL96	asAfsuagUfuAfcfcacuAfaCfggcuasgsa	UCUAGCCGUUAGUGGUAAACUAAU
AD-1661620	asusuguaCfuGfAfcfaagaacuuL96	asAfsggUfuCfuUfugucAfgUfacaasgs	CUAUUGUACUGACAAGAAACCUG
AD-1661639	usgsucgUfaUfaAfaungauuuL96	asCfsuauCfcAfauuuaUfaGfcagacsgsg	CCUGCUGCUAUAAAUUUGGAUAGA
AD-1661670	uscsguuGfuUfUfgfugagcuuuL96	asAfsccagCfuUfCfacaAfaAfacagasusu	AAUCUGUUGUUUGUGAAGCUGUC
AD-1661705	usgsucagAfgAfaGfuuaaagauL96	asUfscuuUfaAfuAfuAfuCfuCfugacasc	GUUGUCAGAGAAGUAUUAAAAGAC
AD-1661748	gsuscaacAfuUfaAfaaagaauuuL96	asAfsaaUfcUfUfguuaAfuGfugacscsu	AGGUCAACAUAACAAGAAUUUA
AD-1661856	asusccagCfaCfaGfaauguuuuL96	asAfsccaAfcAfuCfugUfgCfugcauscsc	GGAUGCAGCACAGAAUGUUUGGUA
AD-1661874	gsusaguuCfaAfaCfuguaauuuL96	asAfsguaAfuAfcfaguuUfgAfacuacscsa	UGGUAGUUCAAAACUGUAUUACUU
AD-1661892	ususuaaUfgAfaGfcaagguuuL96	asGfsaccAfcUfUfgcuuUfuAfuuaaagsu	ACUUUAAUGGAAGCAAGUGGUCC
AD-1661910	uscscaccAfaAfuUfGfaagaauuuL96	asAfsuaaAfuCfuUfucuuUfuGfugggascsc	GGUCCCACAAAUGAAGAUAUUUAUA
AD-1661942	cscsaucaAfuAfgfAfgauuaggaauL96	asGfsuuuUfuAfuUfucuuAfuAfgauggscsa	UGCCAUCUAUAGAGAUAGGAACG
AD-1661995	asgsccgUfuUfGfCfagaugeuuL96	asCfsuagCfaUfCfugcaAfaCfagggcususg	CAAGCCUGUUUGCAGAUUGCUAGG
AD-1662021	asasggagCfaUfGfCfaaagaauuuL96	asAfsuuaUfcUfUfugcaUfgCfuccuuusgsa	UCAAGGAGCAUGCAAAGAUAAUC

AD-1662094	usgsucaaAfaGfUfCfacaugauucuuL96		asGfsaauCfaUfGfugaeUfuUfugacasasg		CUUGUCAAAAAGUCACAUGAUUCA
AD-1662115	csasacagGfuCfGfAfaGaucaauuuL96		asAfsaauGfaUfCfuucgAfcCfuguuugsug		CACAAACAGGUCGAAGAUAUCAAUUU
AD-1662159	cscsaagaAfgAfcAfgccugauuuL96		asUfsaauCfaGfGfugcuCfuUfcuuugssug		CACCAAGAAGACAGCCUGAAUAG
AD-1662184	ascsgauuCfuGfAfafeugaaacuL96		asAfsuguUfcCfAfguucAfgAfacuucscg		CGACAGUUCUGAACUCUGGAACAUG
AD-1662202	asusggcAfuUfGfGfguucaaaL96		asCfsuuuAfgAfccecaAfuGfcccusgsu		ACAUGGGCAUUGGGUUCUAAAAGG
AD-1662220	asggscAfaCfAfuaaaucuguuL96		asAfcagAfuUfufuaugUfuAfguucusus		AAAGGACUAAACAUAAAUCUGUG
AD-1662244	asasgucAfaUfGfCfaauugcuuuL96		asCfsaagAfcAfaugcaUfuGfagcuususu		AAAAGCUCAAUGCAUUGUCUUUGU
AD-1662275	usasgaugUfgAfcfacugagacuL96		asUfsgucUfcAfgfugauCfaCfaucuaususu		AAUAGAUGUGAUCACUGAGACAG
AD-1662297	csusugguUfuUfGfGfcuucuuL96		asUfsgaaAfgAfccecaAfaAfcceagsug		CACUUGGUUUUUGGCUCUUUCAG
AD-1662320	asggscuCfaGfGfUfucuucauuL96		asAfsuggAfaAfgfaaceUfgAfgaccuscu		AGAGGUCUCAGGUUUUUUCCAUUG
AD-1662349	uscsgauuCfuGfAfcacaguuuuL96		asUfsaaaCfuGfUfguucAfgAfcuugsgsg		CCUCAGAUCUGAACACACAGUUUAG
AD-1662368	asgsugcuUfuAfcAfcugugcuuL96		asAfsagcaCfaGfCfauguAfaAfgcacusasa		UUAGUGCUUUACAUGCUGUGCUC
AD-1662386	csuscuuGfaAfgAfgauucaacuL96		asGfsuugAfaAfuCfcuUfcAfaagagsesa		UGCUCUUUGAAGAGAUUUCAAACA
AD-1662405	asgsaauUfuGfUfAfguuuaagcuL96		asGfscuuUfaAfcfaucAfaUfaucusug		CAAGAAUUAUGUAUGUUAAAAGCA
AD-1662428	asgsagauGfgUfAfafuucagcuuL96		asAfsagcuGfuAfgfaauaCfaAfcucugsga		UCAGAGAUGGUAUUCUACAGCUC
AD-1662450	cscscuugAfaGfGfCfaaaauaaL96		asCfsuuaUfaUfufugccUfuCfagaggssug		CACCUCUGAAGGCAAAUAUAAAGC
AD-1662489	ususcungAfaGfUfUfcaugugauuL96		asAfsucaCfcAfuAfgaacUfuCfaagaasusu		AAUUCUUUGAAGUUCUUGGUGAUC
AD-1662508	uscsgagCfaAfuUfGfaccuucuuL96		asGfsagaAfgGfUfcaauUfgCfacugasusc		GAUCAGUGCAAUUGACCUUCUCC
AD-1662537	usgsccagUfuGfAfaaaugauuuuL96		asAfsaauCfcAfuuuucAfaCfuggcagsgg		CCUGCCAGUUUAAAAGGAAUUAUUU

AD-1662550	usasuacuGfuAfgGfcfugaugaaacuL96		asGfsuuuCfaUfCfagcuAfcAfguauasasu		AUUUAUCUGUAGCUGAUGAAACU
AD-1662568	ascnuccuGfaUfuUfufuaguauuuauL96		asUfsuaaCfuAfcfaaaaUfcAfggagususu		AAACUCCUGAUUUUGUAGUUAAU
AD-1662590	gsgsgaugUfaGfAfafeuucaagaauL96		asUfsucuUfgAfafgnuuUfaCfaucssasg		CUGGGAUGUAGAACUUCAAAGAAG
AD-1662618	usasaguuCfuAfaGfuucauuuuuL96		asAfsaacAfuGfAfacuuAfgAfacuuasgsc		GCUAAGUUCUAAAGUUCUUGUUUG
AD-1662649	csusggucUfaUfuUfufuaguuuuugL96		asCfscaaAfaUfcfaaaaUfaGfaceagsesa		UGCUGGUCUUAUUUGAUUUUUUGGG
AD-1662650	gsusaauCfAfaUfuaucuucauL96		asUfsgaaGfaAfaUfaaagCfuGfauuacscsc		GGGUAUCAGCAUUAUUCUUCAG
AD-1662660	usgsuuuuCfuUfCfAfaaggagaauL96		asUfsucuUfcCfuuugaAfgAfaaacasgsg		CCUGUUUCUUCUCAAAGGGAAGAAA
AD-1662680	ascscacUfuAfuUfucccaacuauL96		asUfsaguUfuGfGfgaaUfaGfaguususu		AAACACUCUUAUUCUCCAAACUAC
AD-1662698	usascagaAfuAfaUfuguuuaacuL96		asGfsuuuAfaCfafeuuAfuUfeguuaasgsu		ACUACAGAAUAAUGUGUUAAACA
AD-1662717	csasugcuAfaAfuAfguucauL96		asUfsgauAfgAfcuuUfuAfgcaususu		AACAUGCUAAAAGUUCUUCUUCAG
AD-1662743	csasaauCfuUfGfUfauuuucucL96		asGfsagaUfaAfaUfaaCfuGfauuususu		AACAAUUCACUGUAUUUAUCUCC
AD-1662783	asgsagcuUfuUfGfufuuaauuuL96		asUfsauuUfuUfaaCfaAfaAfgccuscsu		AGAGGCCUUUUGUUUAAAUAUAA
AD-1662806	usugsgcuAfuAfaCfaugucuuuL96		asGfsaaaGfaCfaAfuuuAfuAfgccaasusc		GAUUGGCUUAACAUGUCUUUCA
AD-1662829	asusnaggCfuUfUfUfaagaacacuL96		asGfsugtuUfuCfuUfaaaaAfgCfuaaugsuc		GCAUAGGCCUUUUAAGAAACACA
AD-1662847	ascsgggUfuUfUfGfuaucuuuuL96		asUfsaaaGfaAfaUfaaaAfaCfcucugusgu		ACACAGGGUUUUGUAUUUCUUUAC
AD-1662874	asgsagcuCfuUfAfaUfuguuuucuuL96		asUfsaagCfaAfcfaaaAfgAfgcucugsua		UCAGAGCUCUUAAAUGUUGCUUAG
AD-1662904	ascngucAfaGfUfaAfaagcaagauL96		asUfscuuGfcUfUfuguaCfuGfacagusesa		UGACUGUCAAGUACAAGCAAGAC
AD-1662932	ususaagAfuCfaUfuguaaacuL96		asGfsuuuUfuAfaUfaaagAfuUfucuaasgsg		CCUUAAGAAAUCUUAUUGUAGAAACA
AD-1662973	asusggcuCfuAfaGfccaacuuuuL96		asUfsaaaGfuUfGfgcuuAfgAfgcausgsg		CCAUGUCUCUAAAGCCAAACUUUAA

AD-1663013	asasacugUfaUfCfAfaaucuguaauL96		asAfsuacAfgAfufuugaUfaCfagnuusasc		GUAAACUGUAUCAAAUCUGUAUA
AD-1663030	usgsuauAfaAfAfCfuuaugcuagL96		asCfsuagCfaUfaFaguUfuAfuuaacasasc		GUUGUAUA AAACUU AUGCUAGU
AD-1663053	asusuggaAfgUfGfUfuaagaaauL96		asAfsuuuCfuUfGfaacaCfuUfccaasasa		UUUUUGGAAAGUGUUCAAGAAUA
AD-1663076	csusugUfaCfuUfGfauaaaauL96		asGfsuauUfuUfaFucagUfaCfacaagsusu		AACUUGUGUACUGAUAAAAUACU
AD-1663110	gsasagAfuAfAfUfguuuuuuuL96		asUfsuaaAfgAfafcauuAfuCfuucucsusg		CAGAGAAGAUAAUGUUCUUUAU
AD-1663167	gsasaaggGfaAfAfGfucageuuuuL96		asGfsaaaGfcUfGfcauuUfcCfciuucsesu		AUGAAAGGAAAAGUCAGCUUUCA
AD-1663217	csusagaaCfuGfUfGfccuaguuuuL96		asGfsгааCfuAfGfgcaCfUfucuaagsg		CCCUAGAACUGUGCCUAGUUCU
AD-1663252	gsgsugacUfgAfAfAfaucacauL96		asGfsuguGfaAfUfguuuUfcGfucacsasa		UUGGUGACUGAAACAUCACACA
AD-1663293	gsasagcUfcUfUfAfgcuuuuuL96		asUfsgagAfaAfGfcauaGfaGfciuucgsu		ACGAAACGCCUUAAGCUUUCUCAG
AD-1663301	csasagauUfaUfUfGfaaucuuuuL96		asAfsuuuAfgAfufucaUfaAfcuucgsesu		AGCAGAGUUUAUUGAAUCUUAAUU
AD-1663334	ususaauCfuAfAfUfGcuuaaguuL96		asAfsuuuGfaAfGfcauuAfgAfuguaasusa		UAUUACAUCUAAUGCUUCAAGUG
AD-1663357	gscsuuuGfgAfAfAfgcugauguuL96		asAfsucaUfcAfGfcauuCfcAfagagcsasa		UUGCUUUGGAAAGCUGAUGAUG
AD-1663378	uscscuuGfuAfGfAfgauggacuuL96		asAfsugcCfaUfCfuuuUfcAfagagcsasa		UGUCUUCUUGUAGAAGAUGGACUC
AD-1663439	csusnaguGfaUfUfGfugucugcauuL96		asAfsugcAfgAfCfcaaaUfcAfcuaagsasg		CUCUUAAGUAUUGUGUCUGCAUU
AD-1663464	usususggAfaGfAfUfuuacuuuuuuL96		asAfsaaaGfgUfAfaauCfuUfccaasasa		UAUUUGGGAAGAUUUACCUUUUC
AD-1663486	gsusuguaCfgUfAfAfgcuuaaaauL96		asAfsuuuAfaGfCfuuuuUfcUfacaacsasg		CUGUUGUACGUAAAAGCUUAAAUU
AD-1663505	usususuuUfuGfUfUfugacuuuuuuL96		asAfsaaaGfuCfAfaacAfaAfgcaasusu		AAUUGCUUUUUGUUGUGACUUUUU
AD-1663535	csusuuuuCfuGfAfgcucuuuucauuL96		asCfsaugAfaAfgcucAfgAfaaaagsusc		GACUUUUUCUGAGCUUUUCAUGG
AD-1663583	ususcuuUfuAfGfUfGacuuuuuuL96		asAfsuuuUfcUfcauuAfaAfaugaacsasa		UGUUCAUUUUAGUGACUGUAUU

AD-1663612	gscsuggaUfuAfaUfghuuuuuuuuL96	asGfsuacAfaAfaAfaUuUfaUfccagcsasa	UUGCUGGAUUAAUGUUUUUGUACA
AD-1663641	ususcaguAfaAfuAfaugaaaagguL96	asCfscuuUfuCfaAfuuuuUfuAfcugaasasc	GUUUCAGUAAAUAUUGAAAAGGA

Table 4: Unmodified Sense and Antisense Strand Sequences of HMGCR dsRNA Agents

Duplex Name	Sense Sequence (5' to 3')	SEQ ID NO	Range In NM_008255.2	Antisense Sequence (5' to 3')	SEQ ID NO	Range In NM_008255.2
AD-71284.2	UAUGAUGUCCAUGAACAUUUU		239-259	AACAUGUUCAUGGACAUCAUACA		237-259
AD-71242.2	UAUGAUGUCCAUGAACAUUUU		239-259	AACAUGUUCAUGGACAUCAUACA		237-259
AD-71285.2	CGCUGGAUAGCUGAUCCUUCU		963-983	AGAAGGAUCAGCUAUCCAGCGAC		961-983
AD-71289.2	GGCUGUCAAGUAUUUUUCUU		1145-1165	AAGAAAUAUACUUGACAGCCAA		1143-1165
AD-71281.2	AGAAAAGUUGAGGUUAUAAA		1332-1352	UUUUUAACCCUCAUUUUCUU		1330-1352
AD-71239.2	AGAAAAGUUGAGGUUAUAAA		1332-1352	UUUUUAACCCUCAUUUUCUU		1330-1352
AD-71291.2	UUUUUUGGAAGUUUAAGAA		3692-3712	UUCUUAAACACUUCCAAUAACU		3690-3712
AD-71249.2	UUUUUUGGAAGUUUAAGAA		3692-3712	UUCUUAAACACUUCCAAUAACU		3690-3712
AD-71287.2	GAGUUUGAUCUUAAUUUU		3977-3997	AAAAUUAAGAUUCAACAACUCUG		3975-3997
AD-71245.2	GAGUUUGAUCUUAAUUUU		3977-3997	AAAAUUAAGAUUCAACAACUCUG		3975-3997
AD-71290.2	AGUUUUGUAUAAUUAUAAU		4014-4034	UAUUUUUUUUUAUACAACUCUG		4012-4034
AD-71248.2	AGUUUUGUAUAAUUAUAAU		4014-4034	UAUUUUUUUUUAUACAACUCUG		4012-4034
AD-71286.2	UUUUGUAUAAUUAUAAU		4016-4036	UUUUUUUUUUUAUACAACUCU		4014-4036
AD-71244.2	UUUUGUAUAAUUAUAAU		4016-4036	UUUUUUUUUUUAUACAACUCU		4014-4036
AD-71283.2	AUUACUAAUUGUAUACAUUU		4353-4373	AAUGUAUACAUUUAGUAAUUG		4351-4373
AD-71241.2	AUUACUAAUUGUAUACAUUU		4353-4373	AAUGUAUACAUUUAGUAAUUG		4351-4373
AD-71280.2	UAAUUUGUAUACAUUUUGUUA		4358-4378	UAACAAAUGUAUACAUUUAGU		4356-4378
AD-71282.2	CCUCUACAAUUUUCUCAAA		437-457	UUUGAGAAAUUUGUAGAGGCC		435-457
AD-71288.2	AGUUGAUAAUUUGAAAGA		4394-4414	UCUUUCAUAAUUACUCAACUGG		4392-4414
AD-71246.2	AGUUGAUAAUUUGAAAGA		4394-4414	UCUUUCAUAAUUACUCAACUGG		4392-4414
Duplex Name	Sense Sequence (5' to 3')		Range In Xm_008251139.1	Antisense Sequence (5' to 3')		Range In Xm_008251139.1
AD-71273.2	UCCGAAGUUUGAAGAGGAUGU		195-215	ACAUCCUUCUCAAACUUCGGACA		193-215
AD-71231.2	UCCGAAGUUUGAAGAGGAUGU		195-215	ACAUCCUUCUCAAACUUCGGACA		193-215
AD-71270.2	UCUUUGGGCCUCCCAUCCCU		77-97	AGGGAUGGGAGGCCCAAGAGG		75-97

AD-71268.2	CCUUAGUGGCUGAAACUGACU	1250-1270	AGUCAGUUUCAGCCACUAAAGGGC	1248-1270
AD-71226.2	CCUUAGUGGCUGAAACUGACU	1250-1270	AGUCAGUUUCAGCCACUAAAGGGC	1248-1270
AD-71271.2	AUUAAAUAUCUCAAUAGCAGU	2780-2800	ACUGCAUUUGAGAUUUUUAAUUCA	2778-2800
AD-71229.2	AUUAAAUAUCUCAAUAGCAGU	2780-2800	ACUGCAUUUGAGAUUUUUAAUUCA	2778-2800
AD-71269.2	AUUUAUCUAGCUGAAAAAUCU	3585-3605	AGAUUUUCAGCUAGAUAAAAUUA	3583-3605
AD-71227.2	AUUUAUCUAGCUGAAAAAUCU	3585-3605	AGAUUUUCAGCUAGAUAAAAUUA	3583-3605
AD-71272.2	AGUUUUUUUGUAAAGCAGCUCU	3608-3628	AGAGCUGCUUACAAAAAAACUCU	3606-3628
AD-71230.2	AGUUUUUUUGUAAAGCAGCUCU	3608-3628	AGAGCUGCUUACAAAAAAACUCU	3606-3628
Duplex Name	Sense Sequence (5' to 3')	Range In	Antisense Sequence (5' to 3')	Range In
		XM_011543358.1		XM_011543358.1
AD-71293.2	AGAUCUGUGGUUGGAAUUAUA	319-339	UAUAAUUCCAACCCACAGAUUUUA	317-339
AD-71251.2	AGAUCUGUGGUUGGAAUUAUA	319-339	UAUAAUUCCAACCCACAGAUUUUA	317-339
AD-71274.2	AAGUUUGAAGAGGAUGUUUA	348-368	UAAAACAUCUUCUUAACAAUUUG	346-368
AD-71232.2	AAGUUUGAAGAGGAUGUUUA	348-368	UAAAACAUCUUCUUAACAAUUUG	346-368
AD-71294.2	GAUUUUUGAGCAGUGACAUU	360-380	AAUGUCACUGCUCAAAAACAUCU	358-380
AD-71252.2	GAUUUUUGAGCAGUGACAUU	360-380	AAUGUCACUGCUCAAAAACAUCU	358-380
AD-71292.2	CACUUCUUAGACAAAGAAUUA	528-548	UAUUUUUUUGUCUAAAGAAAGUAA	526-548
AD-71250.2	CACUUCUUAGACAAAGAAUUA	528-548	UAUUUUUUUGUCUAAAGAAAGUAA	526-548
AD-71260.3	ACAGGCUUGAAUAGAGCUUUA	549-569	UAAAGCUUCAUUCAAGCCUGUCA	547-569
AD-71218.4	ACAGGCUUGAAUAGAGCUUUA	549-569	UAAAGCUUCAUUCAAGCCUGUCA	547-569
AD-71296.2	GCUCGUGAAUUGGCAUUUA	666-686	UAAAUUUGCCAUUCCACGAGCAA	664-686
AD-71254.2	GCUCGUGAAUUGGCAUUUA	666-686	UAAAUUUGCCAUUCCACGAGCAA	664-686
AD-71298.2	UUGAAUUUUGUCUGCUUUA	763-783	UAAAGCAGCACAUAAUUUCAAGC	761-783
AD-71256.2	UUGAAUUUUGUCUGCUUUA	763-783	UAAAGCAGCACAUAAUUUCAAGC	761-783
AD-71265.2	CCGAGUUUUAGAAGAAGAAGA	911-931	UCUUUUUUUUUAAAACUCGGGC	909-931
AD-71223.2	CCGAGUUUUAGAAGAAGAAGA	911-931	UCUUUUUUUUUAAAACUCGGGC	909-931
AD-71263.2	CGAGUUUUAGAAGAAGAAGA	912-932	UUUUUUUUUUUAAAACUCGGG	910-932
AD-71221.2	CGAGUUUUAGAAGAAGAAGA	912-932	UUUUUUUUUUUAAAACUCGGG	910-932
AD-71299.2	GAGGUCUAAAGAUUUUUGUA	956-976	UACAUAAUCAUUUGACCCUCUG	954-976
AD-71257.2	GAGGUCUAAAGAUUUUUGUA	956-976	UACAUAAUCAUUUGACCCUCUG	954-976
AD-71267.2	CGAGAAAGAAAAGUUGAGGUU	1371-1391	AACCUCAUUUUUUUUUCUCGGU	1369-1391
AD-71225.2	CGAGAAAGAAAAGUUGAGGUU	1371-1391	AACCUCAUUUUUUUUUCUCGGU	1369-1391
AD-71264.2	GAAAAGUUGAGGUUUUAAAAA	1378-1398	UUUUUUAUAAACCUCAACUUUCUU	1376-1398
AD-71222.2	GAAAAGUUGAGGUUUUAAAAA	1378-1398	UUUUUUAUAAACCUCAACUUUCUU	1376-1398
AD-71275.2	GCAGAGAAAAGGUGCAAAUUA	1557-1577	UAUUUUUGCACCUCUUCUGCAU	1555-1577
AD-71233.2	GCAGAGAAAAGGUGCAAAUUA	1557-1577	UAUUUUUGCACCUCUUCUGCAU	1555-1577
AD-71278.2	UGUUGAGAAUGUUAUUGGA	1770-1790	UCCAAUAACAUUUCACACAAG	1768-1790

AD-71236.2	UGUUGAGAAUGUUAUUGGA	1770-1790	UCCAAUACAUCUCACAACAAG	1768-1790
AD-71266.2	CUUGGUGAGGUGCCAGCAGA	1917-1937	UCUGCUGCACCUCACCAAGAC	1915-1937
AD-71224.2	CUUGGUGAGGUGCCAGCAGA	1917-1937	UCUGCUGCACCUCACCAAGAC	1915-1937
AD-71297.2	CUAUGAUUGAGGUCAACAUA	2374-2394	UAUGUUGAGCCUCAAUCAJAGCC	2372-2394
AD-71255.2	CUAUGAUUGAGGUCAACAUA	2374-2394	UAUGUUGAGCCUCAAUCAJAGCC	2372-2394
AD-71277.2	UUUGAUGAAAUCUUGAAGUU	3178-3198	AAUUCAAAGAAUUUCAUCAAAC	3176-3198
AD-71235.2	UUUGAUGAAAUCUUGAAGUU	3178-3198	AAUUCAAAGAAUUUCAUCAAAC	3176-3198
AD-71276.2	GAUUUUGUAGUUAUUUAUA	3286-3306	UAUUAAAUAUAACUACAAAUCAG	3284-3306
AD-71234.2	GAUUUUGUAGUUAUUUAUA	3286-3306	UAUUAAAUAUAACUACAAAUCAG	3284-3306
AD-71258.3	AAACUCUGUAUUGUUGUAAUA	3858-3878	UAUUACAACAUAUACAGAUUUGA	3856-3878
AD-71216.4	AAACUCUGUAUUGUUGUAAUA	3858-3878	UAUUACAACAUAUACAGAUUUGA	3856-3878
AD-71295.2	AUCUGUAUUGUUGUAAUAAA	3860-3880	UUUAUUACAACAUAUACAGAUUU	3858-3880
AD-71253.2	AUCUGUAUUGUUGUAAUAAA	3860-3880	UUUAUUACAACAUAUACAGAUUU	3858-3880
AD-71279.2	AGGUUGGUGACUGAAACAUA	4097-4117	UAUGUUUCAGUCACCAACUCC	4095-4117
AD-71237.2	AGGUUGGUGACUGAAACAUA	4097-4117	UAUGUUUCAGUCACCAACUCC	4095-4117
AD-71259.4	GAGUUAUUGAAUCUUAUUUU	4174-4194	AAAAUUAAAGAUUCAAUAACUCUG	4172-4194
AD-71217.4	GAGUUAUUGAAUCUUAUUUU	4174-4194	AAAAUUAAAGAUUCAAUAACUCUG	4172-4194
AD-71261.2	UAAUAAAGAACUCCUUAUUUU	4220-4240	AAAAUAAAGGAGUUCUUUAUUUU	4218-4240
AD-71219.2	UAAUAAAGAACUCCUUAUUUU	4220-4240	AAAAUAAAGGAGUUCUUUAUUUU	4218-4240
AD-71262.2	AAUAAAGAACUCCUUAUUUA	4221-4241	UAAAAUAAAGGAGUUCUUUAUUU	4219-4241
AD-71220.2	AAUAAAGAACUCCUUAUUUA	4221-4241	UAAAAUAAAGGAGUUCUUUAUUU	4219-4241

Table 5: Modified Sense and Antisense Strand Sequences of HMGCRC dsRNA Agents

Duplex Name	Sense Sequence (5' to 3')	SEQ ID NO	Antisense Sequence (5' to 3')	SEQ ID NO	mRNA Target Sequence	SEQ ID NO
AD-71258.3	asasaucGfuAfUfAfuguuuuuuuuL96		usAfsuuuAfaAfcfauuAfaAfgauuuugsa		UCAAUUCUGUAUUGUUGUAAUA	
AD-71259.4	gsasguuaUfuGfAfAfucuuuuuuuuL96		asAfsaaUfaAfGfaucAfaUfaucucsuug		CAGAGUUAUUUGAAUCUUAUUUU	
AD-71260.3	ascsaagcUfuGfAfAfugaaeuuuuuL96		usAfsaagCfuUfcfaucAfaGfccugucsa		UGACAGGCUUGAAUGAAGCUUUUG	
AD-71261.2	usasauaaAfgAfAfucuuuuuuuuL96		asAfsaaUfaAfgfaguUfcUfuuuuuasusu		AUAUAUAAAGAACUCCUUAUUUU	
AD-71262.2	asasauaaGfaAfCfUfcuuuuuuuuL96		usAfsaaaUfaAfgfaguUfcUfuuuuuasusu		AUAUAUAAAGAACUCCUUAUUUUUG	
AD-71263.2	csgsaguUfuAfGfAfagaagaagaL96		usUfseuuCfuUfcfuuuAfaAfacuegsgsg		CCCGAGUUUAGAAGAAGAAGAA	
AD-71264.2	gsasaagUfuGfAfGfuuuuuuuuuuL96		usUfsuuuAfuAfaAfcueAfaCfuuuuucsuu		AAGAAAGUUAGGAGUUUAUAAAC	
AD-71265.2	cscsaguUfuUfAfGfagaagaagaL96		usCfsuucUfuCfuUfuuuAfaAfcuegsgsc		GCCCGAGUUUAGAAGAAGAAGA	
AD-71266.2	csusuggUfGfAfGfugccagcagaL96		usCfsugcUfgGfCfaccUfcAfcuagasc		GUCUUGGUGGAGGUGCCAGCAGC	
AD-71267.2	csgsagaAfgAfAfafaguugagguuL96		asAfsccuAfaAfcfuuuuCfuUfucuegsgsu		ACCGAGAAAGAAAGUUGAGGUU	

AD-71268.2	cscsuuagUfgGfCUfgaaacugaculL96	asGfsucaGfUfUfCfagcCfaCfuaaggsgsc	GCCUUAGUGGCUGAAACUGACU
AD-71269.2	asusuuauCfuAfgCfagcuAfgAfuuaaususa	asGfsauuUfuUfCfagcuAfgAfuuaaususa	UAAUUUAUCUAGCUGAAAAAUCU
AD-71270.2	usesuuugUfgGfCfCfueccauccculL96	asGfsggaUfgGfGfagggCfaCfaaagagsg	CCUUUUUGUGGCUCCCAUCUCCCU
AD-71271.2	asasuuuaAfaAfuUfCfuaagcagluL96	asCfsuicAfuUfGfagauUfuUfuuauscsa	UGAAUUAAAAAUCUCAUUGCAGU
AD-71272.2	asgsuuuuUfuUfGfUfaagcagcuculL96	asGfsgcUfgCfUfuacaAfaAfaaacusecu	AGAGUUUUUUUGAAAGCAGCUCU
AD-71273.2	usescgaaGfuUfUfGfagagggauulL96	asCfsaucCfuUfCfuaaAfeUfueggascsa	UGUCCGAAAGUUUGAAAGAGGAUGU
AD-71274.2	asasgnuuGfaAfgfAfggaguuuuuL96	usAfsaaaCfaUfCfucuuUfcAfaaacuusug	CAAAGUUUGAAAGAGGAGUUGUUUG
AD-71275.2	gscsagagAfaAfgfGfugcaaaaualL96	usAfsauuUfuGfCfaccuUfuCfucugesasu	AUGCAGAGAAAAGGUGCAAAAUC
AD-71276.2	gsasuuuuGfuAfgfUfuuaauuuuaL96	usAfsauaAfaUfUfuaaUfcAfaaacscasg	CUGAUUUUGUAGUUAUUUUAAUA
AD-71277.2	ususuugaGfaAfaUfufucuuagauulL96	asAfsuuCfaAfgfaauUfcAfucaaacasc	GUUUUGAUGAAAUCUUGAAAGUU
AD-71278.2	usgsuuugGfaAfgfAfguuuuuuuuL96	usCfscuuUfaAfcfaauUfcAfaaacasag	CUUGUUUGAGAAUGUUAUUUGGA
AD-71279.2	asgsuuugGfuAfgfCfugaaacauuaL96	usAfsaugUfuUfCfagucAfcCfaaacuscsc	GGAGGUUGGUGACUGAAACAUC
AD-71280.2	usasaauuGfuAfuUfAfaauuuuuuaL96	usAfsacaAfaAfuUfguauAfcAfaauuasgsu	ACUAAAUUUGUAACAUUUUUGUUA
AD-71281.2	asgsaaaaGfuUfgfAfguuuaaaaaL96	usUfsuuaUfaAfcfucuaAfcUfuuuucscsu	AGAGAAAAGUUAGAGGUUAUAAAA
AD-71282.2	cscsucuuCfaCfaAfauuuuuucuaaL96	usUfsugaGfaAfaAfaauUfgAfaagggscsc	GGCCUCUCACAAAUUUUUCUCAAG
AD-71283.2	asusuacuAfaAfuUfguaacauuuL96	asAfsaugUfaUfAfaaauUfuAfguaausug	CAUUUAUUUUUUUGUAACAUUU
AD-71284.2	usasugauGfuCfCfAfgaacauguuL96	asAfscauGfuUfCfagggAfcAfucauascsa	UGUAUGAUGUCCAUAGAAACAUUU
AD-71285.2	csgscuugAfuAfgfCfugauccuuL96	asGfsaagGfaUfCfagcuAfuCfagcgsasc	GUCGCUUGAUAGCUGAUCCUUCU
AD-71286.2	ususuuuuAfuAfaAfauaaauuaaL96	usUfsuauUfaUfUfuaauAfuAfcuaaacscsu	AGUUUUUGUUAUUUAUUAAUAAA
AD-71287.2	gsasuuugUfuGfAfaAfaucuuuuuuL96	asAfsauuUfaAfgfaauCfaCfaaucusng	CAGAGUUUUUGAAUCUUAAUUUU
AD-71288.2	asgsuuuagGfuAfaAfauuuuuuuuL96	usCfsuuuUfaUfAfaauUfcUfcaacugsg	CCAGUUAGUAUUUAUGAAAGG
AD-71289.2	gsgscuugCfaAfgfUfauuuuuuuuL96	asAfsagaAfaUfAfaaauUfgAfcagccsasa	UUGGCUUGCAAGUAUUUUUCUU
AD-71290.2	asgsuuuuGfuAfuUfAfaaauuaaL96	usAfsuuaUfuAfuUfuuuAfcAfaaacusgsg	CCAGUUUUUGUUAUUAAUAAUA
AD-71291.2	ususuauuGfgAfgfUuuuuuuuuL96	usUfscuuAfaAfcfaauCfcAfauaaacscsu	AGUUUAUUGGAAGUGUUUAAGAA
AD-71292.2	csascuuuUfuAfgfAfaaagaaauL96	usAfsauuUfuUfUfgucUfaGfaagugsasa	UUCACUUUUAGACAAGAAUUG
AD-71293.2	asgsauuuGfuGfUfguuuuuuuuL96	usAfsuuaUfuCfCfaaccAfcAfaaacususa	UAAGAUCUGUGGUUGGAAUUAUG
AD-71294.2	gsasuuuuUfuGfAfgfaguuuuuuL96	asAfsuugCfaCfUfgucUfaAfaaacscsu	AGGAUUUUUGAGCAGUGACAUU
AD-71295.2	asuscuuAfuAfuUfguuuuuuuuL96	usUfsuauUfaCfaAfaaauUfcAfaaacususu	AAAUUCUGUAUUGUUUGUAAUAAA
AD-71296.2	gscsucuuGfgAfaUfggaaauuuuuL96	usAfsaaaUfuGfCfaauUfcAfcagccsasa	UUGCUCGUGGAUUGGCAUUUUUA
AD-71297.2	csusauuUfuGfAfgfuaaauuuuuL96	usAfsaugUfuGfAfcuuCfaUfcauagcsc	GGCUAUGAUUGAGGUCAACAUA
AD-71298.2	ususuuaaUfuAfuUfgfuguuuuuuL96	usAfsaagCfaGfCfaaauAfaUfuuaagcsc	GCUUGAAUUUAUGUGCUGCUUUG
AD-71299.2	gsasggguCfaAfgfAfguuuuuuuuL96	usAfscauAfaUfCfaucUfgAfcceucscsg	CAGAGGUCACAAGAUGAUUAUGUC
AD-71216.4	AAAUUCUGUAUUGUUGUAAUAd TdT	UAUUACAACAUAUACAGAUUUGA dTdT	UCAAUUCUGUAUUGUUGUAAUA
AD-71217.4	GAGUUAUUGAAUCUUAUUUUd TdT	AAAAUUAAAGAUUCAAUAACUCUG dTdT	CAGAGUUUUUGAAUCUUAAUUUU
AD-71218.4	ACAGGUUGAAUGAAGCUUUAd	UAAAGCUUCAUUAAGCCUGUCA	UGACAGGCUUGAAUGAAGCUUUG

AD-71219.2	UAAUAAAGAACUCCUUAUUUd JpL		AAAUAAAGGAGUUUUUUUU JpLpU		AAUAAAGGAGUUUUUUUUUU		AAUAAAGGAGUUUUUUUUUU
AD-71220.2	AAUAAAGAACUCCUUAUUUAd JpL		UAAAUAAAGGAGUUUUUUUU JpLpU		UAAAUAAAGGAGUUUUUUUU		AUAAUAAAGAACUCCUUAUUUUUG
AD-71221.2	CGAGUUUAGAAAGAAGAAd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		CCCGAGUUUAGAAAGAAGAAGAA
AD-71222.2	GAAAGUUUGAGUUUAUUUUAAA JpLp		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		AAGAAAAGUUUGAGGUUAUUAAAAC
AD-71223.2	CCGAGUUUAGAAAGAAGAAd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		GCCCCGAGUUUAGAAAGAAGAAGA
AD-71224.2	CUUGGUGGAGGUGCCAGCAGAd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		GUCUUUGGUGGAGGUGCCAGCAGC
AD-71225.2	CGAGAAAAGAAAAGUUUGAGGUUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		ACCGAGAAAAGAAAAGUUUGAGGUU
AD-71226.2	CCUAGUGGCUGAAAACUGACUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		GCCUUUAGUGGCUGAAAACUGACU
AD-71227.2	AUUUUUUUAGCUGAAAAAUUCUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		UAAUUUUUUUUUUUUUUUUUUUUUU
AD-71229.2	AAUUAAAAUUCUAAUUGCAGUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		UGAAUUAAAAUUUUUUUUUUUUUUUU
AD-71230.2	AGUUUUUUUUUAGCAGCUCUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		AGAGUUUUUUUUUUUUUUUUUUUUUU
AD-71231.2	UCCGAAAGUUUAGAAAGAGGAUGUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		UGUCCGAAAGUUUUUUUUUUUUUUUU
AD-71232.2	AAGUUUUGAAGAGGAUGUUUUA JpLp		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		CAAAGUUUUGAAGAGGAUGUUUUUG
AD-71233.2	GCAGAGAAAGGUGCAAAAUUAd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		AUGCAGAGAAAGGUGCAAAAUUUC
AD-71234.2	GAUUUUUGUAGUUAAUUUUUUUA JpLp		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		CUGAUUUUUUUUUUUUUUUUUUUUU
AD-71235.2	UUUGAUAAAUUUUUUUGAAGUd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		GUUUUUGAUGAAAUUUUUUUUUUUU
AD-71236.2	UGUUGAGAAUGUUUUUUGGA JpLp		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		CUUUGUUGAGAAUUUUUUUUUUUU
AD-71237.2	AGGUUGGUGACUGAAAACAUUAd JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		GGAGGUUGGUGACUGAAAACAUUUC
AD-71239.2	AGAAAAGUUUGAGGUUAUUAAA JpL		UUUUUUUUUUUUUUUUUUUUUU JpLp		UUUUUUUUUUUUUUUUUUUUUU		AGAGAAAAGUUUGAGGUUUUUUUUU

AD-71241.2	AAUACUAAAUAUGUAUACAUUUd JpL	AAUGUAUACAAUUUAGUAAUU GpLpLp	CAAUACUAAAUAUGUAUACAUUU	
AD-71242.2	UAUGAUGUCCAUGAACAUUUd JpL	AACAUUUCAUGGACAUCAUACA JpLp	UGUAUGAUGUCCAUGAACAUUUU	
AD-71244.2	UUUUGUAUAAAUAUAAUAAA JpLp	UUUAUUUAUUUUUAUACAAAAC UJpLpLp	AGUUUUUGUAUAAAUAUAAUAAA	
AD-71245.2	GAGUUGUUGAAUCUUAAUUUUd JpL	AAAUUAAAGAUUCAACAACUCUG JpLp	CAGAGUUGUUGAAUCUUAAUUUUU	
AD-71246.2	AGUUGAGUAAAUAUGAAAGA JpLp	UCUUCAUAAUUUACUCAACUGG JpLp	CCAGUUUGAGUAAAUAUGAAAGG	
AD-71248.2	AGUUUUGUAUAAAUAUAAUA JpLp	UAUUUUUAUUUAUACAAAACUG GpLpLp	CCAGUUUUGUAUAAAUAUAAUA	
AD-71249.2	UUUAUUUGGAAGUGUUUAAGAA JpLp	UUCUAAAACACUCCAAUAAACU JpLp	AGUUUAUUGGAAGUGUUUAAGAA	
AD-71250.2	CACUUCUUAGACAAAGAAUUAd JpL	UAAUUCUUUGUCUAAGAAUGA ApLpLp	UUCACUUCUUAGACAAAGAAUUG	
AD-71251.2	AGAUCUGUGGUUGGAAUUUAUAd JpL	UAUAAUUCCAACCACAGAUUUUA JpLp	UAAGAUCUGUGGUUGGAAUUUAUG	
AD-71252.2	GAUUUUUUGAGCAGUGACAUUd JpL	AAUGUCACUGCUCAAAACAUCU JpLp	AGGAUUUUUUGAGCAGUGACAUU	
AD-71253.2	AUCUGUAUUGUUGUAAUAAAAd JpL	UUUAUUACAACAUAUACAGAUUU JpLp	AAAUCUGUAUUGUUGUAAUAAA	
AD-71254.2	GCUCGUGAAUUGGCAUUUUAd JpL	UAAAUUUGCCAUUCCACGAGCAA JpLp	UUGCUUGGAAUUGGCAUUUUUA	
AD-71255.2	CUAUGAUUGAGGUCAACAUAUAd JpL	UAAUGUUUGACCUCAAUCAUAGCC JpLp	GGCUAUGAUUGAGGUCAACAUAU	
AD-71256.2	UUGAAAUAUUGUGCUCUUUAd JpL	UAAAGCAGCACAUAAUUUCAAGC JpLp	GCUUGAAAUAUUGUGCUCUUUUG	
AD-71257.2	GAGGGUCAAGAUGAUUAUGUAd JpL	UACAUAUUCUUUGACCCUCUG JpLp	CAGAGGGUCAAGAUGAUUAUGUC	

Table 6. HMGCRC Screen in Hep3B cells.

Data are expressed as percent message remaining relative to AD-1955 non-targeting control.

Duplex ID	10nM AVG	STDEV	0.1nM AVG	STDEV
AD-71258.3	60.0	9.1	84.2	8.8
AD-71259.4	48.2	8.1	80.8	26.1
AD-71260.3	67.3	19.9	116.6	30.3
AD-71261.2	20.9	20.0	106.3	22.6
AD-71262.2	62.8	28.7	104.1	20.0
AD-71263.2	36.7	3.5	108.4	9.1
AD-71264.2	25.8	9.7	102.7	5.3
AD-71265.2	44.3	21.9	99.6	4.3
AD-71266.2	106.0	14.0	93.9	8.3
AD-71267.2	96.2	4.0	112.2	15.5
AD-71268.2	104.8	2.9	99.0	9.3
AD-71269.2	91.7	12.2	106.0	21.8
AD-71270.2	111.3	12.4	94.4	14.6
AD-71271.2	42.3	4.9	65.7	13.1
AD-71272.2	92.0	1.3	106.8	1.8
AD-71273.2	81.1	5.5	88.4	12.2
AD-71274.2	107.6	13.2	108.2	17.0
AD-71275.2	86.7	21.4	126.9	13.7
AD-71276.2	95.0	14.5	84.7	12.7
AD-71277.2	104.1	11.1	68.5	26.9
AD-71278.2	103.2	6.8	84.9	7.5
AD-71279.2	61.7	8.1	61.1	9.0
AD-71280.2	81.8	7.8	92.7	13.2
AD-71281.2	35.5	10.7	66.2	6.7
AD-71282.2	34.9	2.1	85.9	2.9
AD-71283.2	81.2	10.7	104.2	6.3
AD-71284.2	36.3	6.6	93.8	16.8
AD-71285.2	65.7	1.8	48.3	22.9
AD-71286.2	38.7	3.1	44.6	11.3
AD-71287.2	36.5	4.9	59.9	3.6
AD-71288.2	86.0	8.3	92.8	10.4
AD-71289.2	30.2	7.5	98.2	16.3
AD-71290.2	36.3	10.2	91.5	5.8
AD-71291.2	55.6	7.6	115.6	17.7

AD-71292.2	102.2	8.9	76.1	18.8
AD-71293.2	67.1	9.4	95.8	27.5
AD-71294.2	107.7	17.5	130.8	20.1
AD-71295.2	47.7	4.3	96.7	8.9
AD-71296.2	108.8	15.5	108.9	4.2
AD-71297.2	124.2	12.7	120.0	31.3
AD-71298.2	104.4	15.1	98.5	10.5
AD-71299.2	65.8	8.6	104.3	27.2
AD-1955	100.7	12.1		

Table 7. HMGCR Screen in PMH cells.

Data are expressed as percent message remaining relative to AD-1955 non-targeting control.

DuplexID	20nM AVG	STDEV
AD-71216.4	14.5	3.1
AD-71217.4	25.9	7.6
AD-71218.4	12.7	5.1
AD-71219.2	19.8	1.9
AD-71220.2	20.4	1.8
AD-71221.2	20.1	0.7
AD-71222.2	23.6	1.7
AD-71223.2	10.0	1.5
AD-71224.2	29.0	5.6
AD-71225.2	17.6	1.5
AD-71226.2	75.7	9.8
AD-71227.2	62.3	6.9
AD-71229.2	47.0	9.1
AD-71230.2	66.8	5.4
AD-71231.2	16.4	3.6
AD-71232.2	28.1	2.3
AD-71233.2	19.4	1.6
AD-71234.2	16.1	4.2
AD-71235.2	17.7	2.5
AD-71236.2	19.8	2.7
AD-71237.2	41.6	9.1
AD-71239.2	28.9	7.9
AD-71241.2	51.9	6.3
AD-71242.2	16.5	2.4

AD-71244.2	69.8	9.0
AD-71245.2	36.9	7.0
AD-71246.2	69.2	8.9
AD-71248.2	63.4	7.2
AD-71249.2	28.4	2.4
AD-71250.2	23.3	2.0
AD-71251.2	12.7	1.1
AD-71252.2	15.9	2.5
AD-71253.2	8.2	0.6
AD-71254.2	26.4	2.4
AD-71255.2	13.9	1.4
AD-71256.2	35.1	1.8
AD-71257.2	19.1	0.6
AD-1955	101.9	20.8

Table 8. HMGCR Screen in Hep3B cells.

Duplex Name	10 nM Avg	STDEV
AD-1663641.1	57.672	6.861
AD-1663612.1	53.375	8.552
AD-1663583.1	54.097	6.053
AD-1663535.1	43.796	2.203
AD-1663505.1	53.699	8.744
AD-1663486.1	48.801	4.559
AD-1663464.1	43.645	4.094
AD-1663439.1	44.314	4.533
AD-1663378.1	57.011	5.917
AD-1663357.1	46.299	5.845
AD-1663334.1	50.948	6.008
AD-1663301.1	33.240	1.257
AD-1663293.1	35.596	5.548
AD-1663252.1	44.260	4.027
AD-1663217.1	59.459	7.389
AD-1663167.1	28.335	1.391
AD-1663110.1	45.943	2.634
AD-1663076.1	35.310	2.849
AD-1663053.1	41.964	4.842
AD-1663030.1	24.776	3.601
AD-1663013.1	29.006	5.264
AD-1662973.1	24.896	6.064

Duplex Name	10 nM Avg	STDEV
AD-1662932.1	26.437	7.592
AD-1662904.1	25.914	5.084
AD-1662874.1	26.193	2.982
AD-1662847.1	25.382	5.370
AD-1662829.1	28.289	1.547
AD-1662806.1	20.660	0.855
AD-1662783.1	20.357	2.685
AD-1662743.1	22.637	4.372
AD-1662717.1	17.307	2.228
AD-1662698.1	19.148	0.291
AD-1662680.1	19.846	1.095
AD-1662660.1	28.846	3.545
AD-1662650.1	21.079	1.532
AD-1662649.1	24.594	2.898
AD-1662618.1	21.292	5.973
AD-1662590.1	24.771	1.713
AD-1662568.1	24.733	1.080
AD-1662550.1	18.181	1.845
AD-1662537.1	29.220	7.197
AD-1662508.1	26.912	2.615
AD-1662489.1	24.403	8.387
AD-1662450.1	32.388	3.379
AD-1662428.1	27.831	4.373
AD-1662405.1	23.705	0.522
AD-1662386.1	21.437	0.730
AD-1662368.1	21.441	3.637
AD-1662349.1	21.872	4.772
AD-1662320.1	22.357	2.457
AD-1662297.1	21.402	2.427
AD-1662275.1	23.901	4.464
AD-1662244.1	21.420	1.411
AD-1662220.1	16.653	1.753
AD-1662202.1	20.489	1.985
AD-1662184.1	28.673	3.376
AD-1662159.1	21.637	3.685
AD-1662115.1	14.955	2.197
AD-1662094.1	25.473	2.950
AD-1662021.1	21.369	3.510
AD-1661995.1	23.077	4.052
AD-1661942.1	38.333	2.856
AD-1661910.1	20.894	1.680
AD-1661892.1	23.620	0.784
AD-1661874.1	11.922	0.610
AD-1661856.1	16.387	0.931

Duplex Name	10 nM Avg	STDEV
AD-1661748.1	14.259	4.968
AD-1661705.1	19.433	1.117
AD-1661670.1	19.008	0.761
AD-1661639.1	25.001	1.163
AD-1661620.1	24.675	1.373
AD-1661602.1	33.383	5.536
AD-1661584.1	27.889	2.803
AD-1661562.1	22.586	1.697
AD-1661528.1	19.336	2.441
AD-1661513.1	18.882	3.194
AD-1661481.1	19.284	5.449
AD-1661459.1	20.691	1.402
AD-1661429.1	55.638	4.211
AD-1661378.1	16.234	1.956
AD-1661300.1	58.640	2.900
AD-1661258.1	49.592	8.671
AD-1661186.1	32.032	5.136
AD-1661153.1	49.286	9.737
AD-1661120.1	5.872	1.584
AD-1661092.1	15.752	4.496
AD-1661057.1	79.169	11.998
AD-1661014.1	17.541	4.790
AD-1660985.1	23.870	3.669
AD-1660967.1	29.080	11.930
AD-1660949.1	25.885	8.350
AD-1660909.1	25.629	5.659
AD-1660874.1	25.415	5.118
AD-1660844.1	18.994	5.945
AD-1660840.1	29.889	2.610
AD-1660822.1	60.084	13.194
AD-1660805.1	17.624	3.823
AD-1660765.1	24.625	1.738
AD-1660735.1	12.562	1.234
AD-1660693.1	12.422	4.888
AD-1660663.1	41.888	2.943
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AD-1660597.1	26.099	8.731
AD-1660580.1	14.531	4.122
AD-1660558.1	18.663	2.084
AD-1660523.1	70.749	19.905
AD-1660484.1	16.830	3.936
AD-1660462.1	20.470	6.184
AD-1660430.1	38.887	5.109
AD-1660402.1	26.624	6.156

Duplex Name	10 nM Avg	STDEV
AD-1660376.1	32.676	2.184
AD-1660349.1	30.184	2.731
AD-1660293.1	33.666	5.237
AD-1660260.1	19.044	4.557
AD-1660238.1	53.281	8.096
AD-1660211.1	36.870	5.118
AD-1660176.1	16.477	6.067
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AD-1660102.1	28.828	4.602
AD-1660073.1	20.125	5.474
AD-1660047.1	13.401	3.739
AD-1660027.1	14.367	3.923
AD-1659991.1	19.116	3.610
AD-1659973.1	24.443	3.730
AD-1659950.1	12.374	1.770
AD-1659914.1	31.488	7.192
AD-1659887.1	22.568	2.223
AD-1659859.1	25.551	7.236
AD-1659843.1	17.970	2.195
AD-1659812.1	20.625	3.045
AD-1659780.1	23.691	1.736
AD-1659762.1	19.868	0.752
AD-1659693.1	16.410	3.598
AD-1659675.1	19.548	4.470
AD-1659648.1	61.703	2.316

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

5

Informal Sequence Listing

SEQ ID NO: 1

>NM_000859.3 Homo sapiens 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), transcript variant 1, mRNA

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SEQ ID NO: 2

10 >NM_001130996.2 Homo sapiens 3-hydroxy-3-methylglutaryl-CoA reductase
 (HMGCR), transcript variant 2, mRNA

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15 **SEQ ID NO: 3**

>gi|767935799|ref|XM_011543357.1| PREDICTED: Homo sapiens 3-hydroxy-3-
 methylglutaryl-CoA reductase (HMGCR), transcript variant X1, mRNA
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SEQ ID NO: 4

>gi|767935801|ref|XM_011543358.1| PREDICTED: Homo sapiens 3-hydroxy-3-
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 50 CATCAGTACTGGTGACACAGGAACCTGAAAATTGAACTTCCCAGGGAACCTCGGCCTAATGAAGAATGTCT
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 55 CCCCTTTGCTTAGATGAAAAAGAATTTAGGTTCCAATGGCAACAACAGAAGGTTGTCTTGTGGCCAGCA
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 TCGTGGCCAGTTGTGCGTCTTCCACGTGCTTGTGACTCTGCAGAAGTGAAGCCCTGGCTCGAAACATCT
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 CAAGTATAGCTGGACGCAACCTTTATATCCGTTTCCAGTCCAGGTCAGGGGATGCCATGGGGATGAACAT
 60 GATTTCAAAGGGTACAGAGAAAGCACTTTCAAAACTTCACGAGTATTTCCCTGAAATGCAGATTCTAGCC
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 5 TATAGAGATAGGAACGGTGGGTGGTGGGACCAACCTACTACCTCAGCAAGCCTGTTTGCAGATGCTAGGT
 GTTCAAGGAGCATGCAAAGATAATCCTGGGGAAAAATGCCCGGCAGCTTGCCCGAATTGTGTGTGGGACCG
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 10 AATGCATTGTCTTGTGGAGGATGAATAGATGTGATCACTGAGACAGCCACTTGGTTTTTGGCTCTTTCAG
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 CTTTGAAGAGATTTCAACAAGAATATTGTATGTTAAAGCATCAGAGATGGTAATCTACAGCTCACCTCTG
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 TGTCAAGTACAAGCAAGACTGGGACCTTAGAAATCATTGTAGAAACACAGTTTTGAAAGAAAAATACCAT
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 30 GAGAGCCTCTTAGTGATTGTGTCTGCATTTGTGTTGGAAGATTACCTTTTTCTGTTGTGTAAGCT
 TAAATTGCTTTTTGTGTGACTTTTTAGCCAGTGACTTTTTCTGAGCTTTTTCATGGAAGTGGCAGTGAAAA
 ATATGTTGAGTGTTCATTTTAGTGACTGTAATTAATATCTTGTGATTAAATGTTTTGTACAATTACTAA
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35

SEQ ID NO: 5

>gi|767935803|ref|XM_011543359.1| PREDICTED: Homo sapiens 3-hydroxy-3-
 methylglutaryl-CoA reductase (HMGCR), transcript variant X3, mRNA
 TTTAATCCAGTAGACAATGAGAAACGCTGATTTGGGTCTATGGCATAATAGGAAGTAGTATGCCACATAC
 40 TGCATGTGGGAGTTCTGCAGGACAGATTGGGAAGTGCATGCGTGTAAAGACTGGGAAGACCAGTTAGGAGA
 GGCTATTGCACTGTTTTAAGTAAGACATAATAAAGCCCTGCACCAGGCGGAGAGCAGAAGGAACGCACAG
 AAGACGCAGGAGAGGCACATTTTCAGAGAGAATCCAGTATGCAATGGATGTCGCACACAAGAGAGAGAGAC
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 45 TGGTAACAATAAGATCTGTGGTTGGAATTATGAATGTCCAAAGTTTTGAAGAGGATGTTTTGAGCAGTGAC
 ATTATAATTCTGACAATAACACGATGCATAGCCATCCTGTATATTTACTTCCAGTTCCAGAATTTACGTC
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 55 CGCTGGATAGCTGATCCTTCTCCTCAAAACAGTACAGCAGATACTTCTAAGTTTTATTAGGACTGGATG
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 60 GACAGGGATAAACCGAGAAAGAAAAGTTGAGGTTATAAAAACCTTAGTGGCTGAAACAGATACCCCAAAC
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 5 CCGAGTCCCTGCAGATGGGATGACTCGTGGCCAGTTGTGCGTCTTCCACGTGCTTGTGACTCTGCAGAA
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 15 ATATATCAGCTGCACCATGCCATCTATAGAGATAGGAACGGTGGTGGTGGGACCAACCTACTACCTCAG
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 25 AGCTAAGTTCTAAGTTTATGTTTGTAAATTAATACTTCAATTTGGTGCTGGTCTATTTTTGATTTTTGGGGG
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 40 ACCTTTTCTGTTGTACGTAAAGCTTAAATGCTTTTGTGTGACTTTTTAGCCAGTGACTTTTTCTGAGC
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45 **SEQ ID NO: 6**

>gi|544437803|ref|XM_005557178.1| PREDICTED: *Macaca fascicularis*
 uncharacterized LOC101926249 (LOC101926249), transcript variant X3, mRNA
 CCGTGTCTCCGTTGACTGGAGACGGGCGGAGCACCGGCGCCGGGTTTTGGTGGCCTCTAGTGAGATCTGG
 AGGTGAGGATCCACGGATTCTGTATCTACAATGTTGTCAAGACTTTTTCGAATGCATGGCCTCTTCGTGG
 50 CCTCCCATCCCTGGGAAGTCATAGTGGGAACAGTGACACTGACCATCTGCATGATGTCCATGAACATGTT
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 TACAGTTGTCATTTCACTTCTTAGACAAAAGAAATGACAGGCTTGAATGAAGCTTTGCCCTTTTTCTACTT
 55 CTGATTGACCTTTCCAGAGCAAGTGCATTAGCAAAGTTTGGCCTCAGTTCCAACCTCACAGGATGAAGTAA
 GGGAAAATATTGCTCGTGGAAATGGCAATTTTAGTCCAACGTTTACCCTCGATGCTCTTGTGTAATGTCT
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 60 TAAGCCAAATCCTGTAACCTCAGAGGGTCAAGATGATTATGTCTCTAGGCTTGGTCTTGTTCATGCTCAC
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5 ATGAAAATGTGTCTAAGAGAATTGAACCAAGTGTTCCTCTGGCAATTTTATCTCTCTAAAATGATCAG
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SEQ ID NO: 7

>gi|160358777|ref|NM_008255.2| Mus musculus 3-hydroxy-3-methylglutaryl-
 Coenzyme A reductase (Hmgcr), mRNA
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 60 GTCAGGCGTCCGCCAGCTGGAGATCATGTGCTGCTTCGGCTGCATGTCAGTGTGGCCAACCTACTTTGTG
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SEQ ID NO: 8

>gi|40538851|ref|NM_013134.2| Rattus norvegicus 3-hydroxy-3-methylglutaryl-CoA reductase (Hmgcr), mRNA
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SEQ ID NO:10 REVERSE COMPLEMENT OF SEQ ID NO: 2

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SEQ ID NO:11 Reverse Complement of SEQ ID NO:3

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 40 GACTGTGGCACAGCTAAAGGCCTCTCTGAACAAAAGACTGAGATGAACACAGCGACTCTTT
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 AGCTTTTCCAGAACGAGCTGCAGTACATCTGTGGTGCTCAGAGCACCATGACATCCACAT
 GAGATCTTTCTAGAACACGGCACGGAAAGAACCATAGTTCCCACGCTCTGAGGAGTCTGTG
 CAAAGGACCCGACAGCTCTCTAAAGAGCCAGAAACCAAGCAGGTGCCTCACTGATC
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 50 CTGGAGAACCCAATGCCCGTGTTCAGTCCAGTATGTCAGGATGCTCAAGCTGCCTTCTT
 GGTGCATGTTCCCTGCAGATCTTGTAATTTATCTTTGATCTGTTGTGAACCATGTGACT
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 55 GGTTCGGATCTCTATAGACGGCATGGTACAGCTGATGTATAAGTCTTCATTTGTGGGACC
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5 AAGGACCCGGCTGCTGGCACCTCCACCAAGACTGATCGCTCTGCAGCCTCTGTTTCGTGCT
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GATCTTGTGTTGCGGGTGAACATGTTTCATGGACATCATAAGATAGTAAGTGTACCGT
TCCCACAATTACCTCCCAGGGATGGGAGGCCACAAAAGAGGCCATGCATACGGAAAAGTCT
TGACAACATTGTAGCTACACAGTCTTGGATCCTCCGGATCTCAATGGAGGCCACCAAG
35

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises
5 at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of any one of SEQ ID NOs:1-8, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the corresponding portion of the nucleotide sequence of any one of SEQ ID NOs:9-16.
- 10 2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HMGCR, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides
15 from any one of the antisense nucleotide sequences in any one of Tables 2-5.
3. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand
20 comprising at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.
4. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the
25 nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than two nucleotides from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.
5. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising
30 at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the sense strands in any one of Tables 2-5 and an antisense strand comprising at least 15 contiguous nucleotides differing by no more than one nucleotide from any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.
- 35 6. The dsRNA agent of claim 1, wherein the dsRNA agent comprises a sense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of

the sense strands in any one of Tables 2-5 and an antisense strand comprising a nucleotide sequence selected from the group consisting of any one of the nucleotide sequences of the antisense strands in any one of Tables 2-5.

- 5 7. The dsRNA agent of any one of claims 1-6, wherein the dsRNA agent comprises at least one modified nucleotide.
8. The dsRNA agent of any one of claims 1-7, wherein substantially all of the nucleotides of the sense strand are modified nucleotides; substantially all of the nucleotides of the antisense strand
10 are modified nucleotides; or substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand are modified nucleotides.
9. The dsRNA agent of any one of claims 1-8, wherein all of the nucleotides of the sense strand are modified nucleotides; all of the nucleotides of the antisense strand are modified nucleotides; or
15 all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides.
10. The dsRNA agent of any one of claims 7-9, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythymidine (dT)
20 nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a 2'-5'-linked ribonucleotide (3'-RNA), a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified
25 nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, a nucleotide comprising a 5'-phosphate mimic, a thermally destabilizing nucleotide, a glycol nucleic acid (GNA), a glycol nucleic
30 acid S-Isomer (S-GNA), a nucleotide comprising a 2' phosphate, and a 2-O-(N-methylacetamide) modified nucleotide; and combinations thereof.
11. The dsRNA agent of any one of claims 7-9, wherein at least one of the modified nucleotides is selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl,
35 2'-C- allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

12. The dsRNA agent of any one of claims 7-9, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, GNA, S-GNA, 3'-RNA, a nucleotide comprising a 2' phosphate, a nucleotide comprising a phosphorothioate group, and a vinyl-phosphonate nucleotide; and combinations thereof.
13. The dsRNA agent of any one of claims 7-9, wherein at least one of the modified nucleotides is a nucleotide modified with a thermally destabilizing nucleotide modification.
14. The dsRNA agent of claim 13, wherein the thermally destabilizing nucleotide modification is selected from the group consisting of an abasic modification; a mismatch with the opposing nucleotide in the duplex; a destabilizing sugar modification, a 2'-deoxy modification, an acyclic nucleotide, an unlocked nucleic acid (UNA), and a glycerol nucleic acid (GNA).
15. The dsRNA agent of any one of claims 1-14, wherein the double stranded region is 19-30 nucleotide pairs in length.
16. The dsRNA agent of claim 15, wherein the double stranded region is 19-25 nucleotide pairs in length.
17. The dsRNA agent of claim 15, wherein the double stranded region is 19-23 nucleotide pairs in length.
18. The dsRNA agent of claim 15, wherein the double stranded region is 23-27 nucleotide pairs in length.
19. The dsRNA agent of claim 15, wherein the double stranded region is 21-23 nucleotide pairs in length.
20. The dsRNA agent of any one of claims 1-19, wherein each strand is independently no more than 30 nucleotides in length.
21. The dsRNA agent of any one of claims 1-20, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.
22. The dsRNA agent of any one of claims 1-21, wherein the region of complementarity is at least 17 nucleotides in length.

23. The dsRNA agent of any one of claims 1-22, wherein the region of complementarity is between 19 and 23 nucleotides in length.

24. The dsRNA agent of any one of claims 1-23, wherein the region of complementarity is 19 nucleotides in length.

25. The dsRNA agent of any one of claims 1-24, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

26. The dsRNA agent of any one of claims 1-24, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

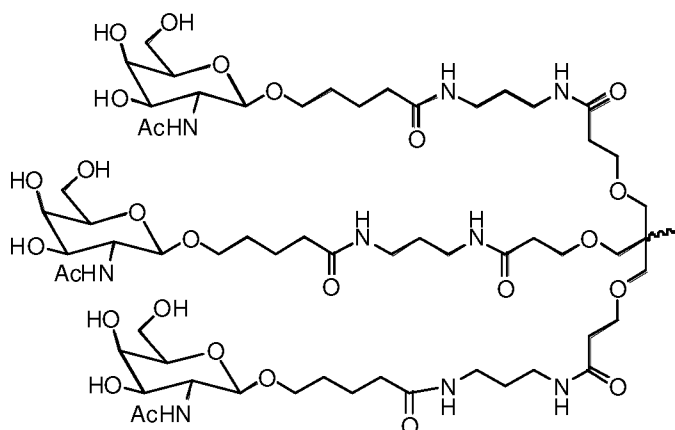
27. The dsRNA agent of any one of claims 1-26, further comprising a ligand.

28. The dsRNA agent of claim 27, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

29. The dsRNA agent of claim 27 or 28, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

30. The dsRNA agent of any one of claims 27-29, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

31. The dsRNA agent of claim 29 or 30, wherein the ligand is



32. The dsRNA agent of claim 31, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic

43. The dsRNA agent of any one of claims 1-42, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.
44. A cell containing the dsRNA agent of any one of claims 1-43.
- 5
45. A pharmaceutical composition for inhibiting expression of a gene encoding 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) comprising the dsRNA agent of any one of claims 1-43 and a pharmaceutically acceptable carrier.
- 10 46. The pharmaceutical composition of claim 45, wherein dsRNA agent is in an unbuffered solution.
47. The pharmaceutical composition of claim 46, wherein the unbuffered solution is saline or water.
- 15
48. The pharmaceutical composition of claim 45, wherein said dsRNA agent is in a buffer solution.
49. The pharmaceutical composition of claim 48, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.
- 20
50. The pharmaceutical composition of claim 49, wherein the buffer solution is phosphate buffered saline (PBS).
- 25 51. A method of inhibiting expression of a 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-43, or the pharmaceutical composition of any one of claims 45-50, thereby inhibiting expression of the HMGCR gene in the cell.
- 30 52. The method of claim 51, wherein the cell is within a subject.
53. The method of claim 52, wherein the subject is a human.
54. The method of claim 52, wherein the subject has an HMGCR-associated disorder.

55. The method of claim 54, wherein the HMGCR-associated disorder is a disorder of lipid metabolism.
56. The method of claim 55, wherein the disorder of lipid metabolism is hyperlipidemia.
- 5 57. The method of claim 56, wherein the hyperlipidemia is selected from the group consisting of mixed hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia.
58. The method of any one of claims 51-57, wherein contacting the cell with the dsRNA agent
10 inhibits the expression of HMGCR by at least 50%, 60%, 70%, 80%, 90%, or 95%.
59. The method of any one of claims 51-58, wherein inhibiting expression of HMGCR decreases HMGCR protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.
- 15 60. A method of treating a subject having a disorder that would benefit from reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-43, or the pharmaceutical composition of any one of claims 45-50, thereby treating the subject having the disorder that would benefit from reduction in HMGCR expression.
- 20 61. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-43, or the pharmaceutical composition of any one of claims 45-50, thereby
25 preventing at least one symptom in the subject having the disorder that would benefit from reduction in HMGCR expression.
62. The method of claim 60 or 61, wherein the disorder is an HMGCR-associated disorder.
- 30 63. The method of claim 62, wherein the HMGCR-associated disorder is a disorder of lipid metabolism.
64. The method of claim 63, wherein the disorder of lipid metabolism is hyperlipidemia.
- 35 65. The method of claim 64, wherein the hyperlipidemia is selected from the group consisting of mixed hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia.

66. The method of claim 60 or 61, wherein the subject is a human.
67. The method of claim 60 or 61, wherein administration of the dsRNA agent to the subject causes a decrease in HMGCR protein accumulation in the subject.
- 5
68. The method of any one of claims 60-67, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.
69. The method of any one of claims 60-68, wherein the dsRNA agent is administered to the subject subcutaneously.
- 10
70. The method of any one of claims 60-69, further comprising determining the level of HMGCR in a sample(s) from the subject.
71. The method of claim 70, wherein the level of HMGCR in the subject sample(s) is an HMGCR protein level in a blood or serum or liver tissue sample(s).
- 15
72. The method of any one of claims 60-71, further comprising administering to the subject an additional therapeutic agent for treatment of an HMGCR-associated disorder.
- 20
73. The method of claim 72, wherein the additional therapeutic agent is selected from the group consisting of statins, a bile sequestering agent, a VLDL secretion inhibitor, a lipophilic antioxidant, an acyl-CoA cholesterol acyl transferase inhibitor, a farnesoid X receptor antagonist, a sterol regulatory binding protein cleavage activating protein (SCAP) activator, a microsomal triglyceride transfer protein (MTP) inhibitor, an ApoE-related peptide, a cholesteryl ester transfer protein (CETP) inhibitor, a therapeutic antibody against HMGCR, and a combination of any of the foregoing.
- 25
74. A kit comprising the dsRNA agent of any one of claims 1-43 or the pharmaceutical composition of any one of claims 45-50.
- 30
75. A vial comprising the dsRNA agent of any one of claims 1-43 or the pharmaceutical composition of any one of claims 45-50.
76. A syringe comprising the dsRNA agent of any one of claims 1-43 or the pharmaceutical composition of any one of claims 45-50.
- 35

77. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of claims 1-43.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/038613

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/009346 A2 (MIRUS CORP [US]) 3 February 2005 (2005-02-03) example 16; table 7 -----	1-77
A	WO 2004/005460 A2 (ISIS PHARMACEUTICALS INC [US]; DEAN NICHOLAS M [US] ET AL.) 15 January 2004 (2004-01-15) page 125; table 3; sequence 217 -----	1-77

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

2 November 2022

10/01/2023

Name and mailing address of the ISA/
 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040,
 Fax: (+31-70) 340-3016

Authorized officer

Romano, Alper

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/038613

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed.
 - b. furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)).
 - accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/038613

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:
1-77 (partially)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US2022 /038613

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-77 (partially)

A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) in a cell, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HMGCR, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the targeting positions 60-82 according to table 2.

2-100. claims: 1-77 (partially)

double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of HMGCR in a cell, wherein the dsRNA agents comprises a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises a region of complementarity to an mRNA encoding HMGCR, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the other non-overlapping targeting positions according to tables 2 and 4.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2022/038613

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005009346 A2	03-02-2005	EP 1636385 A2	22-03-2006
		US 2006063731 A1	23-03-2006
		WO 2005009346 A2	03-02-2005

WO 2004005460 A2	15-01-2004	AU 2003281324 A1	23-01-2004
		US 2004006031 A1	08-01-2004
		WO 2004005460 A2	15-01-2004
