# BETA-CATENIN (CTNNB1) iRNA COMPOSITIONS AND METHODS OF USE THEREOF

## Related Applications

This application claims the benefit of priority to U.S. Provisional Applicaton No. 63/224,901, filed on July 23, 2021, and U.S. Provisional Applicaton No. 63/293,851, filed on December 27, 2021. The entire contents of each of the foregoing applications are incorporated herein by reference.

## Background of the Invention

Wnt/β-catenin signaling is an evolutionarily conserved and versatile pathway that is known to be involved in embryonic development, tissue homeostasis and a wide variety of human diseases. Aberrant activation of this pathway gives rise to the accumulation of β-catenin in the nucleus and promotes the transcription of many oncogenes such as c-Myc and CyclinD-1. As a result, it contributes to carcinogenesis and tumor progression of several cancers, including hepatocellular carcinoma, colon cancer, pancreatic cancer, lung cancer and ovarian cancer (Khramtsov AI, *et al. Am J Pathol.* 2010;176:2911–2920; Tao J, *et al. Gastroenterology*. 2014;147:690–701; Kobayashi M, *et al. Br J Cancer*. 2000;82:1689–1693; Damsky WE, *et al. Cancer Cell.* 2011;20:741–754; Gekas C, *et al. Leukemia*. 2016;30:2002–2010).

β-catenin, encoded by the CTNNB1 gene, is a multifunctional protein with a central role in physiological homeostasis. β-catenin acts both as a transcriptional co-regulator and an adaptor protein for intracellular adhesion. Wnt is the chief regulator of β-catenin, which is a family of 19 glycoproteins to regulate both the β-catenin-dependent (canonical Wnt) and -independent (non-canonical Wnt) signaling pathways (van Ooyen A, Nusse R. *Cell*. 1984;39:233–240).

In canonical Wnt pathway, Dsh, β-catenin, Glycogen Synthase Kinase 3 beta (GSK3β), adenomatous polyposis coli (APC), AXIN, and T-cell factor (TCF)/lymphoid enhancement factor (LEF) have been identified as signal transducers of the canonical Wnt pathway, in which β-catenin is a core molecule (Behrens J, *et al. Nature*. 1996;382:638–642; Peifer M, *et al. Dev Biol.* 1994;166:543–556; Rubinfeld B, *et al. Science*. 1996;272:1023–1026; Yost C, *et al., Genes Dev.* 1996;10:1443–1454). In the absence of Wnt ligands, β-catenin is kept at a low level through the ubiquitin proteasome system (UPS) which results in the ubiquitylation and proteasomal degradation of β-catenin. Upon Wnt activation or genetic mutations of Wnt components, β-catenin accumulates in the cytoplasm and then translocates into the nucleus. Consequently, it binds to other proteins, such as LEF-1/TCF4, to promote the transcription of target genes, such as Jun, c-Myc and CyclinD-1 in a tissue specific manner, most of which encode oncoproteins. As a result, aberrant high expression of β-catenin leads to various diseases including cancer.

In addition, a high-level cytoplasm expression and nuclear localization of β-catenin also induces tumorigenic traits and promotes cancer cell proliferation and survival (Valkenburg KC, *et al.* Cancers (Basel) 2011;3:2050–2079). Moreover, β-catenin promotes the progression of tumors via suppressing the T-cell responses (Hong Y, *et al. Cancer Res.* 2015;75:656–665).

Current treatments for cancer include surgery, radiation and chemotherapy. However, these methods are not fully effective and may result in serous side effects. Accordingly, there is a need in the art for alternative treatments for subjects having a CTNNB1-associated disorder, such as cancer, *e.g.,* hepatocellular carcinoma.

## 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 beta-catenin (CTNNB1). The CTNNB1 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 a CTNNB1 gene and/or for treating a subject who would benefit from inhibiting or reducing the expression of a CTNNB1 gene, *e.g.*, a subject suffering or prone to suffering from a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

Accordingly, in an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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, or 20, contiguous nucleotides differing by no more than 0, 1, 2, or 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 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 SEQ ID NO:2.

In another aspect, the present invention provides a double stranded ribonucleic acid (dsRNA) for inhibiting expression of beta-catenin (CTNNB1) 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 CTNNB1, and wherein the region of complementarity comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23 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, 3, 5, or 6.

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 nucleotides from any one of the nucleotide sequences of the sense strands in any one of Tables 2, 3, 5, or 6 and an antisense strand comprising at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 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, 3, 5, or 6.

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, 3, 5, or 6 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, 3, 5, or 6.

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, 5, and 6 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, 5, and 6.

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, 3, 5, or 6 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, 3, 5, or 6.

In one aspect, the present inventionprovides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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 three, *e.g.,* 3, 2, 1, or 0, nucleotides from any one of the nucleotide sequence of nucleotides 603-625, 1489-1511, or 1739-1761 of SEQ ID NO: 1, 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 three, *e.g.,* 3, 2, 1, or 0, nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:2.

In one embodiment, the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1548393, AD-1548488, and AD-1548459.

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 deoxythimidine (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.

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.

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).

In some embodiments, the modified nucleotide comprises a short sequence of 3'-terminal deoxythimidine 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 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 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.

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.

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.

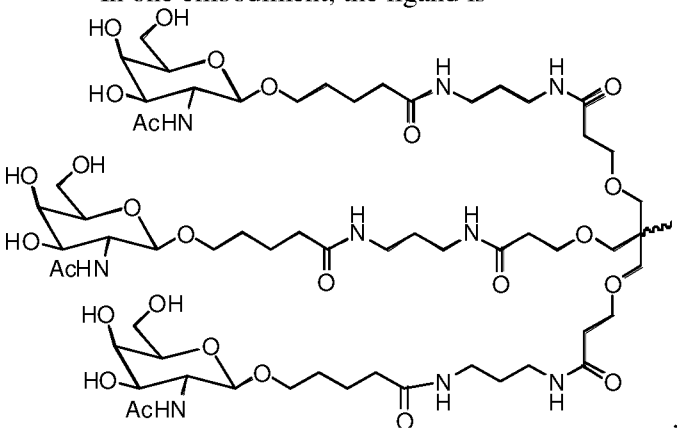
In one embodiment, the dsRNA agent further comprises a ligand.

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

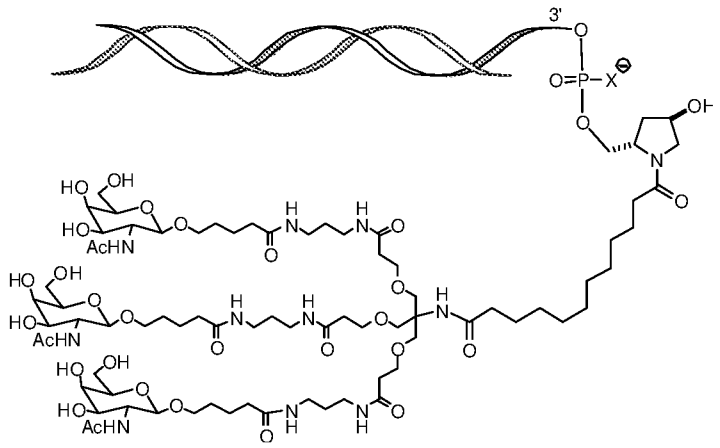
In one embodiment, the ligand is an N-acetylgalactosamine (GalNAc) derivative.

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 the 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 antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5'- UACUGUUGGAUUGAUUCGAAA -3' 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 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand comprises the nucleotide sequence 5'-UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment,the sense strand differs by no more than 4, *e.g.,* 4, 3, 2, 1, or 0, bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4, *e.g.,* 4, 3, 2, 1, or 0, bases from the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

In one aspect, the present invention provides a double stranded RNA (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) in a cell, comprising a sense strand comprising the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and an antisense strand comprising the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3',
wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

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

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

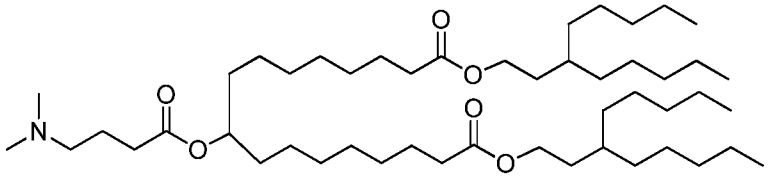
include the dsRNA agent is in a buffer solution, *e.g.,* a buffer solution comprising acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof; or phosphate buffered saline (PBS).

In one aspect, the present invention provides a pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a lipid.

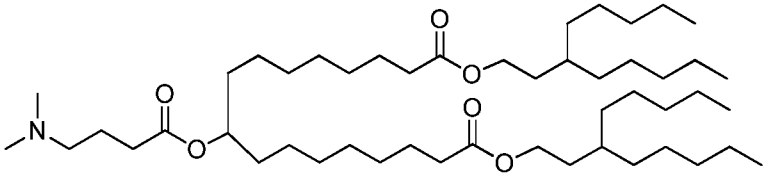
In one embodiment, the lipid is a cationic lipid.

In one embodiment, the cationic lipid comprises one or more biodegradable groups.

In one embodiment, the lipid comprises the structure



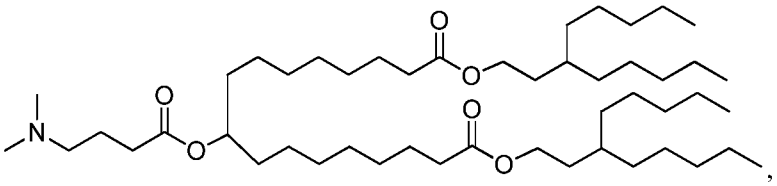
In one embodiment, the pharmaceutical composition comprises

(a) ;

(b) cholesterol;

(c) DSPC; and

(d) PEG-DMG.

In one embodiment, the ,

DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

In one aspect, the present invention provides a pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising, a dsRNA agent for inhibiting expression of a gene encoding beta-catenin (CTNNB1), comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3', and a lipid.

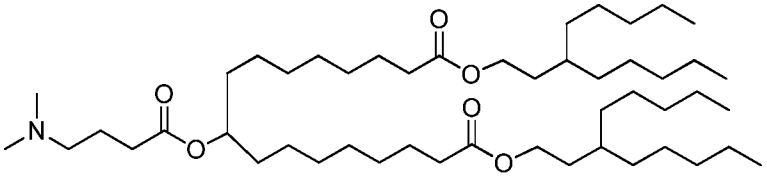
In one embodiment, the sense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, or 21, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5'- UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises at least 15, *e.g.,* 15, 16, 17, 18, 19, 20, 21, 22, or 23, contiguous nucleotides differing by no more than 3, *e.g.,* 3, 2, 1, or 0, nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

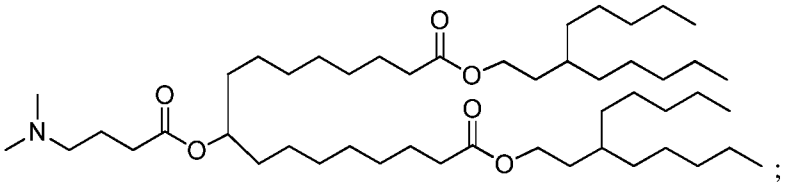
In one embodiment, the sense strand comprises the nucleotide sequence 5'-UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

In one embodiment, the sense strand differs by no more than 4, *e.g.,* 4, 3, 2, 1, or 1, bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4, *e.g.,* 4, 3, 2, 1, or 0, bases from the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3', wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

In one embodiment, the lipid comprises the structure



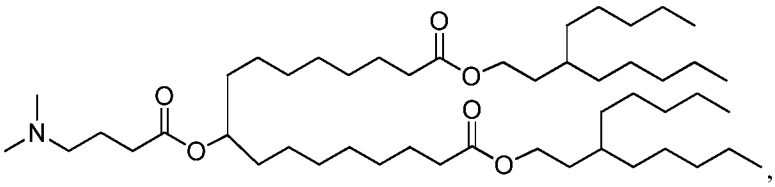
In one embodiment, the pharmaceutical composition comprises

(a) ;

(b) cholesterol;

(c) DSPC; and

(d) PEG-DMG.

In one embodiment, the , DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

In one aspect, the present invention provides a method of inhibiting expression of a beta-catenin (CTNNB1) 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 CTNNB1 gene in the cell.

In one embodiment, the cell is within a subject, *e.g.,* a human subject, *e.g.,* a subject having a beta-catenin (CTNNB1)-associated disorder, such as cancer, *e.g.*, hepatocellular carcinoma.

In certain embodiments, the CTNNB1 expression is inhibited by at least about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%. In one embodiment, inhibiting expression of CTNNB1

decreases CTNNB1 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 beta-catenin (CTNNB1) 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 CTNNB1 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 beta-catenin (CTNNB1) 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 CTNNB1 expression.

In certain embodiments, the disorder is a beta-catenin (CTNNB1)-associated disorder, *e.g.* a cancer.

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma.

In certain embodiments, administration of the dsRNA to the subject causes a decrease CTNNB1 protein accumulation in the subject.

In a further aspect, the present invention also provides methods of inhibiting the expression of CTNNB1 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 CTNNB1 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 dsRNA agent is administered to the subject intravenously.

In one embodiment, the methods of the invention include further determining the level of CTNNB1 in a sample(s) from the subject.

In one embodiment, the level of CTNNB1 in the subject sample(s) is a CTNNB1 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 chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent, an anti-neoplastic composition 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

CTNNB1 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 CTNNB1 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.

## Brief Description of the Drawings

FIG. 1A is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously administered dose of AD-1548393 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

FIG. 1B is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously administered dose of AD-1548459 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

FIG. 1C is a graph depicting the effect of a single 0.1 mg/kg or 0.3 mg/kg intravenously administered dose of AD-1548488 at Days 5, 15, and 29 post-dose. The percent of CTNNB1 mRNA remaining relative to pre-dose levels of CTNNB1 mRNA are shown.

## Detailed Description of the Invention

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a beta-catenin (CTNNB1) 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 (CTNNB1) in mammals.

The iRNAs of the invention have been designed to target the human beta-catenin (CTNNB1) gene, including portions of the gene that are conserved in the CTNNB1 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 efficacy, stability, potency, durability, and safety.

Accordingly, the present invention provides methods for treating and preventing a beta-catenin (CTNNB1)-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CTNNB1 gene.

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 a CTNNB1 gene.

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 a CTNNB1 gene. In some embodiments, such iRNA agents having longer length antisense strands may, for example, include a second RNA strand (the sense 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 (CTNNB1 gene) in mammals. Using *in vitro* assays, the present inventors have demonstrated that iRNAs targeting a CTNNB1 gene can potently mediate RNAi, resulting in significant inhibition of expression of a CTNNB1 gene. Thus, methods and compositions including these iRNAs are useful for treating a subject having a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

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 a CTNNB1 gene, *e.g.*, a beta-catenin (CTNNB1)-associated disease, such as cancer, *e.g.*, hepatocellular carcinoma, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a CTNNB1 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 a CTNNB1 gene, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of a CTNNB1 gene as well as compositions, uses, and methods for treating subjects that would benefit from inhibition and/or reduction of the expression of a CTNNB1 gene, *e.g.*, subjects susceptible to or diagnosed with a CTNNB1-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 ±10%. In certain embodiments, about means ±5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

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

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

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

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

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

As used herein, "beta-catenin," used interchangeably with the term "CTNNB1," refers to a structure protein in the cadherin mediated cell-cell adhesive system, and is also known as a pivotal transcriptional activator of the Wnt signaling pathway. The Wnt/β-catenin signaling pathway, also called the canonical Wnt signaling pathway, is a conserved signaling axis participating in diverse physiological processes such as proliferation, differentiation, apoptosis, migration, invasion and tissue homeostasis (Choi B, *et al., Cell Rep.* 2020;31(5):107540). Dysregulation of the Wnt/β-catenin cascade contributes to the development and progression of some solid tumors and hematological malignancies, such as hematocellular carcinoma (HCC) (Ge X, *et al. Journal of hematology & oncology.* 2010;3:33; He S, *et al., Biomed Pharmacother.* 2020;132:110851; Gajos-Michniewicz A, *et al., Int J Mol Sci* 2020, 21(14); Suzuki T, *et al., J Gastroenterol Hepatol.* 2002;17:994–1000). Indeed, beta-catenin plays important roles in promoting tumor progression by stimulating tumor cell proliferation and reducing the activity of cell adhesion systems and is associated with a poor prognosis, especially in patients with poorly differentiated HCCs (Inagawa S, *et al., Clin Cancer Res.* 2002;8:450–456). CTNNB1 is also known as catenin beta, Armadillo, NEDSDV; MRD19; or EVR7.

The sequence of a human CTNNB1 mRNA transcript can be found at, for example, GenBank Accession No. GI: 1519314571 (NM_001904.4; SEQ ID NO:1; reverse complement, SEQ ID NO: 2). The sequence of mouse CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 260166638 (NM_007614.3; SEQ ID NO:3; reverse complement, SEQ ID NO:4). The sequence of

rat CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 46048608 (NM_053357.2; SEQ ID NO:5; reverse complement, SEQ ID NO: 6). The sequence of Macaca fascicularis CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 985482040 (NM_001319394.1; SEQ ID NO:7; reverse complement, SEQ ID NO: 8). The sequence of Macaca mulatta CTNNB1 mRNA can be found at, for example, GenBank Accession No. GI: 383872646 (NM_001257918.1; SEQ ID NO:9; reverse complement, SEQ ID NO:10).

Additional examples of CTNNB1 mRNA sequences are readily available through publicly available databases, *e.g.*, GenBank, UniProt, OMIM, and the *Macaca* genome project web site.

Further information on CTNNB1 can be found, for example, at www.ncbi.nlm.nih.gov/gene/?term=CTNNB1.

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

The term CTNNB1, as used herein, also refers to variations of the CTNNB1 gene including variants provided in the SNP database. Numerous seuqnce variations within the CTNNB1 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=CTNNB1, the entire contents of which is incorporated herein by reference as of the date of filing this application.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CTNNB1 gene, including mRNA that is a product of RNA processing of a primary transcription product. In one embodment, the target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a CTNNB1gene.

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.

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

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, *e.g.*, Table 1). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement

moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

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 a CTNNB1 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.,* a CTNNB1 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.,* a CTNNB1 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.,* a CTNNB1 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 embodiment, at least one strand comprises a 3' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In other embodiments, at least one strand of the RNAi agent comprises a 5' overhang of at least 1 nucleotide. In certain embodiments, at least one strand comprises a 5' overhang of at least 2 nucleotides, *e.g.*, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In still other embodiments, both the 3' and the 5' end of one strand of the RNAi agent comprise an overhang of at least 1 nucleotide.

In 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.*, a CTNNB1 gene, to direct cleavage of the target RNA.

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, *e.g.*, a CTNNB1 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 overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

In one embodiment, 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 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 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.,* a CTNNB1 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.,* a CTNNB1 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 a CTNNB1 gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an RNAi agent containing a mismatch to a target sequence is effective in inhibiting the expression of a CTNNB1 gene. Consideration of the efficacy of RNAi agents with mismatches in inhibiting expression of a CTNNB1 gene is important, especially if the particular region of complementarity in a CTNNB1 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 base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, *e.g.*, inhibition of gene expression, in vitro or in vivo. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

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

The terms "complementary," "fully complementary" and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between two oligonucletoides 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 a CTNNB1 gene). For example, a polynucleotide is complementary to at least a part of a CTNNB1 mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding a CTNNB1 gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target CTNNB1 sequence. In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target CTNNB1 sequence and comprise a 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, 3, 5, 7, or 9, or a fragment of any one of SEQ ID NOs:1, 3, 5, 7, or 9, 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 CTNNB1 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, 3, 5, or 6, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 2, 3, 5, or 6, 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 CTNNB1 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: 2, 4, 6, 8, or 10, or a fragment of any one of SEQ ID NOs:2, 4, 6, 8, or 10, 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 CTNNB1 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, 3, 5, or 6, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 2, 3, 5, or 6, 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 "cationic lipid" includes those lipids having one or two fatty acid or fatty aliphatic chains and an amino acid containing head group that may be protonated to form a cationic lipid at physiological pH. In some embodiments, a cationic lipid is referred to as an "amino acid conjugate cationic lipid."

The term "biodegradable cationic lipid" refers to a cationic lipid having one or more biodegradable groups located in the mid- or distal section of a lipidic moiety (e.g., a hydrophobic chain) of the cationic lipid. The incorporation of the biodegradable group(s) into the cationic lipid results in faster metabolism and removal of the cationic lipid from the body following delivery of the active pharmaceutical ingredient to a target area.

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 CTNNB1 expression; a human at risk for a disease or disorder that would benefit from reduction in CTNNB1 expression; a human having a disease or disorder that would benefit from reduction in CTNNB1 expression; or human being treated for a disease or disorder that would benefit from reduction in CTNNB1 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 a CTNNB1-associated disorder in a subject. Treatment also includes a reduction of one or more sign or symptoms associated with unwanted CTNNB1 expression; diminishing the extent of unwanted CTNNB1 activation or stabilization; amelioration or palliation of unwanted CTNNB1 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 CTNNB1 in a subject or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 10%, 15%, 20%, 25%, 30%, %, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In certain embodiments, a decrease is at least 20%. In certain embodiments, the decrease is at least 50% in a disease marker, *e.g.*, protein or gene expression level. "Lower" in the context of the level of CTNNB1 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 a CTNNB1-associated disorder towards or to a level in a normal subject not suffering from a CTNNB1-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 a CTNNB1 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 a CTNNB1-associated disorder, *e.g.,* cancer, *e.g.*, hepatocellular carcinoma. 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 "beta-catenin-associated disorder" or "CTNNB1-associated disorder," is a disease or disorder that is caused by, or associated with, CTNNB1 gene expression or CTNNB1 protein production. The term "CTNNB1-associated disorder" includes a disease, disorder or condition that would benefit from a decrease in CTNNB1 gene expression, replication, or protein activity. In some embodiments, the CTNNB1-associated disorder is cancer, *e.g.*, hepatocellular carcinoma.

The term "cancer" is used herein to refer to a group of cells that exhibit abnormally high levels of proliferation and growth. A cancer may be benign (also referred to as a benign tumor), pre-malignant, or malignant. Cancer cells may be solid cancer cells or leukemic cancer cells. The term "cancer growth" is used herein to refer to proliferation or growth by a cell or cells that comprise a cancer that leads to a corresponding increase in the size or extent of the cancer.

Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, myeloma and leukemia. In some embodiments, the cancer comprises a solid tumor cancer. In other embodiments, the cancer comprises a blood based cancer, *e..g.*, leukemia, lymphoma or myeloma. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, hepatocellular carcinoma, hepatoblastomas, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma (HCC). As used herein, the term "hepatocellular carcinoma" refers to a major type of primary liver cancer and one of the rare human neoplasms etiologically linked to viral factors. Chronic infections with the hepatitis B virus (HBV) and the hepatitis C virus (HCV) have been implicated in about 80% of cases worldwide (Wang W, *et al.*, J Gastroenterol. 2017 Apr; 52(4):419-431). Genetic mutations and abnormal activation of signal transduction pathways involved in cell proliferation, apoptosis, metabolism, splicing, and the cell cycle are known to contribute to the development of HCC. In particular, the Wnt/β-catenin signaling pathway was known to be activated in up to 50% of HCC (Lee JM, *et al. Cancer Lett.* 2014 Feb 1; 343(1):90-7; Vilchez V, *et al. World J Gastroenterol.* 2016 Jan 14; 22(2):823-32). The Wnt/β-catenin pathway regulates multiple cellular processes that are involved in the initiation, growth, survival, migration, differentiation, and apoptosis of HCC (Wang Z, *et al., Mol Clin Oncol.* 2015 Jul; 3(4):936-940). Mutations in β-catenin have been identified in these tumors, and β-catenin mutation has also been shown to affect the prognosis of HCC. (Prange W, *et al., J Pathol.* 2003;201:250–259; Torbenson M, *et al., Am J Clin Pathol.* 2004;122:377–382).

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a CTNNB1-associated disorder, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the subject to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject having a CTNNB1-associated disorder, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "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 treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, 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, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (*e.g.*, lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. 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 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

(*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.

## II. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of a CTNNB1 gene. In certain embodiments, the iRNA includes double stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a CTNNB1 gene in a cell, such as a cell within a subject, *e.g.*, a mammal, such as a human susceptible to developing a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.*, hepatocellular carcinoma. 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 a CTNNB1 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 CTNNB1 gene, the iRNA inhibits the expression of the CTNNB1 gene (*e.g.*, a human, a primate, a non-primate, or a rat CTNNB1 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 a CTNNB1 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 CTNNB1 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, 3, 5, and 6, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 2, 3, 5, and 6. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of a CTNNB1 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, 3, 5, or 6, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 2, 3, 5, or 6.

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 2, are not described as modified or conjugated sequences, the RNA of the iRNA 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, 3, 5, or 6 that is un-modified, un-conjugated, or modified or conjugated differently than described therein. In other words, the invention encompasses dsRNA of Tables 2, 3, 5, or 6 which are un-modified, un-conjugated, modified, or conjugated, as described herein. For example, although the sense strands of the agents of the invention shown in Table 5 are conjugated to an L96 ligand, these agents may be unconjugated 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, 3, 5, or 6. 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, 3, 5, or 6 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, 3, 5, or 6, and differing in their ability to inhibit the expression of a CTNNB1 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, 3, 5, or 6 identify a site(s) in a CTNNB1 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, 3, 5, or 6 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in a CTNNB1 gene.

## III. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention *e.g.*, a dsRNA, is un-modified, 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 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.

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, 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 embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified 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, 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 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 phosphorothiotate 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 phosphorothiotate 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 $CH_2$ 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 --$CH_2$--NH--$CH_2$-, --$CH_2$--N($CH_3$)--O--$CH_2$--[known as a methylene (methylimino) or MMI backbone], --$CH_2$--O--N($CH_3$)--$CH_2$--, --$CH_2$--N($CH_3$)--N($CH_3$)--$CH_2$-- and --N($CH_3$)--$CH_2$--$CH_2$-- 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 $C_1$ to $C_{10}$ alkyl or $C_2$ to $C_{10}$ alkenyl and alkynyl. Exemplary suitable modifications include O[($CH_2$)$_n$O]$_m$$CH_3$, O($CH_2$)$_n$OCH$_3$, O($CH_2$)$_n$NH$_2$, O($CH_2$)$_n$CH$_3$, O($CH_2$)$_n$ONH$_2$, and O($CH_2$)$_n$ON[($CH_2$)$_n$CH$_3$)]$_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: $C_1$ to $C_{10}$ lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, $SCH_3$, OCN, Cl, Br, CN, $CF_3$, $OCF_3$, $SOCH_3$, $SO_2CH_3$, $ONO_2$, $NO_2$, $N_3$, $NH_2$, 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--$CH_2CH_2OCH_3$, 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'-dimethylaminooxyethoxy, *i.e.*, a O($CH_2$)$_2$ON($CH_3$)$_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), *i.e.*, 2'-O--$CH_2$--O--$CH_2$--N($CH_3$)$_2$. 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'-$OCH_3$), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) 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.

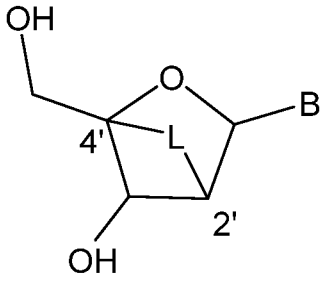
An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxythimidine (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; 5,130,30; 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'-$CH_2$-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′-($CH_2$)—O-2′ (LNA); 4′-($CH_2$)—S-2′; 4′-($CH_2$)$_2$—O-2′ (ENA); 4′-$CH(CH_3)$—O-2′ (also referred to as "constrained ethyl" or "cEt") and 4′-$CH(CH_2OCH_3)$—O-2′ (and analogs thereof; see, *e.g.*, U.S. Patent No. 7,399,845); 4′-$C(CH_3)(CH_3)$—O-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,283); 4′-$CH_2$—$N(OCH_3)$-2′ (and analogs thereof; see *e.g.*, U.S. Patent No. 8,278,425); 4′-$CH_2$—O—$N(CH_3)$-2′ (see, *e.g.*, U.S. Patent Publication No. 2004/0171570); 4′-$CH_2$—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′-$CH_2$—$C(H)(CH_3)$-2′ (see, *e.g.*, Chattopadhyaya *et al., J. Org. Chem.*, 2009,

74, 118-134); and 4′-$CH_2$—C(=$CH_2$)-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'-$CH(CH_3)$-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 Fluiter *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'-0-deoxythymidine (ether), N-

(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"- phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

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.*, CTNNB1 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 no 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, $GalNAc_3$).

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 from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when 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.

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 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 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.

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; 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.

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 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 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 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.

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' 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 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…," "AAABAAABAAAB…," "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 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 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 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 different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "…$N_a$YYY$N_b$…," 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 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 nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage

modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, 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 deoxythimidine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxythimidine (dT). For example, there is a short sequence of deoxythimidine 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):

$5'\ n_p\text{-}N_a\text{-}(X\ X\ X)_i\text{-}N_b\text{-}Y\ Y\ Y\text{-}N_b\text{-}(Z\ Z\ Z)_j\text{-}N_a\text{-}n_q\ 3'$ (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 Nb 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:

$5'\ n_p\text{-}N_a\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q\ 3'$ (Ib);

$5'\ n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_a\text{-}n_q\ 3'$ (Ic); or

$5'\ n_p\text{-}N_a\text{-}XXX\text{-}N_b\text{-}YYY\text{-}N_b\text{-}ZZZ\text{-}N_a\text{-}n_q\ 3'$ (Id).

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:

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):

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;

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

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.

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' (IId).

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 (IId), 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):

sense: 5' $n_p$ -$N_a$-(X X X)$_i$ -$N_b$- Y Y Y -$N_b$ -(Z Z Z)$_j$-$N_a$-$n_q$ 3'

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

(III)

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;

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

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.

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

5' $n_p$ - $N_a$ -Y Y Y -$N_a$-$n_q$ 3'

3' $n_p$'-$N_a$'-Y'Y'Y' -$N_a$'$n_q$' 5'

(IIIa)

5' $n_p$ -$N_a$ -Y Y Y -$N_b$ -Z Z Z -$N_a$-$n_q$ 3'

3' $n_p$'-$N_a$'-Y'Y'Y'-$N_b$'-Z'Z'Z'-$N_a$'$n_q$' 5'

(IIIb)

5' $n_p$-$N_a$- X X X -$N_b$ -Y Y Y - $N_a$-$n_q$ 3'

3' $n_p$'-$N_a$'-X'X'X'-$N_b$'-Y'Y'Y'-$N_a$'-$n_q$' 5'

(IIIc)

5' $n_p$ -$N_a$ -X X X -$N_b$-Y Y Y -$N_b$- Z Z Z -$N_a$-$n_q$ 3'

3' $n_p$'-$N_a$'-X'X'X'-$N_b$'-Y'Y'Y'-$N_b$'-Z'Z'Z'-$N_a$-$n_q$' 5'

(IIId)

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 $N_a$ 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 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIId), 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 of alternating pattern.

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

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

When the dsRNAi agent is represented as formula (IIIc) or (IIId), 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.

In certain embodiments, when the dsRNAi agent is represented by formula (IIId), the $N_a$ modifications are 2′-O-methyl or 2′-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), 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 a *via* phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIId), 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, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other

embodiments, when the RNAi agent is represented by formula (IIId), 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 (IIIa), 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, the dsRNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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 more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), 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.

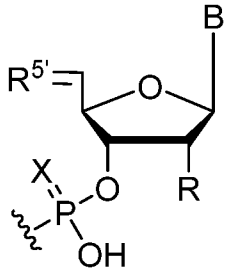
In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to a ligand. Each of the agents can target the same gene or two different genes;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

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 phosphonate (VP) modification of an RNAi agent as described herein. In exemplary embodiments, a 5' vinyl phosphonate modified nucleotide of the disclosure has the structure:




wherein X is O or S;

R is hydrogen, hydroxy, fluoro, or $C_{1-20}$alkoxy (*e.g.*, methoxy or n-hexadecyloxy);

$R^{5'}$ is $=C(H)-P(O)(OH)_2$ and the double bond between the C5' carbon and $R^{5'}$ is in the *E* or *Z* orientation (*e.g.*, *E* orientation); and

B is a nucleobase or a modified nucleobase, optionally where B is adenine, guanine, cytosine, thymine, or uracil.

A vinyl phosphonate of the instant disclosure may be attached to either the antisense or the sense strand of a dsRNA of the disclosure. In certain embodiments, a vinyl phosphonate of the instant disclosure is attached to the antisense strand of a dsRNA, optionally at the 5' end of the antisense strand of the dsRNA.

Vinyl phosphonate modifications are also contemplated for the compositions and methods of the instant disclosure. An exemplary vinyl phosphonate structure includes the preceding structure, where R5' is =C(H)-OP(O)(OH)2 and the double bond between the C5' carbon and R5' is in the E or Z orientation (*e.g.*, E orientation).

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, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin. In one embodiment, the acyclic group is a serinol backbone or diethanolamine backbone.PCT/US12/068491, ,

*i. Thermally Destabilizing Modifications*

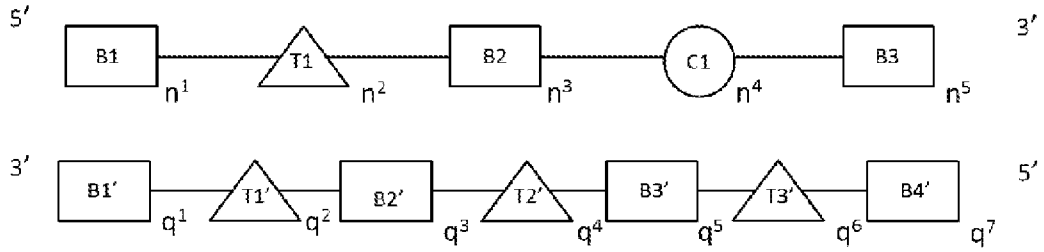
In certain embodiments, a dsRNA molecule can be optimized for RNA interference by incorporating thermally destabilizing modifications in the seed region of the antisense strand. As used herein "seed region" means at positions 2-9 of the 5'-end of the referenced strand or at positions 2-8 of the 5'-end of the refrenced 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.

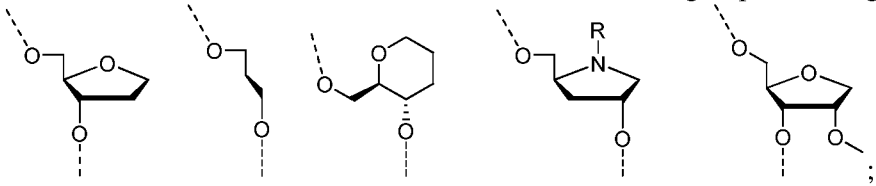
An iRNA agent comprises a sense strand and an antisense strand, each strand having 14 to 40 nucleotides. The RNAi agent may be represented by formula (L):



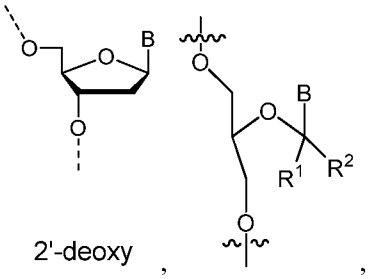

(L),

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.

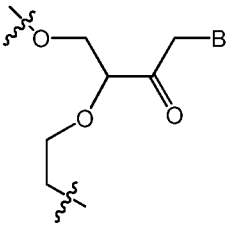
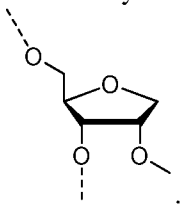
C1 is a thermally destabilizing nucleotide placed at a site opposite to the seed region of the antisense strand (*i.e.*, at positions 2-8 of the 5'-end of the antisense strand, or at positions 2-9 of the 5'-end of the refrenced strand). For example, C1 is at a position of the sense strand that pairs 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:




; and iii) sugar modification selected from the group consisting of:



, and


, wherein B is a modified or unmodified nucleobase, $R^1$ and $R^2$ independently are H, halogen, $OR_3$, or alkyl; and $R_3$ is H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar. In 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 .

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 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.

$n^4$, $q^2$, 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.

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).

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

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.

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).

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.

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,

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 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.

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 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 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.

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; 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 6, 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 7, 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.

In one embodiment, B1 is 2'-OMe or 2'-F, $n^1$ is 6, 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 7, 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; 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, 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.

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 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 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 5, T2' is 2'-F, $q^4$ is 1, 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; optionally with at least 2 additional TT at the 3'-end of the antisense strand.

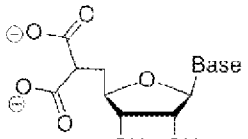
In one embodiment, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'-OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 5, T2' is 2'-F, $q^4$ is 1, B3' is 2'-OMe or 2'-F, $q^5$ is 5, T3' is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

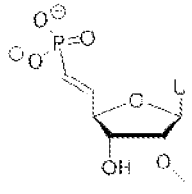
In one embodiment, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'-OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 4, $q^4$ is 0, B3' is 2'-OMe or 2'-F, $q^5$ is 7, T3' is 2'-F, $q^6$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

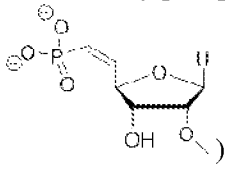
In one embodiment, B1 is 2'-OMe or 2'-F, $n^1$ is 8, T1 is 2'F, $n^2$ is 3, B2 is 2'-OMe, $n^3$ is 7, $n^4$ is 0, B3 is 2'-OMe, $n^5$ is 3, B1' is 2'-OMe or 2'-F, $q^1$ is 9, T1' is 2'-F, $q^2$ is 1, B2' is 2'-OMe or 2'-F, $q^3$ is 4, $q^4$ is 0, B3' is 2'-OMe or 2'-F, $q^5$ is 7, T3' is 2'-F, $q^6$ 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'-$PS_2$), 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'-$PS_2$. In one embodiment, the RNAi agent comprises a 5'-$PS_2$ in the antisense strand.

In one embodiment, the RNAi agent comprises a 5'-$PS_2$. 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, $n^1$ 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. The RNAi agent also comprises a 5'-PS.

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. The RNAi agent also comprises a 5'-P.

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. 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, $n^1$ 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. The RNAi agent also comprises a 5'- $PS_2$.

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. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

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; 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, $n^1$ 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; 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, $n^1$ 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; 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, $n^1$ 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; 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'- $PS_2$.

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; 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, $n^1$ 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. The RNAi agent also comprises a 5'-P.

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. The dsRNA agent also comprises a 5'-PS.

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. 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, $n^1$ 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. The RNAi agent also comprises a 5'- $PS_2$.

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. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'- $PS_2$.

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 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, $n^1$ 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. The RNAi agent also comprises a 5'- P.

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. The RNAi agent also comprises a 5'- PS.

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. 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, $n^1$ 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. The dsRNAi RNA agent also comprises a 5'- $PS_2$.

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. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'- $PS_2$.

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 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, $n^1$ 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. The RNAi agent also comprises a 5'- P.

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. The RNAi agent also comprises a 5'- PS.

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. 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, $n^1$ 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. The RNAi agent also comprises a 5'- $PS_2$.

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. The RNAi agent also comprises a 5'-deoxy-5'-*C*-malonyl.

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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'- $PS_2$.

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 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, $n^1$ 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; 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, $n^1$ 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; 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, $n^1$ 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; 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, $n^1$ 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; 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'- $PS_2$ and a targeting ligand. In one embodiment, the 5'- $PS_2$ 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, $n^1$ 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; 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'-$PS_2$ and a targeting ligand. In one embodiment, the 5'-$PS_2$ 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'-$PS_2$ and a targeting ligand. In one embodiment, the 5'-$PS_2$ 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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, $n^1$ 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 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'- $PS_2$ and a targeting ligand. In one embodiment, the 5'-$PS_2$ 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, $n^1$ 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 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, 3, 5, or 6. 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. Acid. Sci. USA*, 1989, 86: 6553-6556). In other embodiments, the ligand is cholic acid (Manoharan *et al.*, *Biorg. Med. Chem. Let.*, 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. Let.*, 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.*, di-hexadecyl-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-carbonyloxycholesterol 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-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic 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)cholenic 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-κB.

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 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, 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 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 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, 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 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 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 antennopedia. 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: 14). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP (SEQ ID NO:15) 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:16) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:17) 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 peptidiomimemtics 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).

*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 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 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 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 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 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 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.

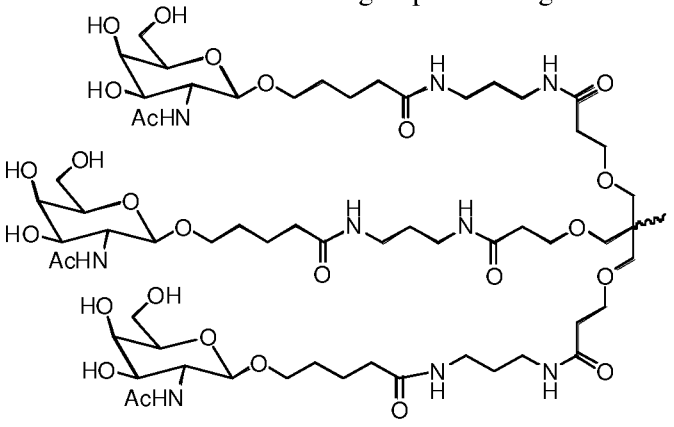
In certain embodiments, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In certain embodiments, the double stranded RNAi agents of the invention comprise a plurality (*e.g.*, 2, 3, 4, 5, or 6) GalNAc or GalNAc

derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

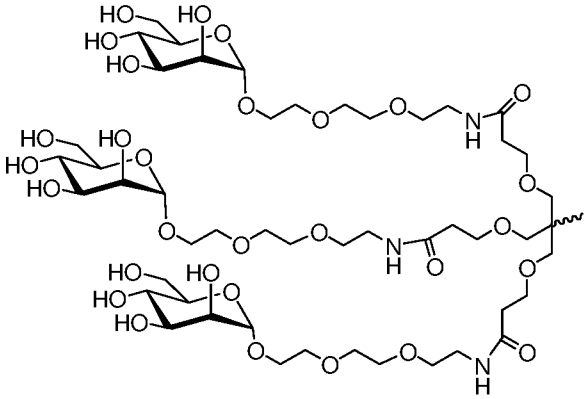
In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of 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.

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.

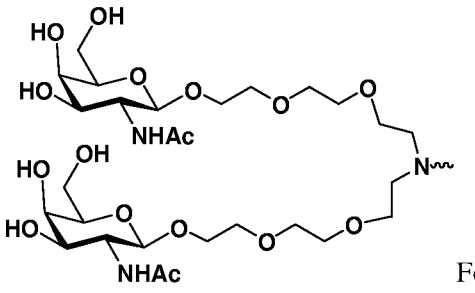
In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:



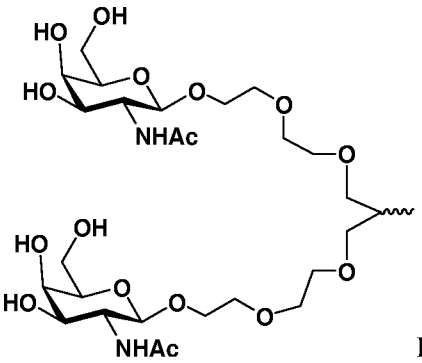
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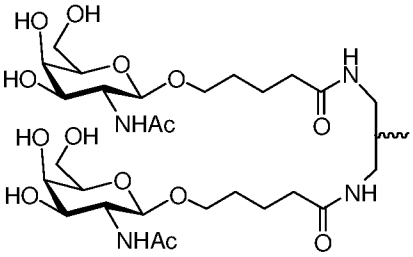
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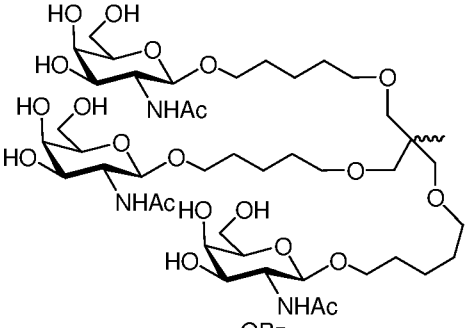
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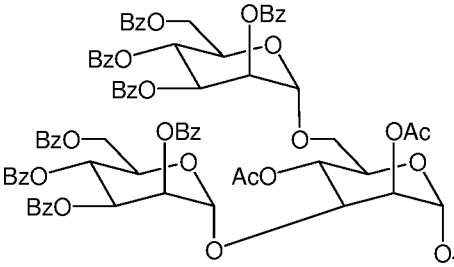
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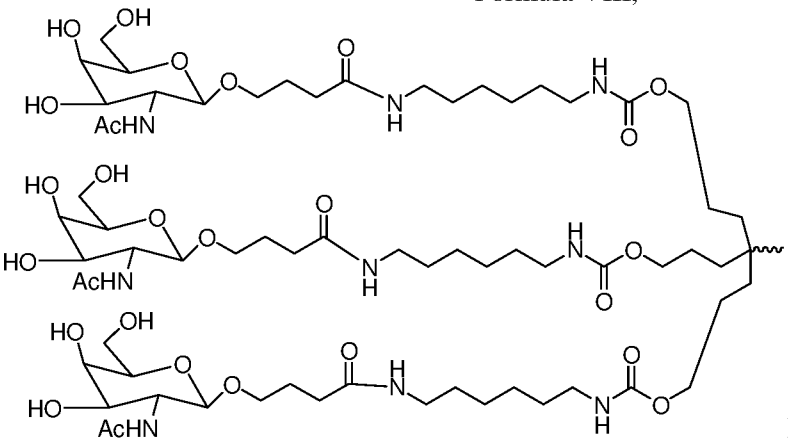
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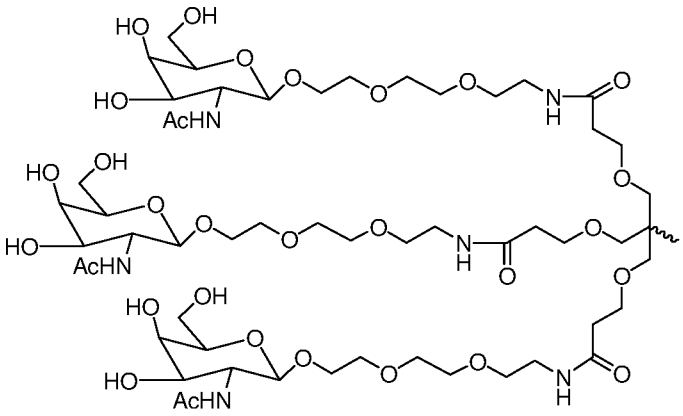
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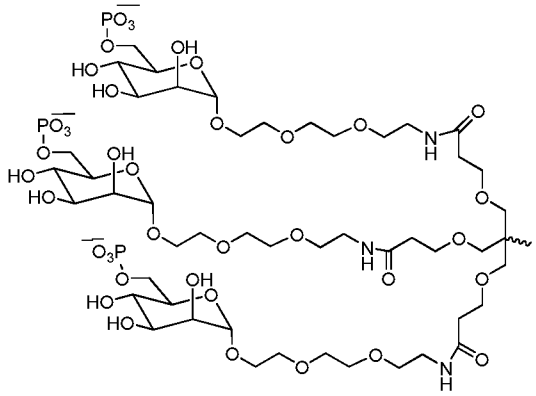
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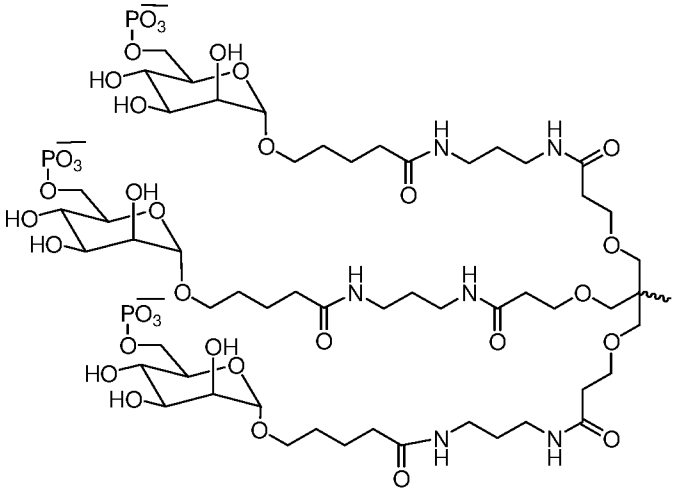
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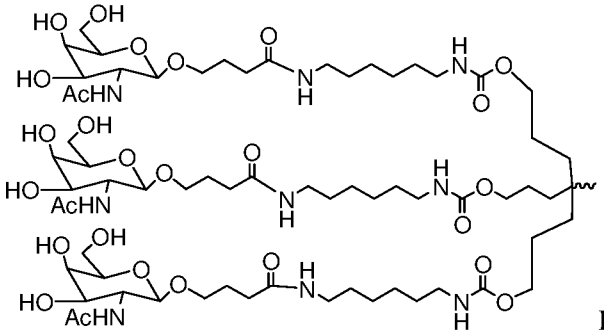
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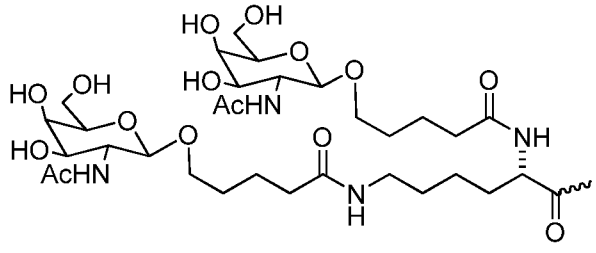
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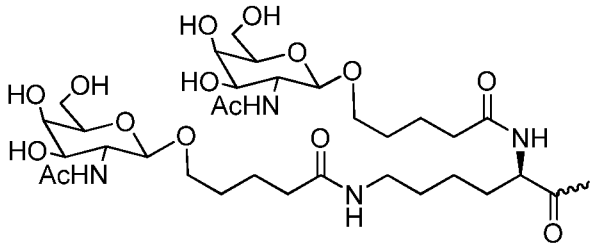
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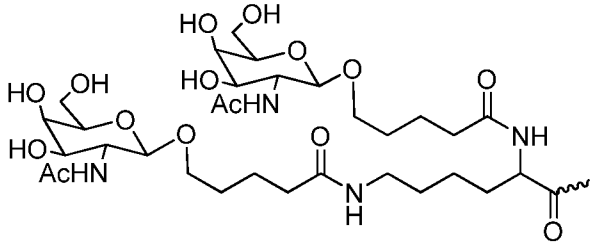
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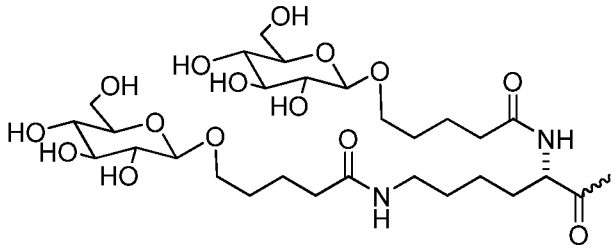
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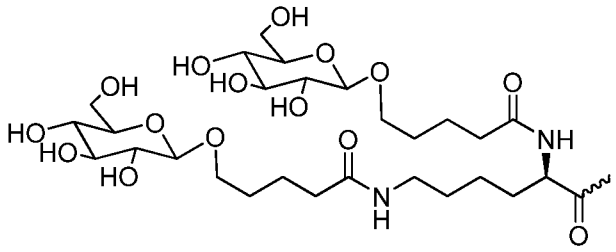
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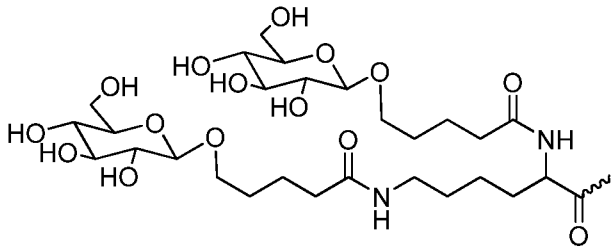
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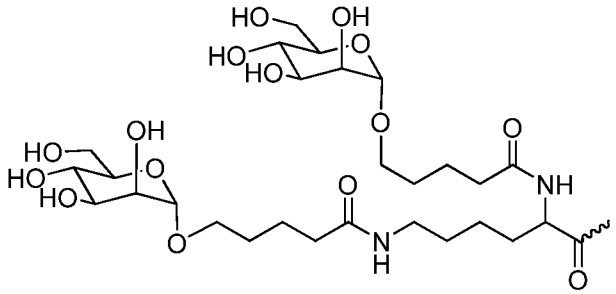
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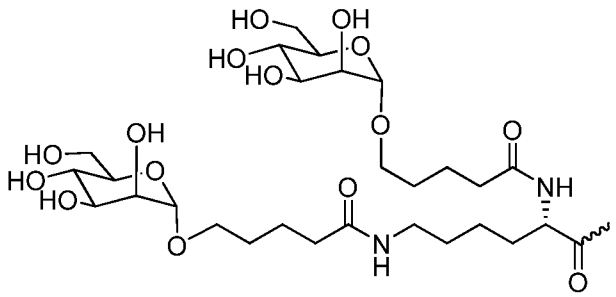
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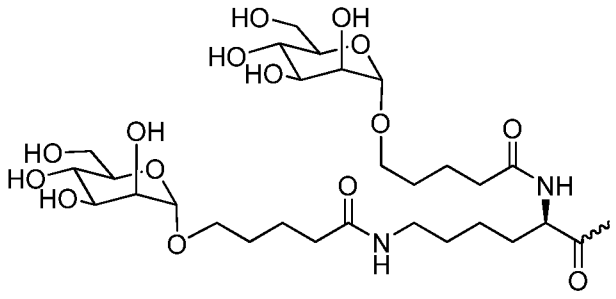
Formula XIX,



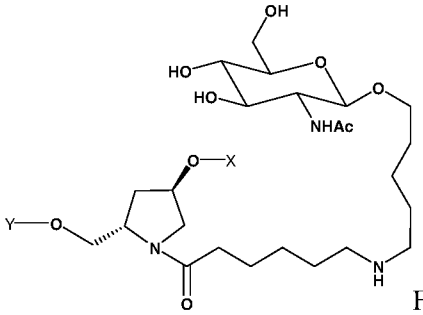
Formula XX,



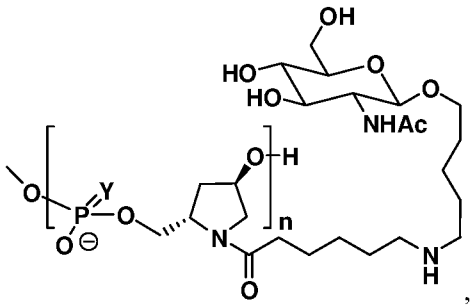
Formula XXI,



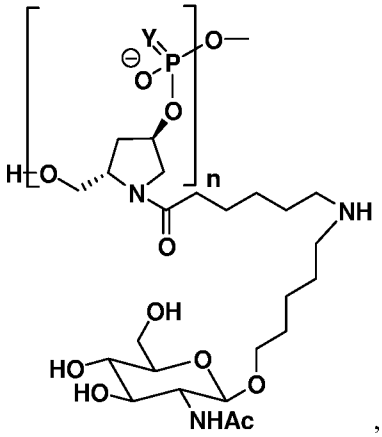
Formula XXII,



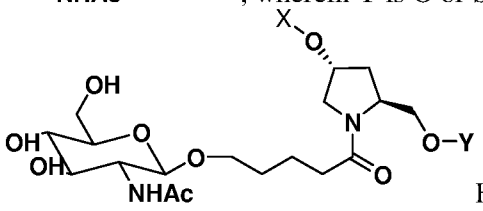
Formula XXIII;



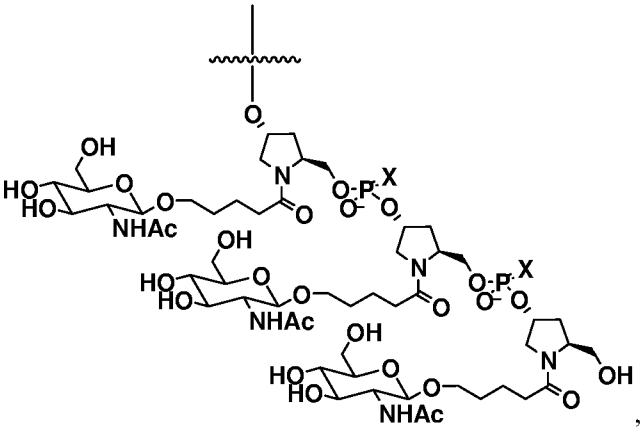
, wherein Y is O or S and n is 3 -6 (Formula XXIV);



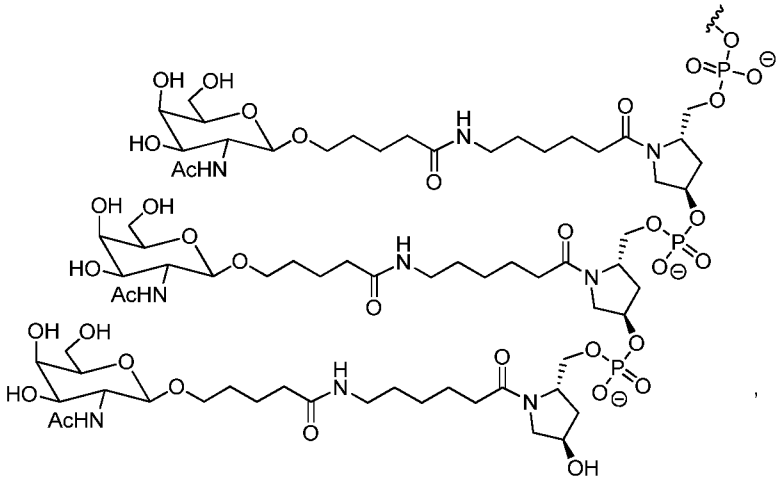
, wherein Y is O or S and n is 3-6 (Formula XXV);



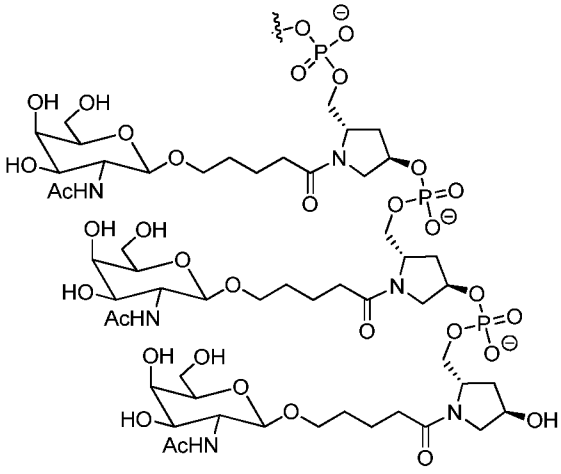
Formula XXVI;



, wherein X is O or S (Formula XXVII);

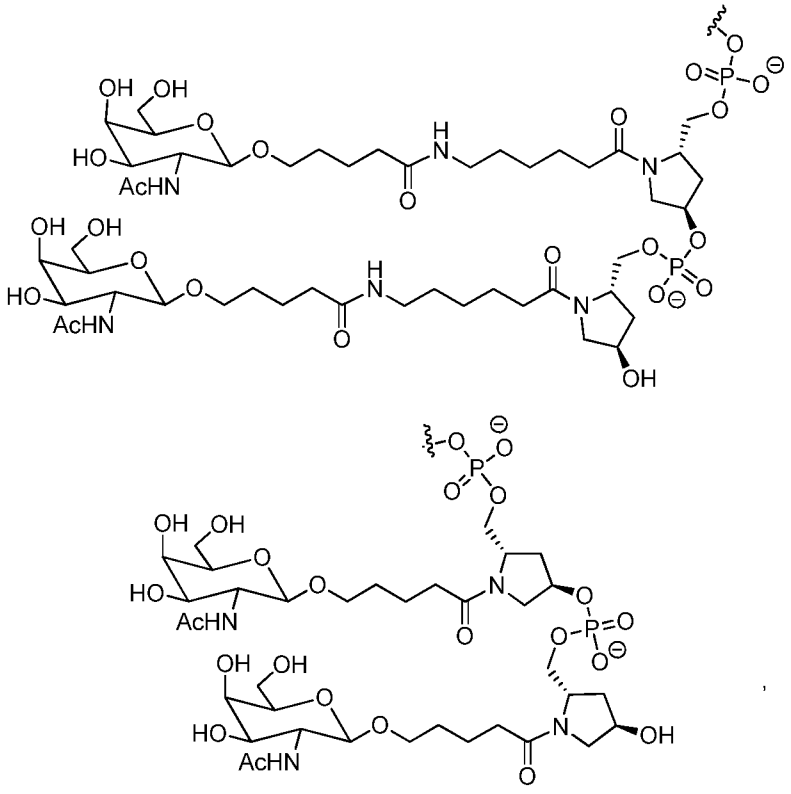


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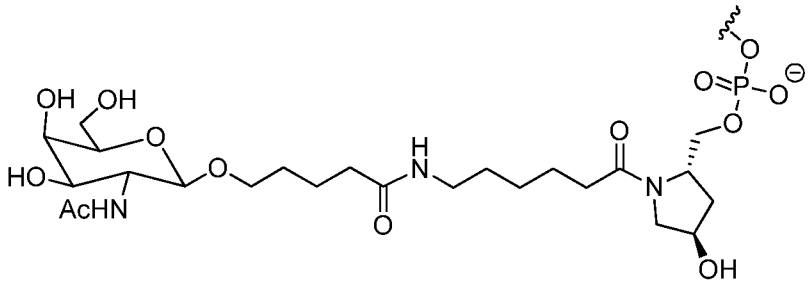
,

Formula XXVII; Formula XXIX;

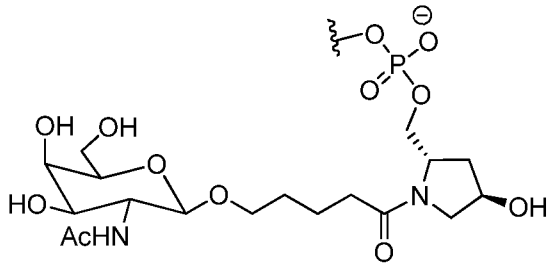


Formula XXX;

Formula XXXI;

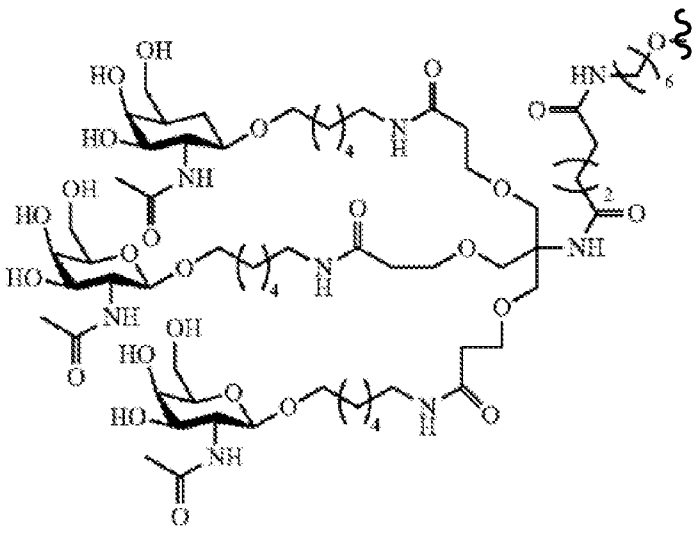


, and



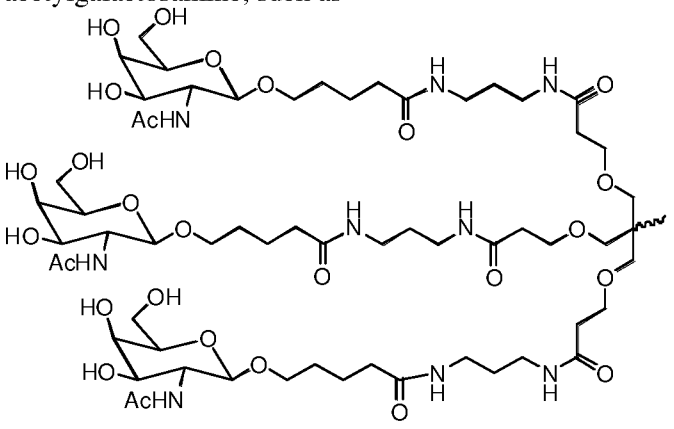
Formula XXXII;

Formula XXXIII.



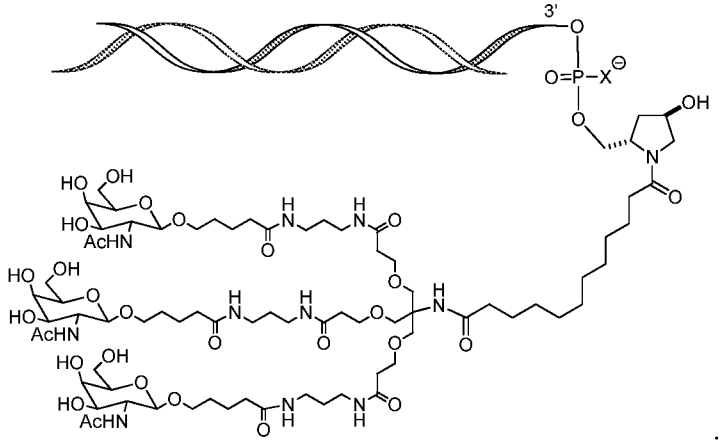
Formula XXXIV.

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

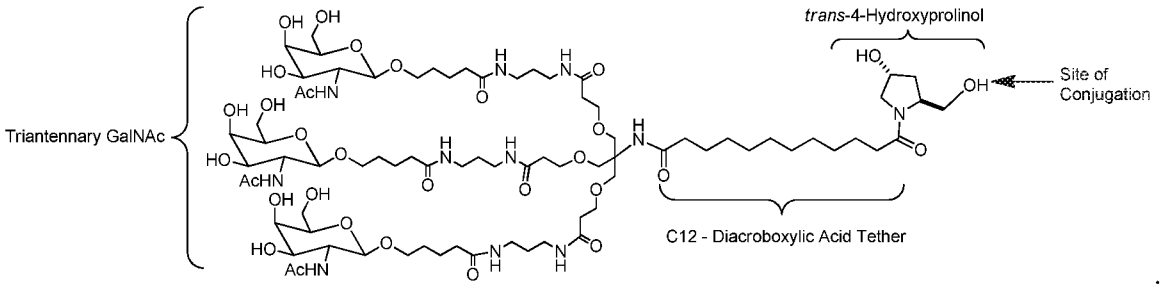


Formula II.

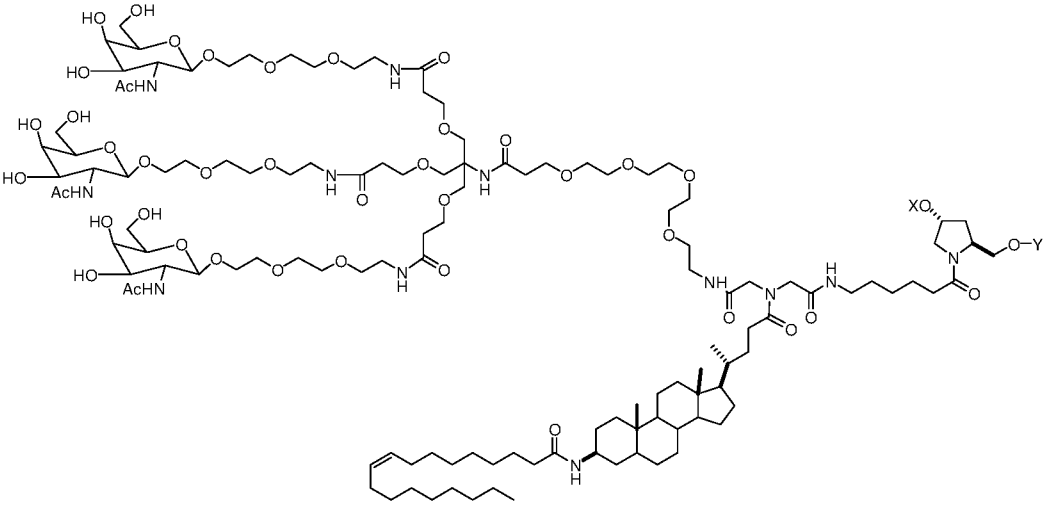
In some embodiments, the RNAi agent is attached to the carbohydrate conjugate *via* a linker as shown in the following schematic, wherein X is O or S.



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

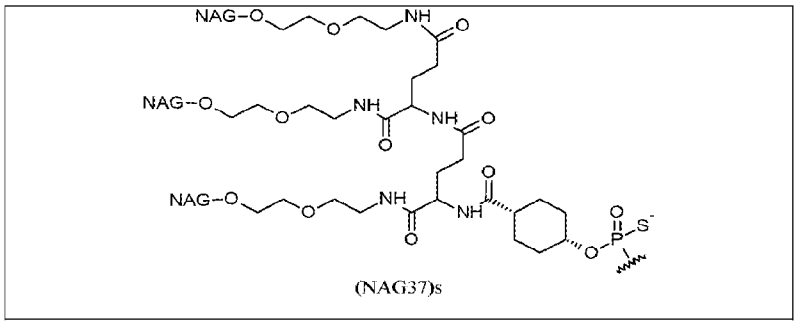



Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(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:




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 embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the 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 antsisense 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' – 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.

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.

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.

*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 bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, $SO_2$, $SO_2NH$ 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, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl,

alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), $SO_2$, 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.

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).

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

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some 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.

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 know in the art, which mimic the rate of cleavage which would be observed in a cell, *e.g.*, a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In 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.\

*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.

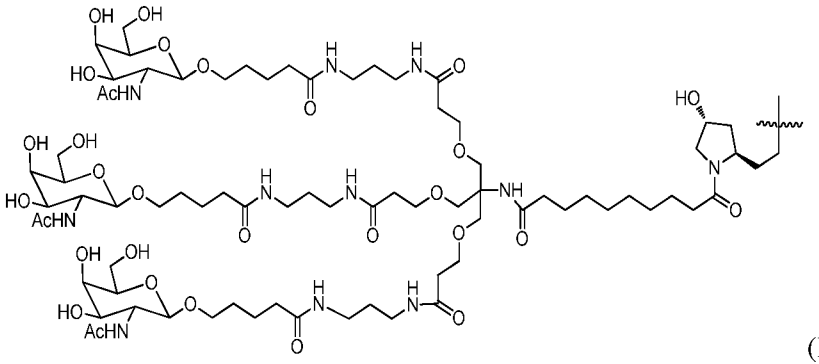
*iv. Ester-based linking groups*

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.

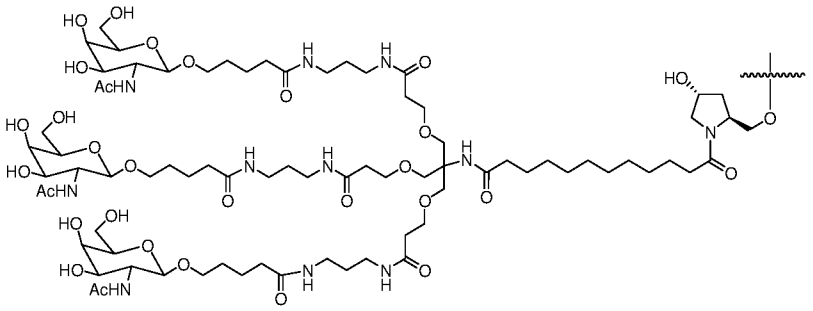
*v. Peptide-based cleaving groups*

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 alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (*i.e.*, the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-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.

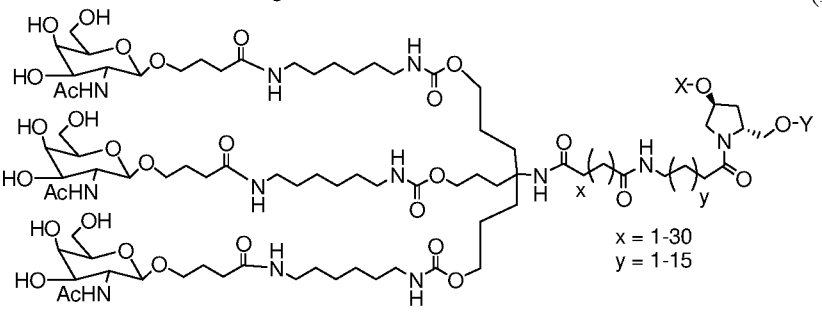
In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,



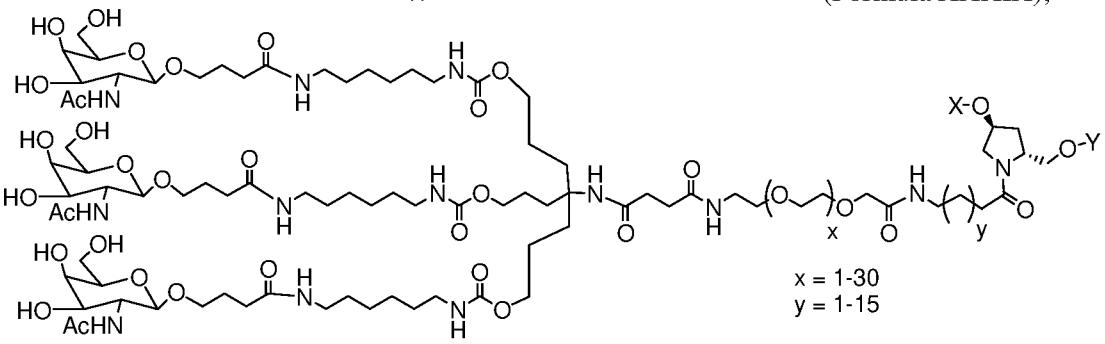
(Formula XXXVII),



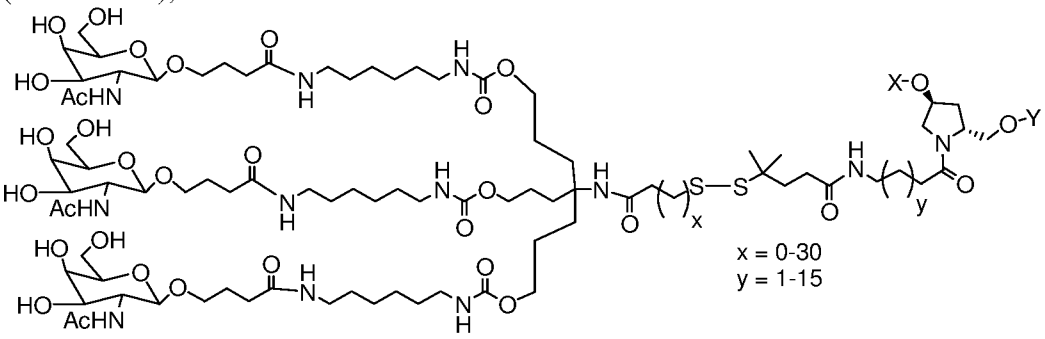
(Formula XXXVIII),



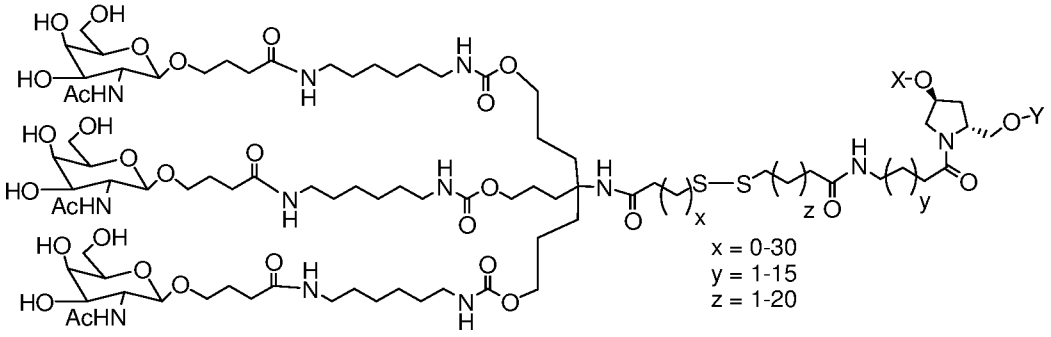

(Formula XXXIX),



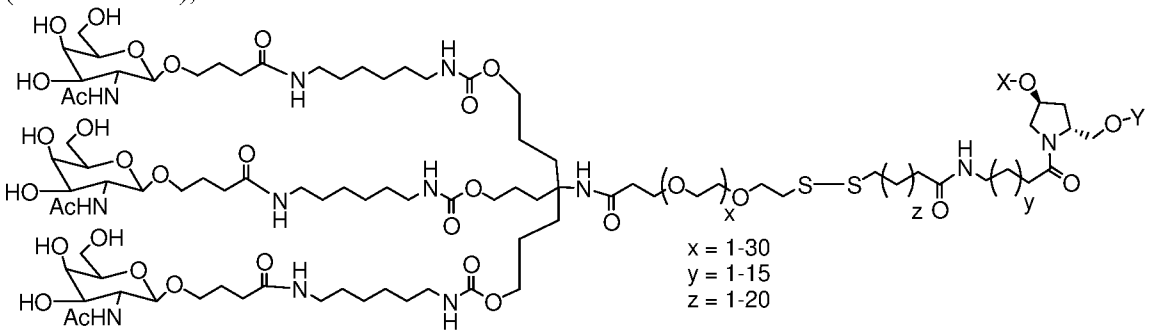

(Formula XL),



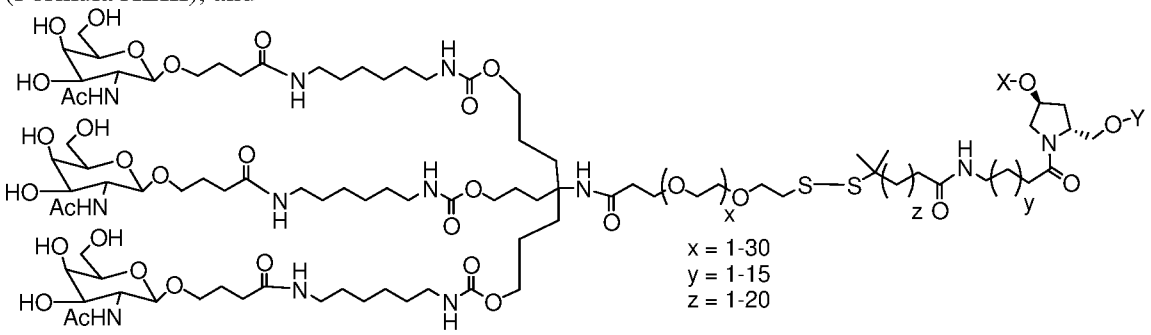

(Formula XLI),




(Formula XLII),




(Formula XLIII), and

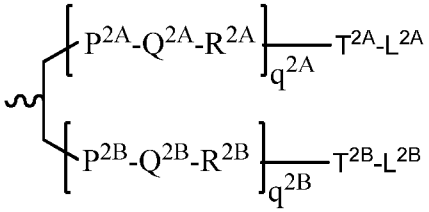



(Formula XLIV), when one of X or Y is an oligonucleotide, the other is a hydrogen.

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.

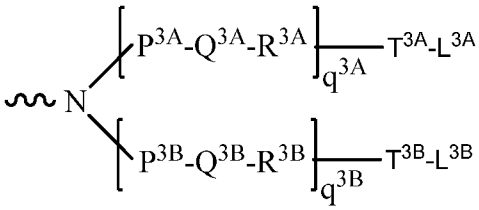
In one embodiment, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XLV) – (XLVI):

Formula XXXXV

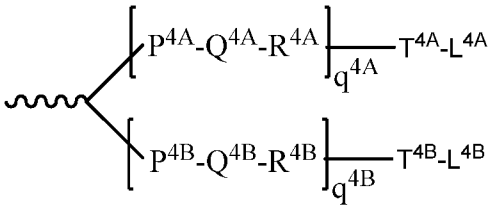


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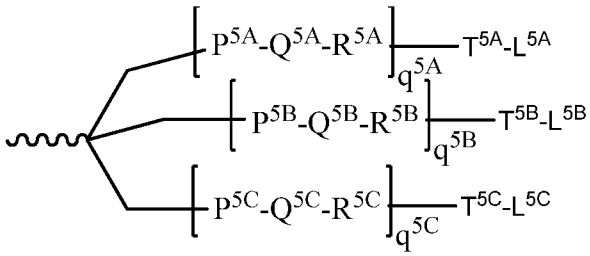
Formula XLVI



,



, or



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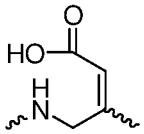
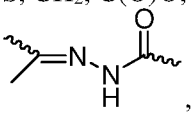
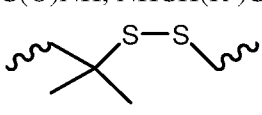
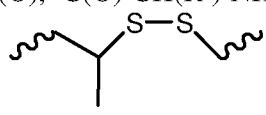
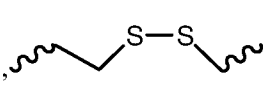
Formula XL` Formula XLVIII

wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

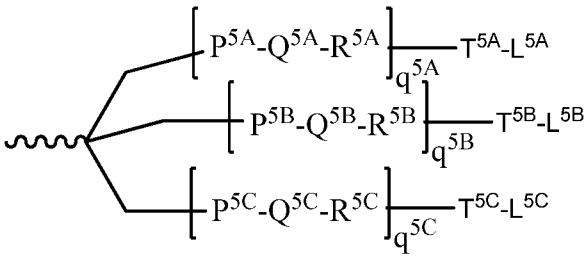
$P^{2A}$, $P^{2B}$, $P^{3A}$, $P^{3B}$, $P^{4A}$, $P^{4B}$, $P^{5A}$, $P^{5B}$, $P^{5C}$, $T^{2A}$, $T^{2B}$, $T^{3A}$, $T^{3B}$, $T^{4A}$, $T^{4B}$, $T^{4A}$, $T^{5B}$, $T^{5C}$ are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), $CH_2$, $CH_2NH$ or $CH_2O$;

$Q^{2A}$, $Q^{2B}$, $Q^{3A}$, $Q^{3B}$, $Q^{4A}$, $Q^{4B}$, $Q^{5A}$, $Q^{5B}$, $Q^{5C}$ 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), $SO_2$, $N(R^N)$, 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, S, $CH_2$, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, $-C(O)-CH(R^a)-NH-$, CO, CH=N-O, , , , ,  or heterocyclyl;

$L^{2A}$, $L^{2B}$, $L^{3A}$, $L^{3B}$, $L^{4A}$, $L^{4B}$, $L^{5A}$, $L^{5B}$ 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 target gene, such as those of formula (XLIX):

Formula XLIX



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

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; 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; 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 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 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 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. Let.*, 1993, 3:2765), a thiocholesterol (Oberhauser *et*

*al.*, *Nucl. Acids Res.*, 1992, 20:533), an aliphatic chain, *e.g.*, dodecandiol or undecyl residues (Saison-Behmoaras *et al.*, *EMBO J.*, 1991, 10:111; Kabanov *et al.*, *FEBS Lett.*, 1990, 259:327; Svinarchuk *et al.*, *Biochimie*, 1993, 75:49), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan *et al.*, *Tetrahedron Lett.*, 1995, 36:3651; Shea *et al.*, *Nucl. Acids Res.*, 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan *et al.*, *Nucleosides & Nucleotides*, 1995, 14:969), 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.

## 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 a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.,* hepatocellular carcinoma) 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.* 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

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 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) J*ournal 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 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 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 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 a CTNNB1 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 hepatocyte. In one embodiment, the cell is an extrahepatic cell.

*A. Vector encoded iRNAs of the Invention*

iRNA targeting the CTNNB1 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 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.*, *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 vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. Constructs for the recombinant expression of 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

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 a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.,* hepatocellular carcinoma.

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 a CTNNB1 gene.

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 a CTNNB1 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 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.

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 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, 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), 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 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 agents and surfactants. Suitable lipids and liposomes include neutral (*e.g.*, dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl 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.

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.

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, monolein, 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.

In another embodiment, the topical penetration enhancer is a bile salt. The bile salt can be cholic acid, dehydrocholic acid, deoxycholic acid, glucholic 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.

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

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.

In another embodiment, the penetration enhancer can be selected from a group consisting of unsaturated cyclic ureas, 1-alkyl-alkones, 1-alkenylazacyclo-alakanones, 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.

iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs 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 $C_{1-20}$ alkyl ester (*e.g.,* isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Patent No. 6,747,014, which is incorporated herein by reference.

*A. iRNA Formulations Comprising Membranous Molecular Assemblies*

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, *e.g.*, a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, *e.g.*, one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA composition. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate RNAi. In some cases the liposomes are also specifically targeted, *e.g.*, to direct the iRNA to particular cell types.

A liposome containing a RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic.

Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, *e.g.*, by dialysis, to yield a liposomal preparation of RNAi agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, *e.g.*, by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (*e.g.*, spermine or spermidine). pH can also adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Felgner, P. L. *et al., Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; U.S. Pat. No. 4,897,355; U.S. Pat. No. 5,171,678; Bangham, *et al. M. Mol. Biol.* 23:238, 1965; Olson, *et al. Biochim. Biophys. Acta* 557:9, 1979; Szoka, *et al. Proc. Natl. Acad. Sci.* 75: 4194, 1978; Mayhew, e*t al. Biochim. Biophys. Acta* 775:169, 1984; Kim, *et al. Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, *et al. Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, *e.g.,* Mayer, *et al. Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, *et al. Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging RNAi agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang *et al.*, *Biochem. Biophys. Res. Commun.*, 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou *et al.*, *Journal of Controlled Release*, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic

liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Examples of other methods to introduce liposomes into cells *in vitro* and *in vivo* include U.S. Pat. No. 5,283,185; U.S. Pat. No. 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Felgner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu *et al. S.T.P.Pharma. Sci.*, 1994, 4(6) 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside $G_{M1}$, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen *et al.*, *FEBS Letters*, 1987, 223, 42; Wu *et al.*, *Cancer Research*, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos *et al.* (*Ann. N.Y. Acad. Sci.*, 1987, 507, 64) reported the ability of monosialoganglioside $G_{M1}$, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon *et al.* (*Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen *et al.*, disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside $G_{M1}$ or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb *et al.*) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim *et al*).

In one embodiment, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are taken up by macrophages in vivo and can be used to deliver RNAi agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated RNAi agents in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of RNAi agent (see, e.g., Felgner, P. L. et al., Proc. Natl. Acad. Sci., USA 8:7413-7417, 1987 and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis(oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Indiana) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, California) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Maryland). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer RNAi agent into the skin. In some implementations, liposomes are used for delivering RNAi agent to epidermal cells and also to enhance the penetration of RNAi agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, *e.g.*, Weiner *et al., Journal of Drug Targeting,* 1992, vol. 2,405-410 and du Plessis *et al., Antiviral Research*, 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. *et al. Gene* 56:267-276. 1987; Nicolau, C. *et al. Meth. Enz.* 149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, L., P*roc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with RNAi agent are useful for treating a dermatological disorder.

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include RNAi agent can be delivered, for example, subcutaneously by infection in order to deliver RNAi agent to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, *e.g.*, in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in United States provisional application serial Nos. 61/018,616, filed January 2, 2008; 61/018,611, filed January 2, 2008; 61/039,748, filed March 26, 2008; 61/047,087, filed April 22, 2008 and 61/051,528, filed May 8, 2008. PCT application no PCT/US2007/080331, filed October 3, 2007 also describes formulations that are amenable to the present invention.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, *e.g.*, they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add

surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The iRNA for use in the methods of the invention can also be provided as micellar formulations. “Micelles” are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal $C_8$ to $C_{22}$ alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include

lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the siRNA composition and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the siRNA composition, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol and/or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol and/or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.,* there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.,* through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, *e.g.,* at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. *Lipid particles*

iRNAs, *e.g.,* dsRNAs of in the invention may be fully encapsulated in a lipid formulation, *e.g.,* a LNP, *e.g.*, to form a SPLP, pSPLP, SNALP, or other nucleic acid-lipid particle.

As used herein, the term "SNALP" refers to a stable nucleic acid-lipid particle, including SPLP. As used herein, the term "SPLP" refers to a nucleic acid-lipid particle comprising plasmid DNA encapsulated within a lipid vesicle. SNALPs and SPLPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (*e.g.*, a PEG-lipid conjugate).

SNALPs and SPLPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (*e.g.*, sites physically separated from the administration site). SPLPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid- lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, *e.g.*, U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; U.S. Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (*e.g.*, lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(I -(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(I -(2,3- dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3- dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino)acetoxypropane (DLin-DAC), 1,2-Dilinoleoyl-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.Cl), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.Cl), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), l,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In another embodiment, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles. Synthesis of 2,2-Dilinoleyl-4-dimethylaminoethyl-

[1,3]-dioxolane is described in United States provisional patent application number 61/107,998 filed on October 23, 2008, which is herein incorporated by reference.

In one embodiment, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0 ± 20 nm and a 0.027 siRNA/Lipid Ratio.

The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l- carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1 -trans PE, 1 -stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl ($Ci_2$), a PEG-dimyristyloxypropyl ($Ci_4$), a PEG-dipalmityloxypropyl ($Ci_6$), or a PEG- distearyloxypropyl (C)$_8$ ~~(C)$_8$~~. The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, *e.g.*, about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98·4HCl (MW 1487) (see U.S. Patent Application No. 12/056,230, filed 3/26/2008, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (*i.e.*, LNP01 particles).

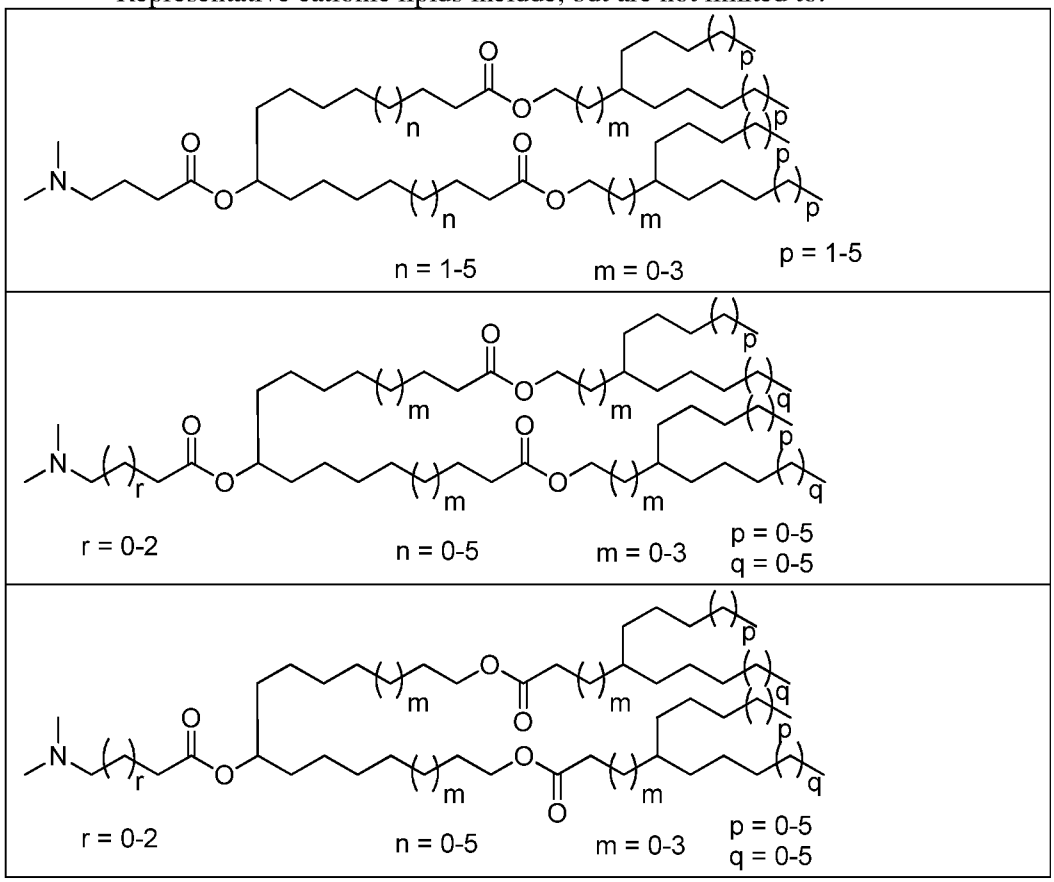
In certain embodiments of the invention, suitable cationic lipids suitable for use in the compositions of the invention are those described in U.S. Patent No. 9,061,063, and PCT Publication No. WO 2013/086354, the entire contents of each of which are incorporated herein by reference. In some embodiments, suitable cationic lipids include one or more biodegradable groups. The biodegradable group(s) include one or more bonds that may undergo bond breaking reactions in a biological environment, *e.g.*, in an organism, organ, tissue, cell, or organelle. Functional groups that contain a biodegradable bond include, for example, esters, dithiols, and oximes. Biodegradation can be a factor that influences the clearance of the compound from the body when administered to a subject. Biodegredation can be measured in a cell based assay, where a formulation including a cationic lipid is exposed to cells, and samples are taken at various time points. The lipid fractions can

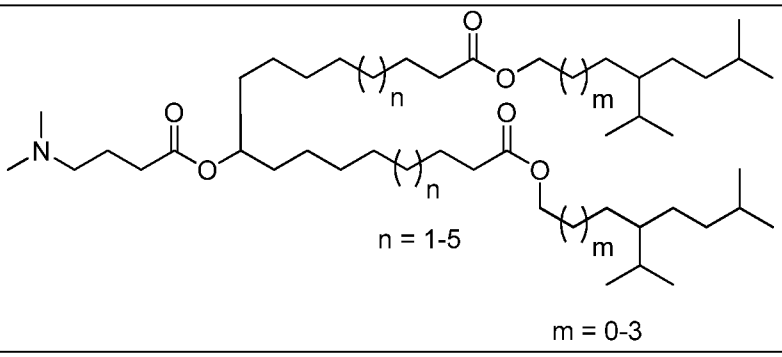
be extracted from the cells and separated and analyzed by LC-MS. From the LC-MS data, rates of biodegradation (e.g., as t1/2 values) can be measured. the cationic lipd comprises a biodegradable group.

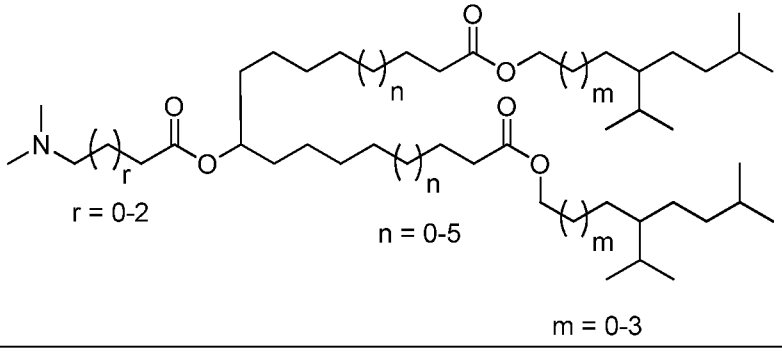
In one embodiment, a cationic lipid of any of the embodiments described herein has an in vivo half life (t1/2) (e.g., in the liver, spleen or plasma) of less than about 3 hours, such as less than about 2.5 hours, less than about 2 hours, less than about 1.5 hours, less than about 1 hour, less than about 0.5 hour or less than about 0.25 hours. The cationic lipid preferably remains intact, or has a half-life sufficient to form a stable lipid nanoparticle which effectively delivers the desired active pharmaceutical ingredient (e.g., a nucleic acid) to its target but thereafter rapidly degrades to minimize any side effects to the subject. For instance, in mice, the cationic lipid preferably has a t1/2 in the spleen of from about 1 to about 7 hours.

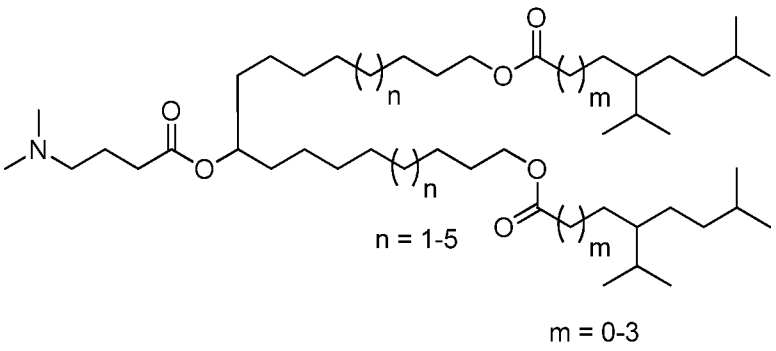
In another embodiment, a cationic lipid of any of the embodiments described herein containing a biodegradable group or groups has an in vivo half life (t1/2) (e.g., in the liver, spleen or plasma) of less than about 10% (e.g., less than about 7.5%, less than about 5%, less than about 2.5%) of that for the same cationic lipid without the biodegrable group or groups.

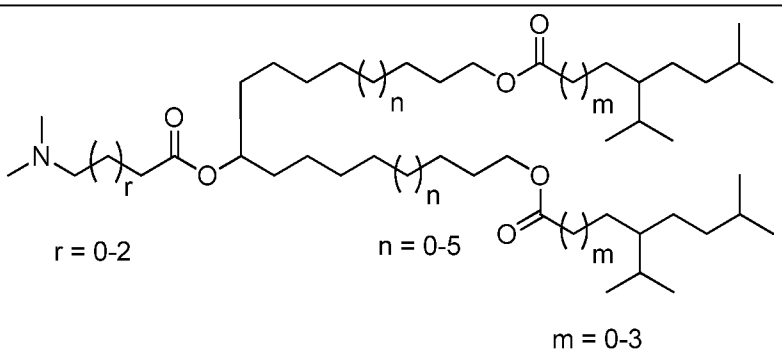
Representative cationic lipids include, but are not limited to:

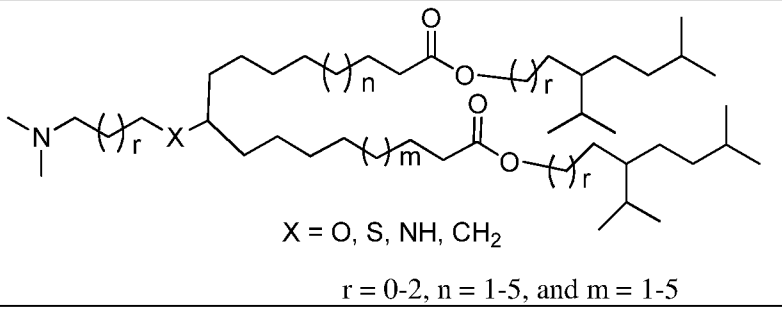


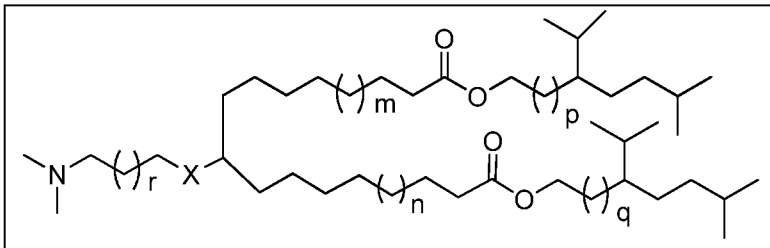

n = 1-5
m = 0-3


r = 0-2
n = 0-5
m = 0-3

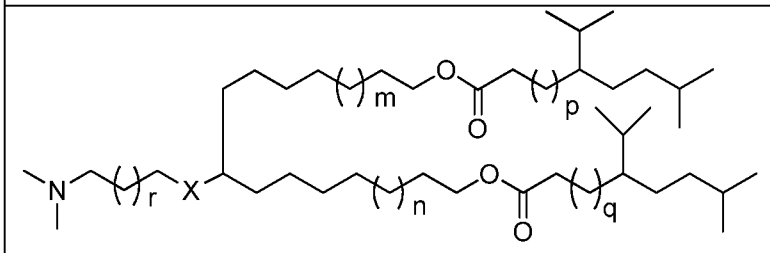

n = 1-5
m = 0-3


r = 0-2
n = 0-5
m = 0-3

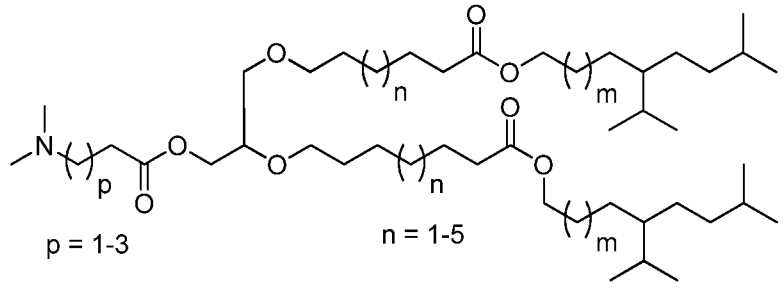

X = O, S, NH, CH2
r = 0-2, n = 1-5, and m = 1-5



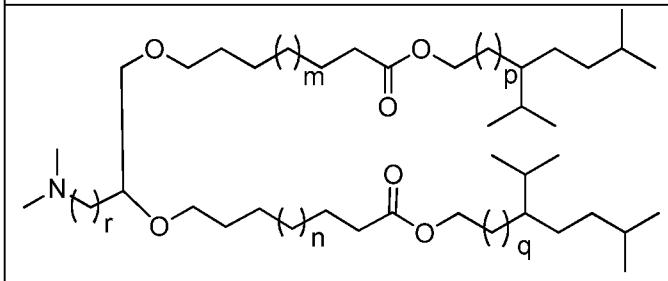
r = 0-2 X = O, S, NH, $CH_2$ m = 0-5 p = 0-3
n = 0-5 q = 0-3



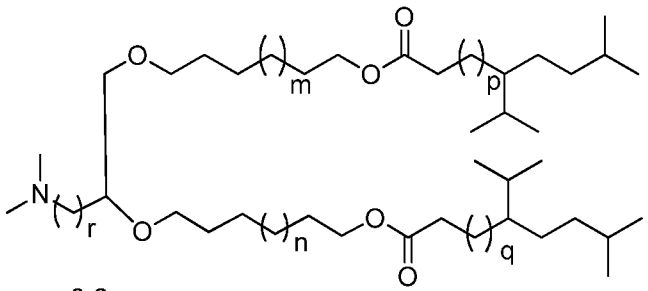
r = 0-2 X = O, S, NH, $CH_2$ m = 0-5 p = 0-3
n = 0-5 q = 0-3



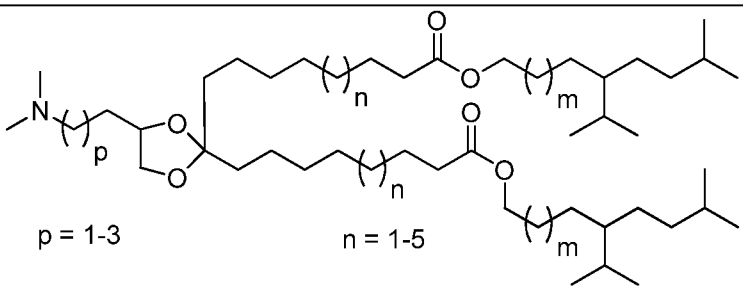
p = 1-3 n = 1-5 m = 0-3

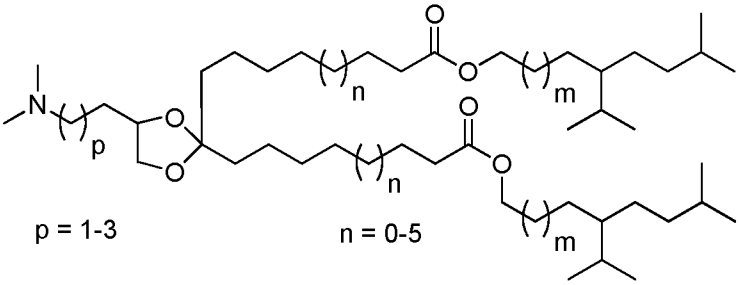


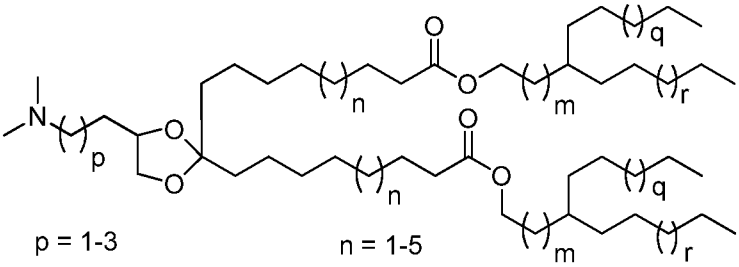
r = 0-2 m = 0-5 p = 0-3
n = 0-5 q = 0-3

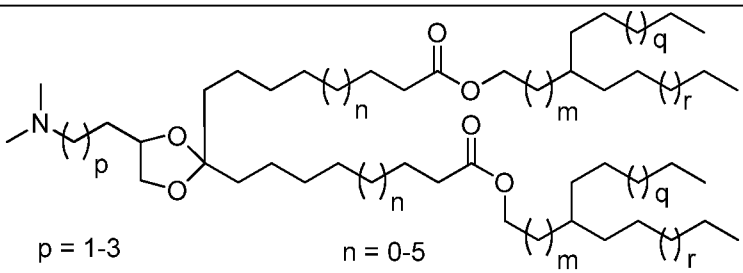


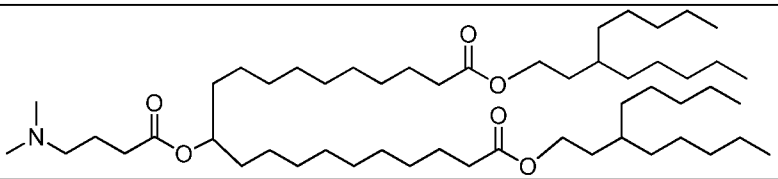
r = 0-2 m = 0-5 p = 0-3
n = 0-5 q = 0-3

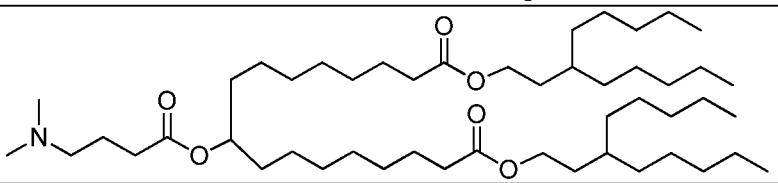

p = 1-3
n = 1-5
m = 0-3

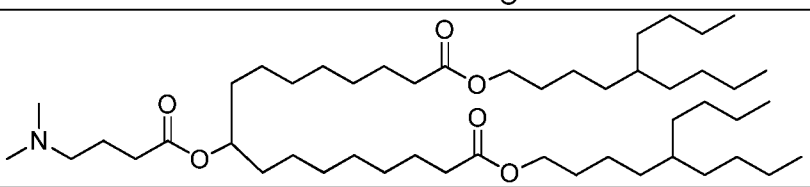

p = 1-3
n = 0-5
m = 0-3

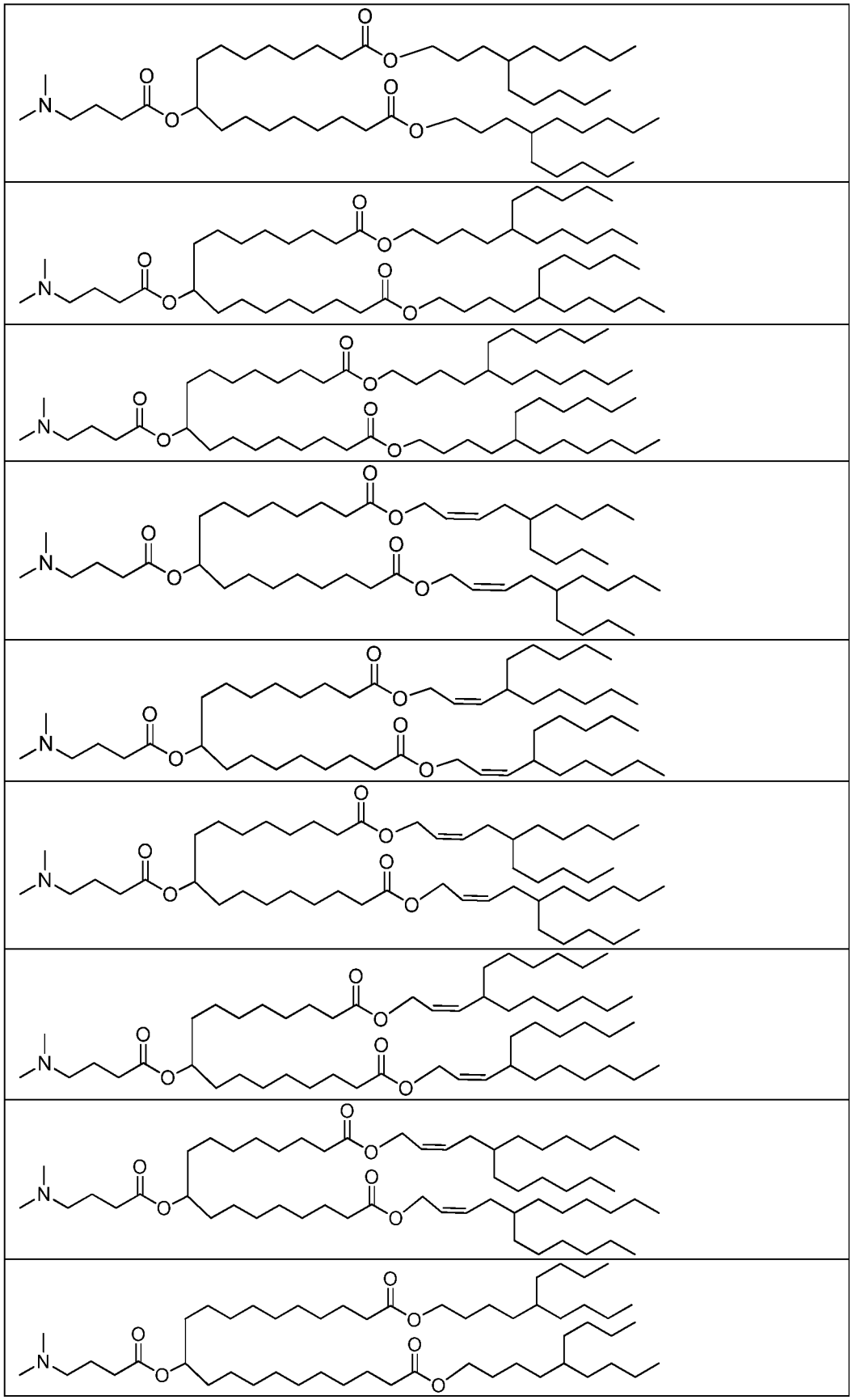

p = 1-3
n = 1-5
m = 0-3; q = 0-4; r = 0-4

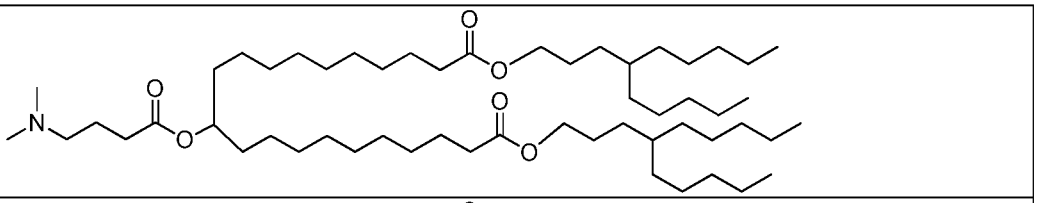

p = 1-3
n = 0-5
m = 0-3; q = 0-4; r = 0-4

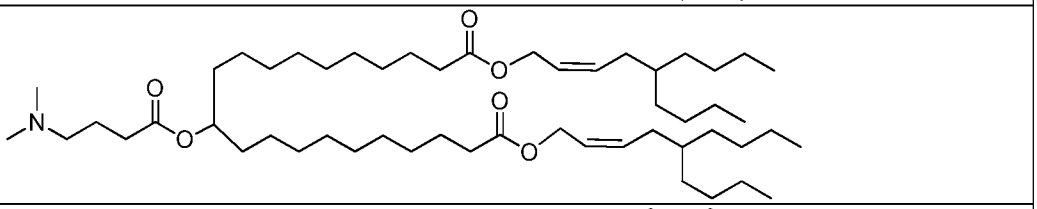


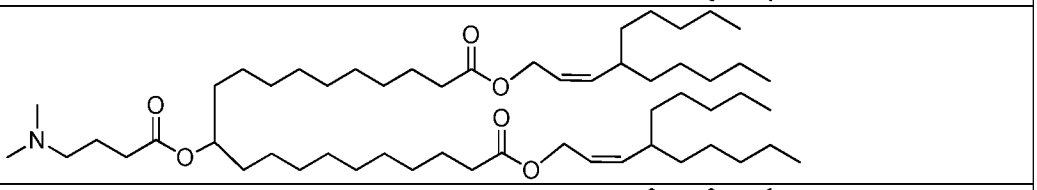


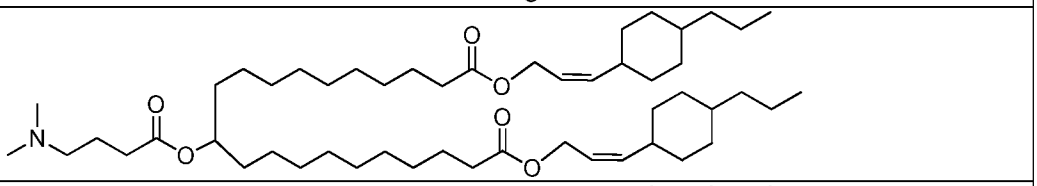


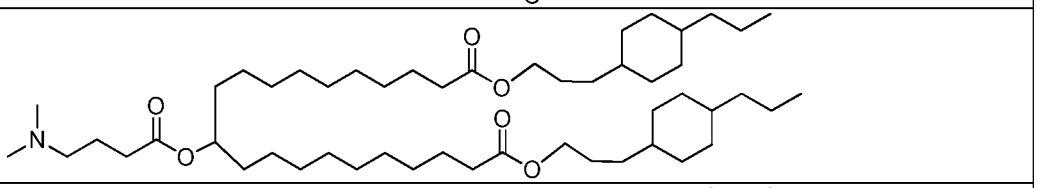


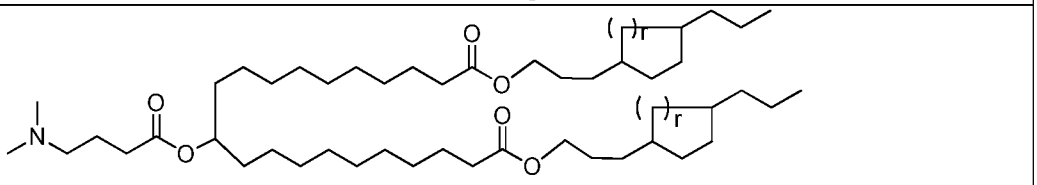




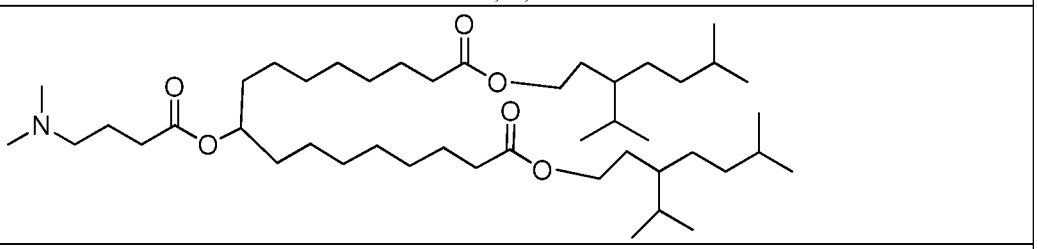




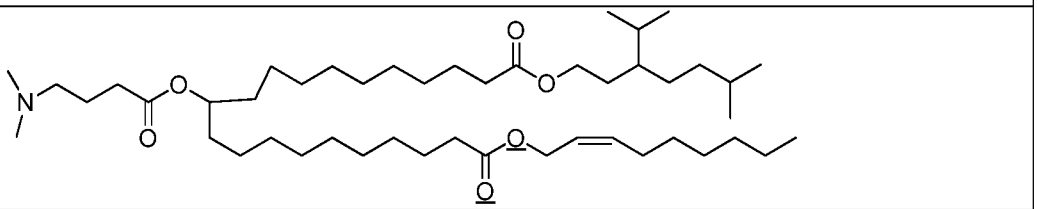


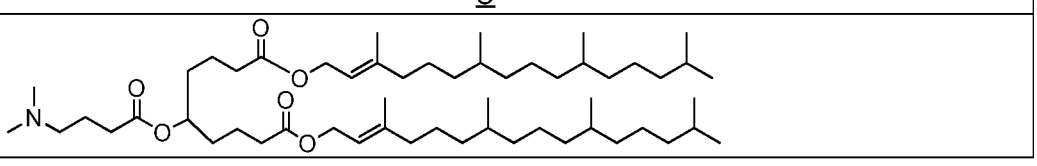


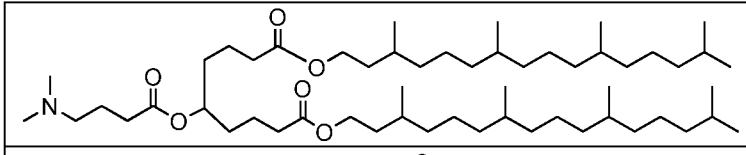
r = 0, 1, or 2

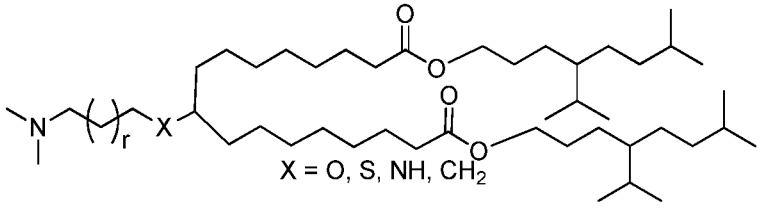


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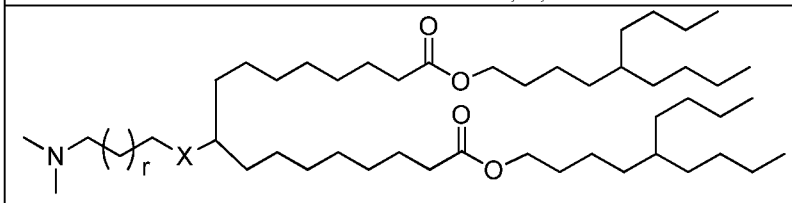




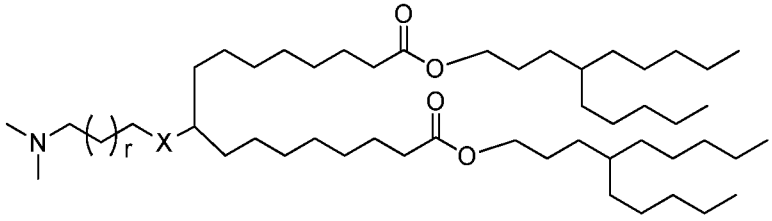




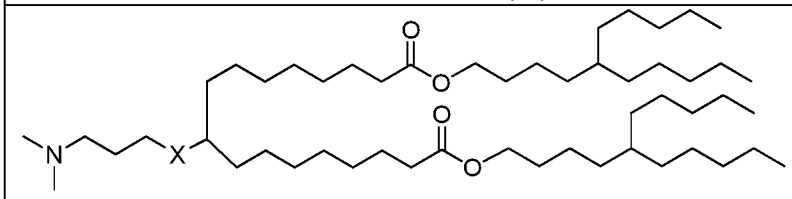

r = 0, 1, or 2

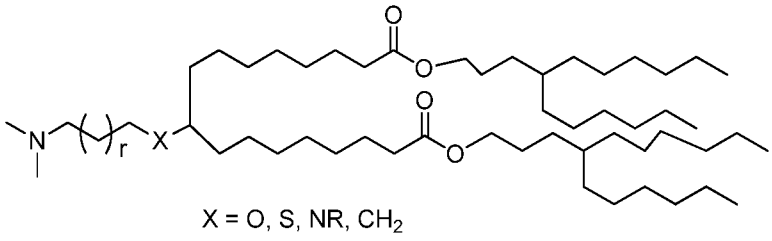



r = 0, 1, or 2

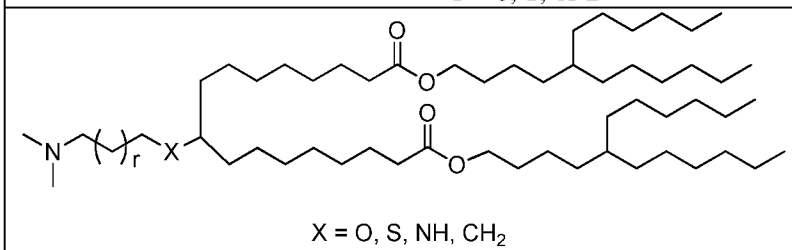



r = 0, 1, or 2

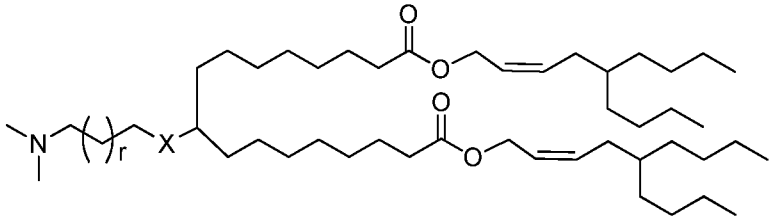



r = 0, 1, or 2

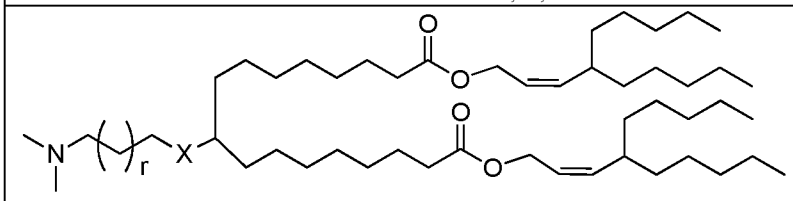



r = 0, 1, or 2



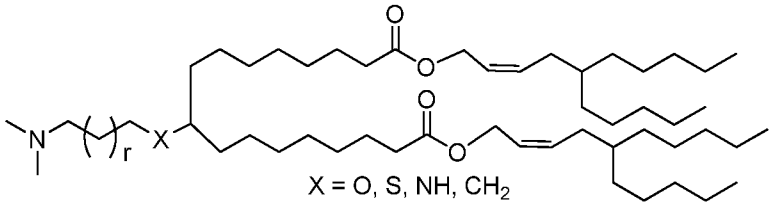
X = O, S, NH, $CH_2$

r = 0, 1, or 2



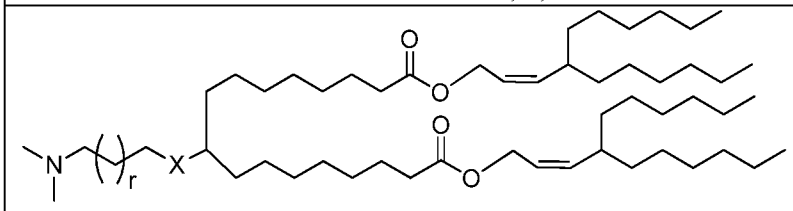
X = O, S, NH, $CH_2$

r = 0, 1, or 2



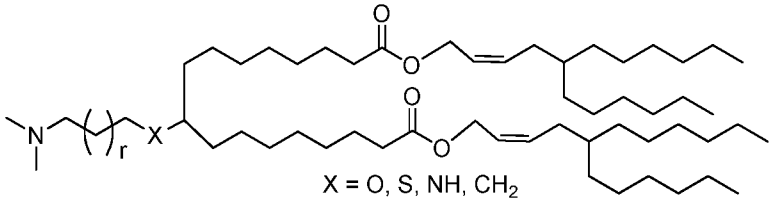
X = O, S, NH, $CH_2$

r = 0, 1, or 2



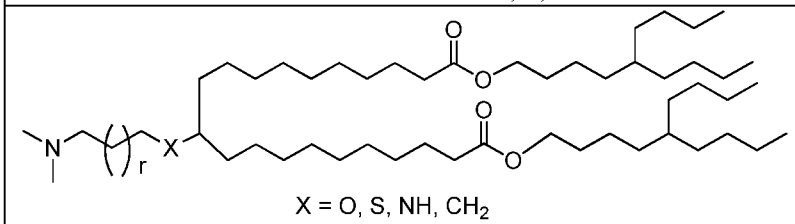
X = O, S, NH, $CH_2$

r = 0, 1, or 2



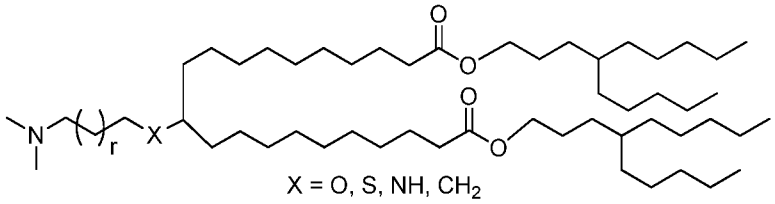
X = O, S, NH, $CH_2$

r = 0, 1, or 2

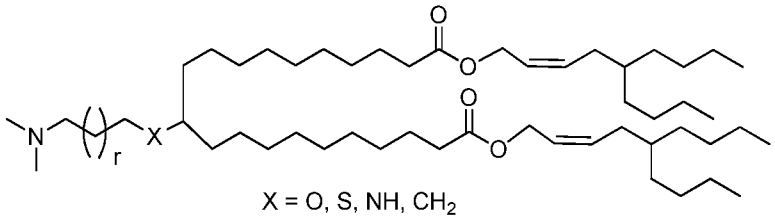


X = O, S, NH, $CH_2$

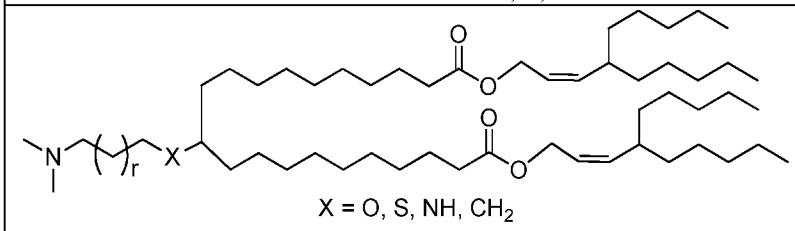
r = 0, 1, or 2



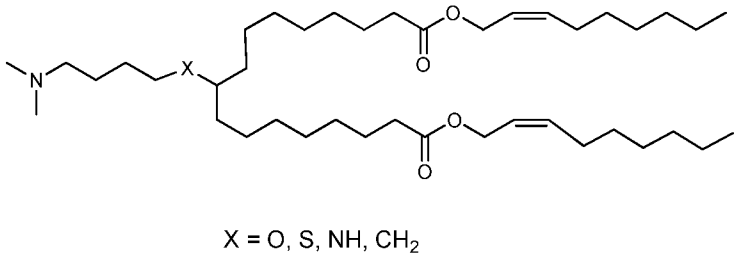

r = 0, 1, or 2

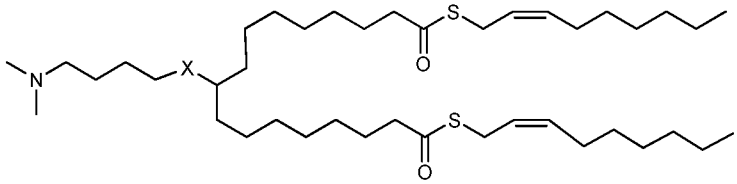


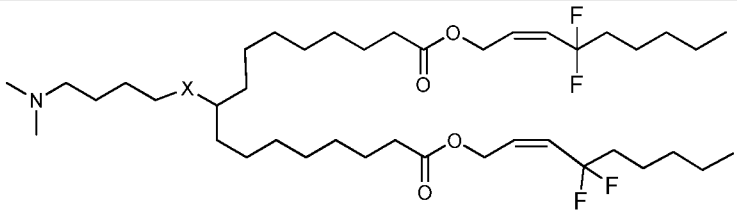

r = 0, 1, or 2

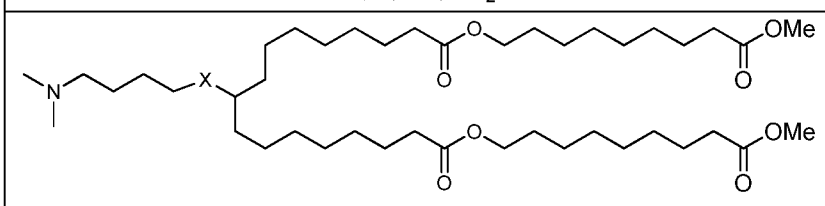


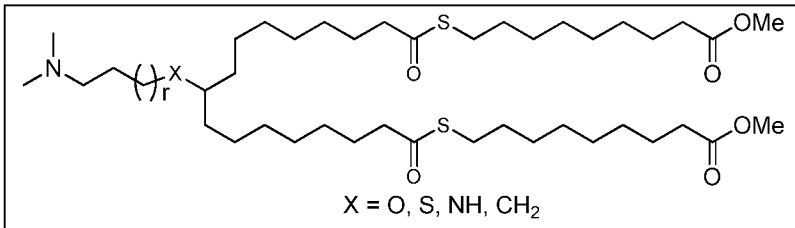

r = 0, 1, or 2



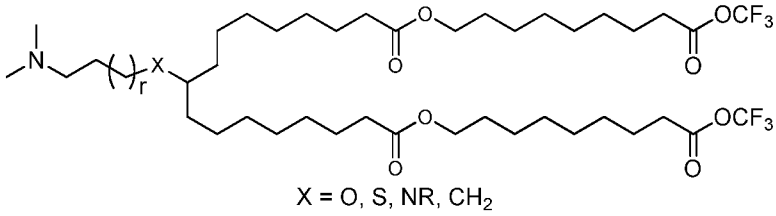



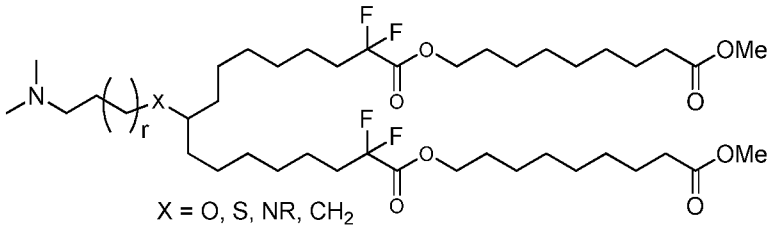





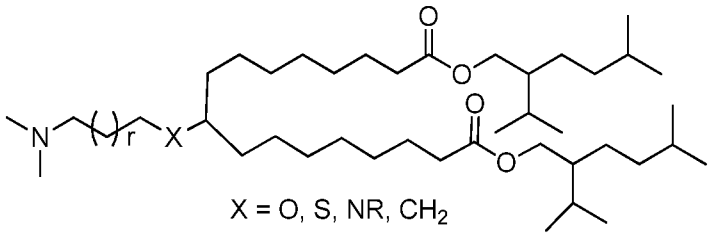

r = 0, 1, or 2



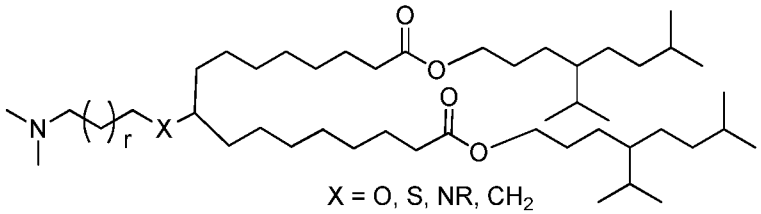

r = 0, 1, or 2



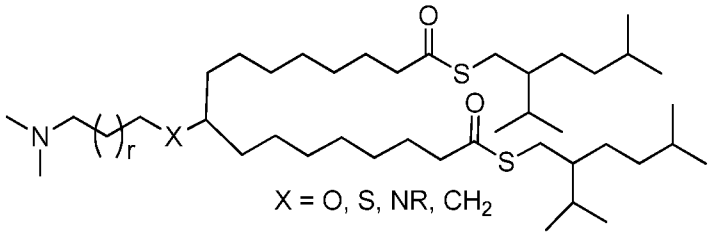

r = 0, 1, or 2



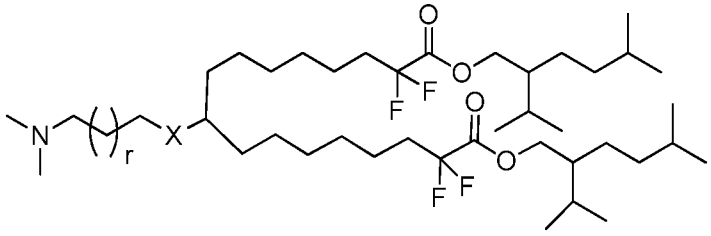

r = 0, 1, or 2



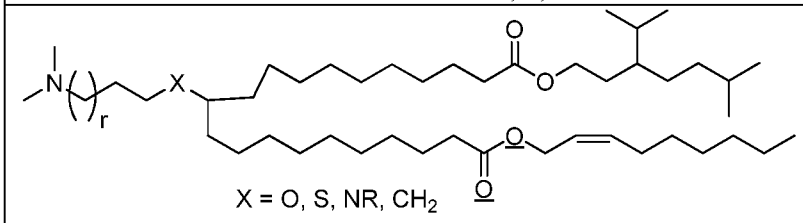

r = 0, 1, or 2




r = 0, 1, or 2

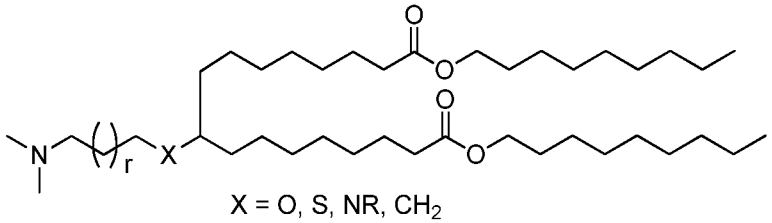


r = 0, 1, or 2



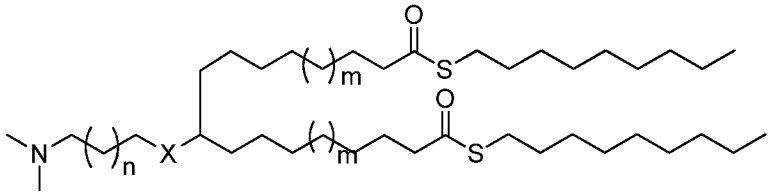
X = O, S, NR, $CH_2$

r = 0, 1, or 2

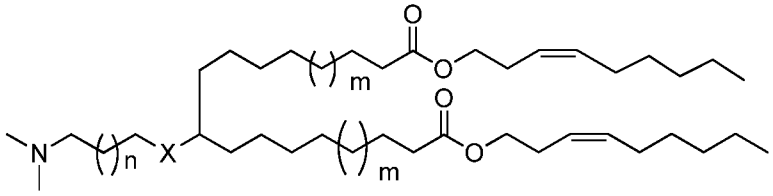


X = O, S, NR, $CH_2$

r = 0, 1, or 2

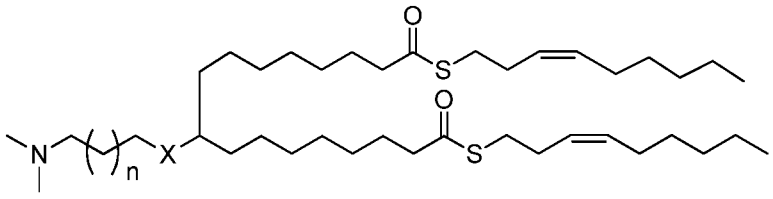


m = 0-5, n = 0, 1, or 2

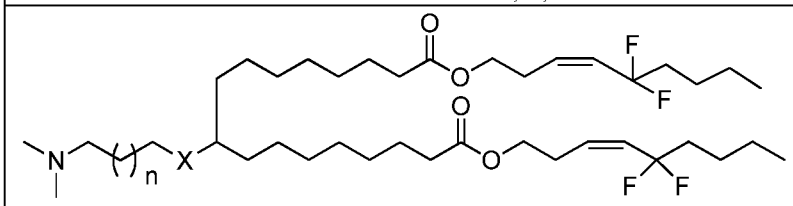


X = O, S, NR, $CH_2$

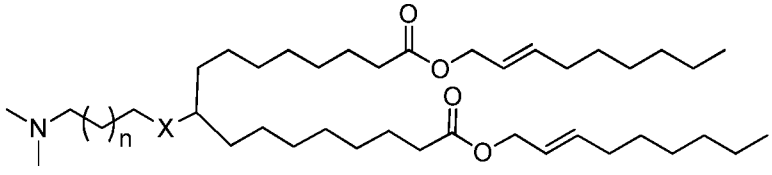
m = 0-5, n = 0, 1, or 2



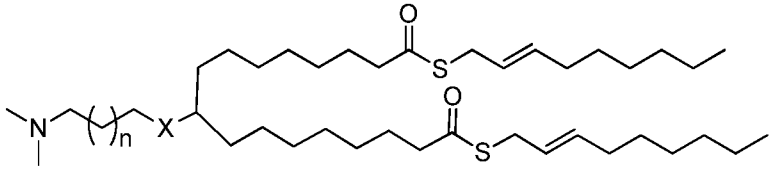
n = 0, 1, or 2



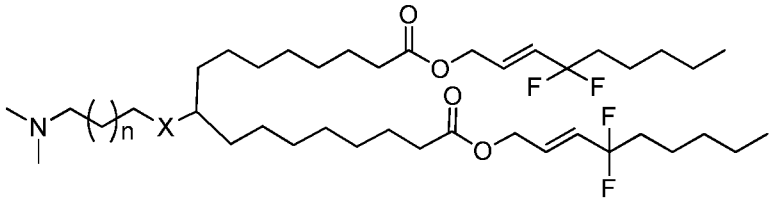
n = 0, 1, or 2



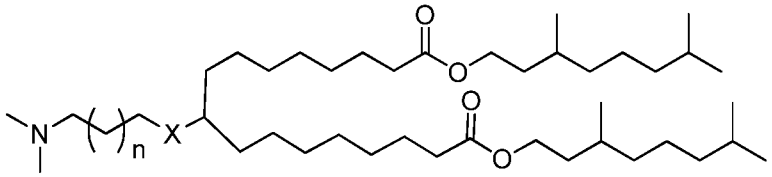
n = 0, 1, or 2



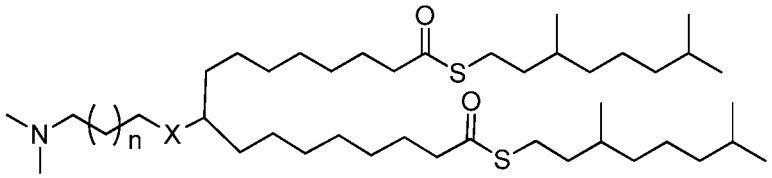
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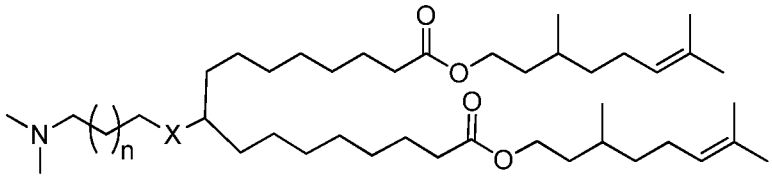
n = 0, 1, or 2



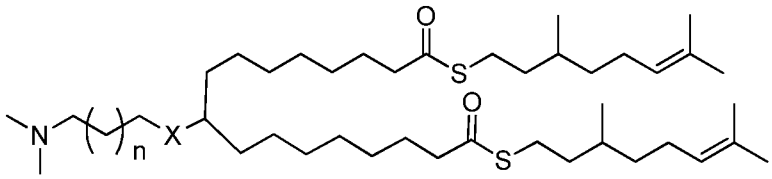
n = 0, 1, or 2



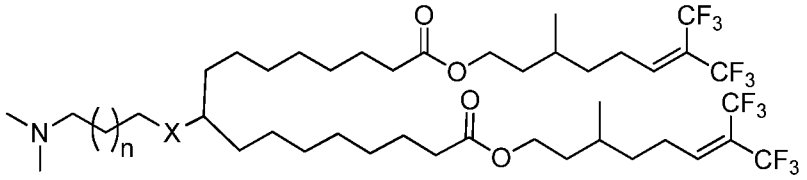
n = 0, 1, or 2



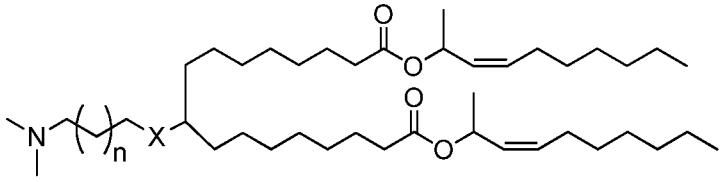
n = 0, 1, or 2



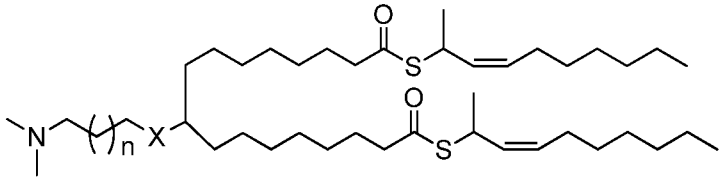
n = 0, 1, or 2



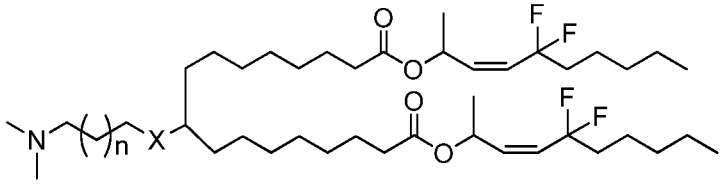
n = 0, 1, or 2



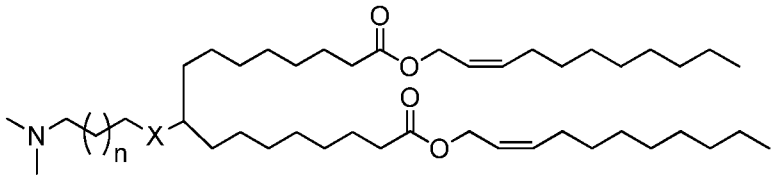
n = 0, 1, or 2



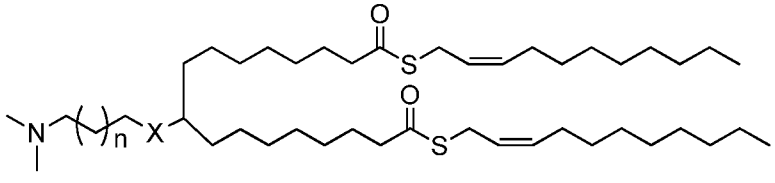
n = 0, 1, or 2



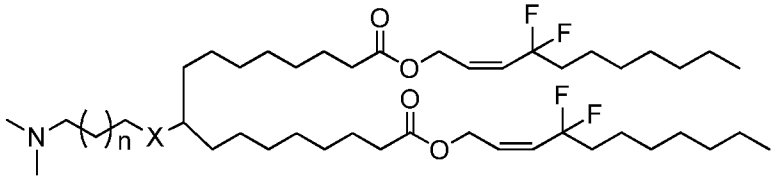
n = 0, 1, or 2



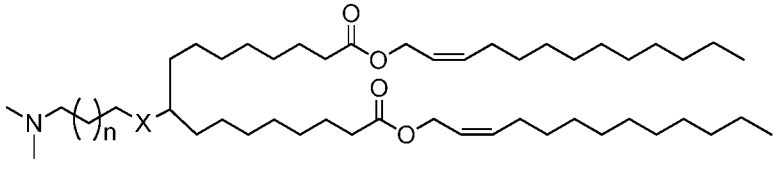
n = 0, 1, or 2



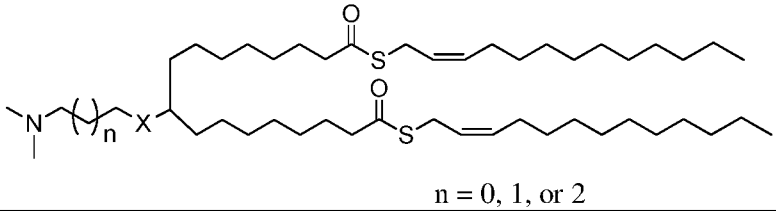
n = 0, 1, or 2



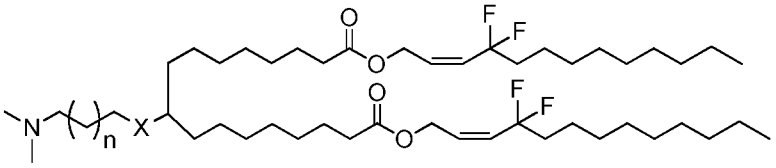
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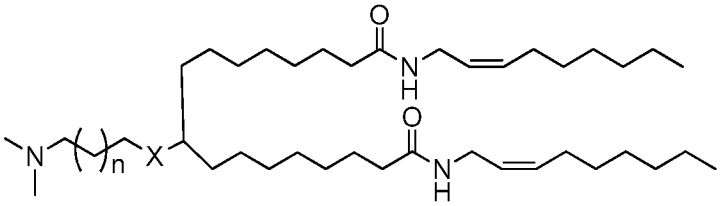
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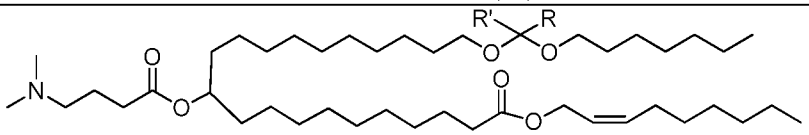
n = 0, 1, or 2



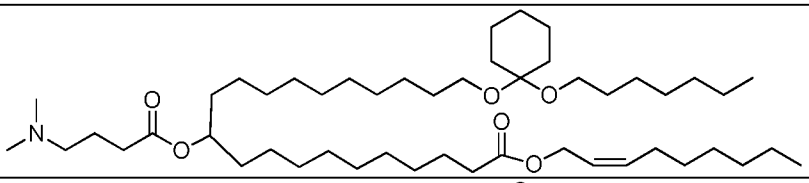
n = 0, 1, or 2

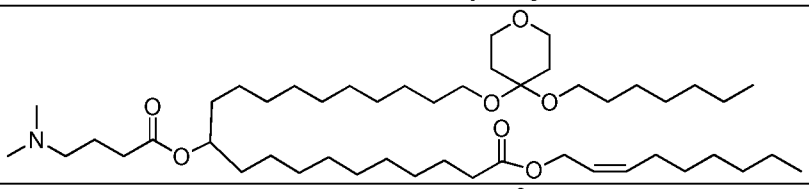


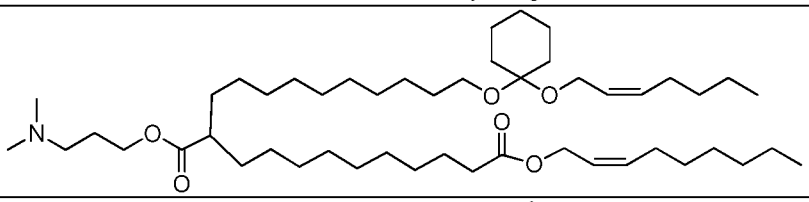
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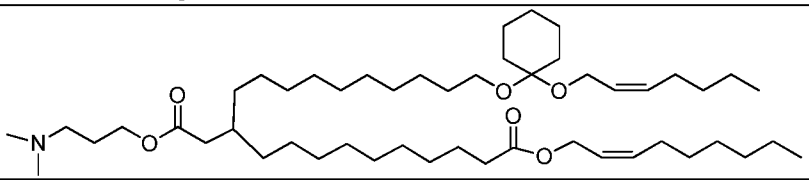


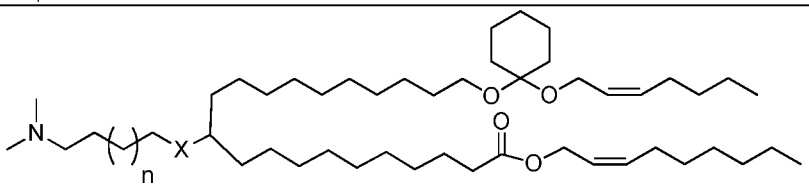
R, R' = $CH_3$, Cylcopentyl etc





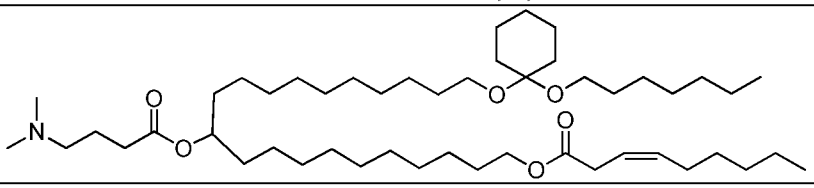


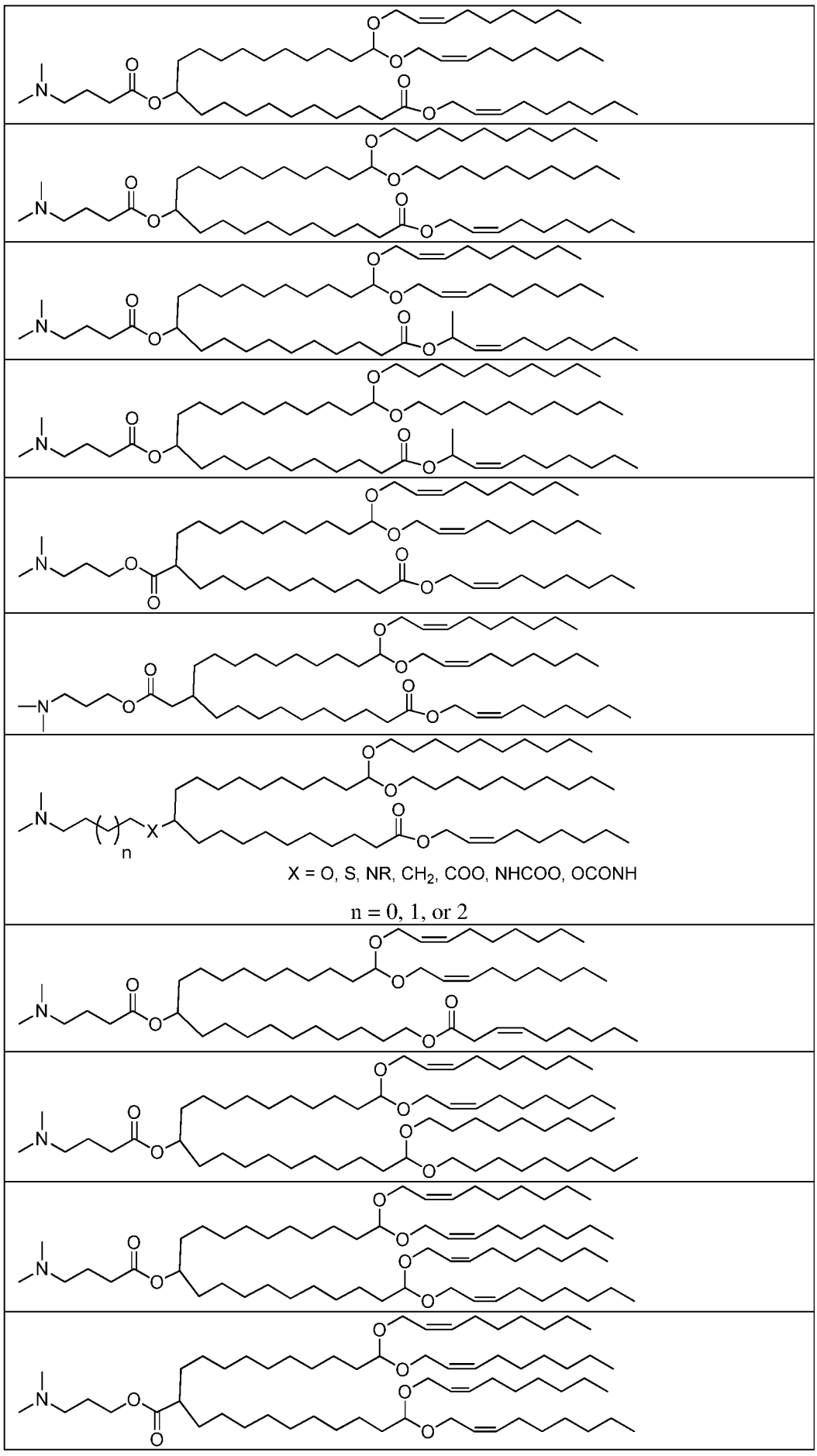


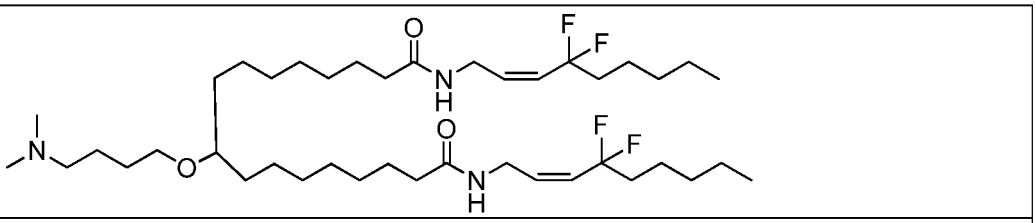


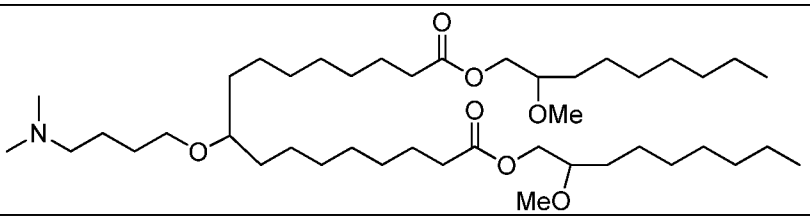
X = O, S, NR, $CH_2$, COO, NHCOO, OCONH

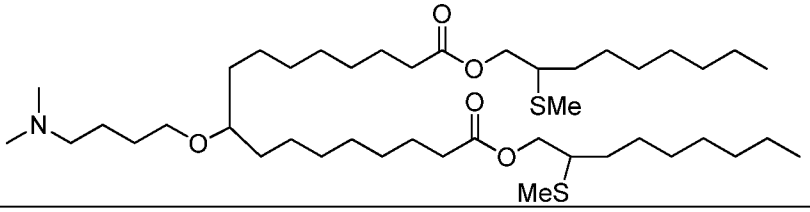
n = 0, 1, or 2

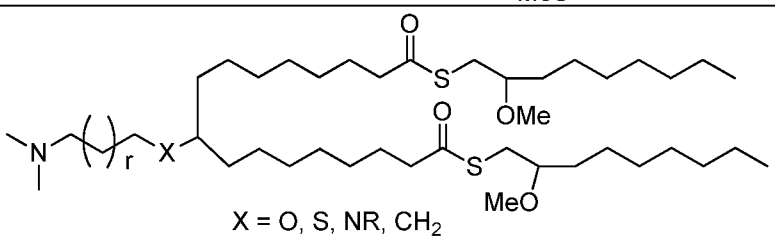



X = O, S, NR, CH2, COO, NHCOO, OCONH
n = 0, 1, or 2



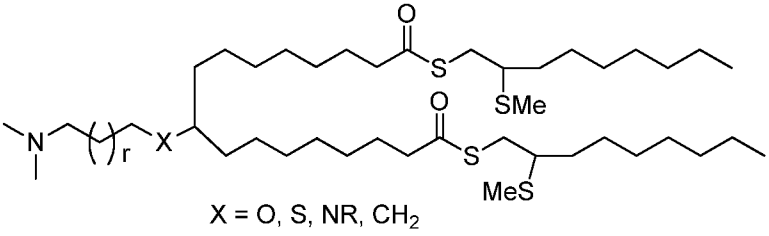






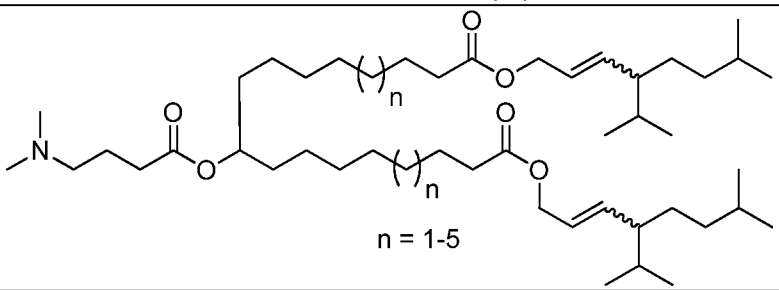
X = O, S, NR, $CH_2$

r = 0, 1, or 2

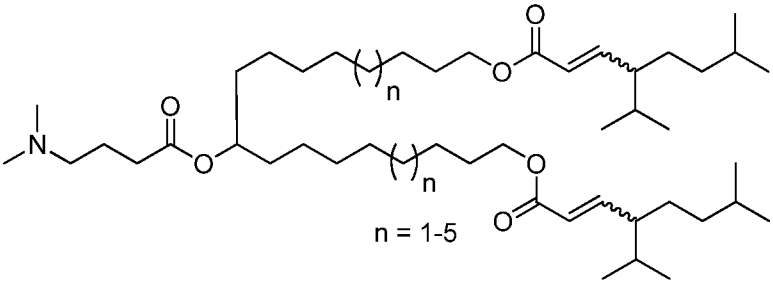


X = O, S, NR, $CH_2$

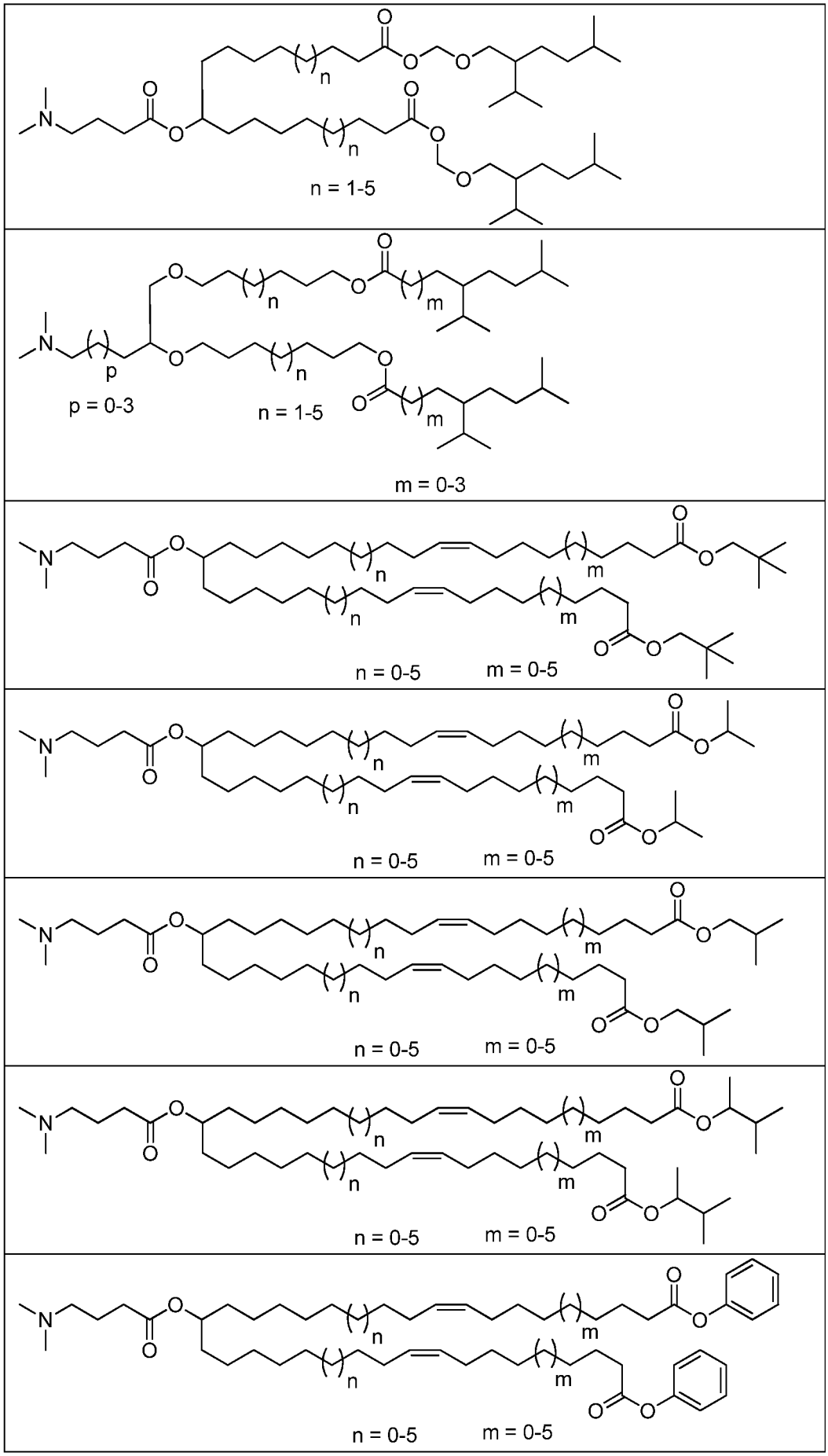
r = 0, 1, or 2

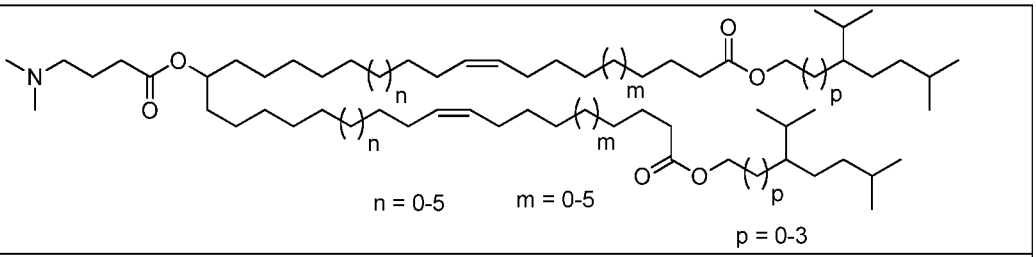


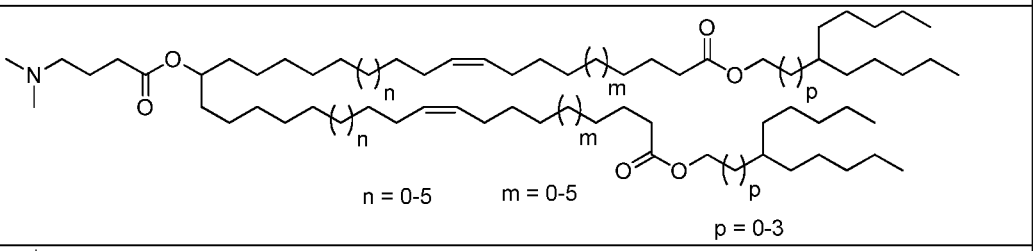
n = 1-5

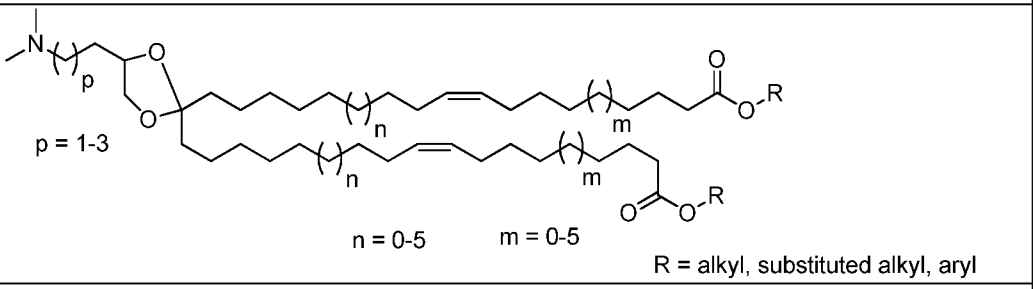


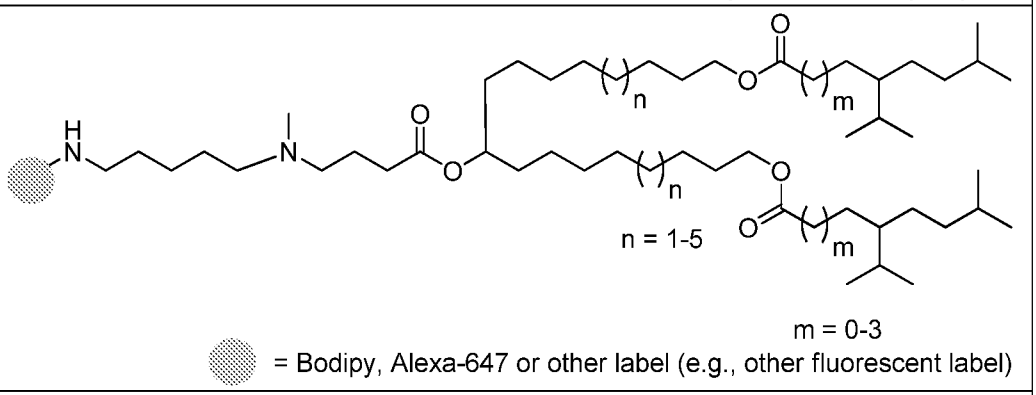
n = 1-5

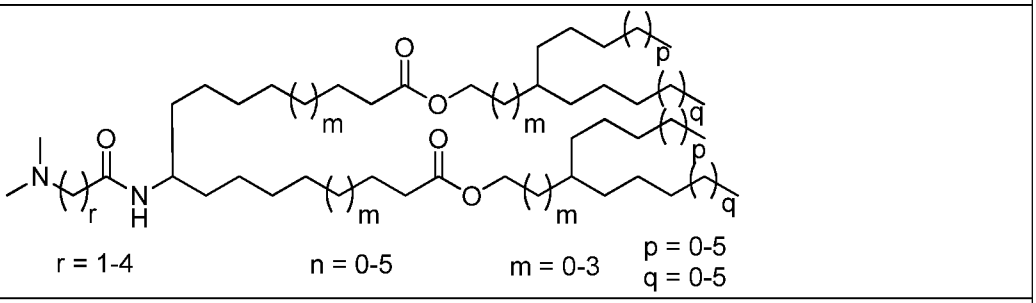

n = 1-5
p = 0-3
n = 1-5
m = 0-3
n = 0-5
m = 0-5
n = 0-5
m = 0-5
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n = 0-5
m = 0-5

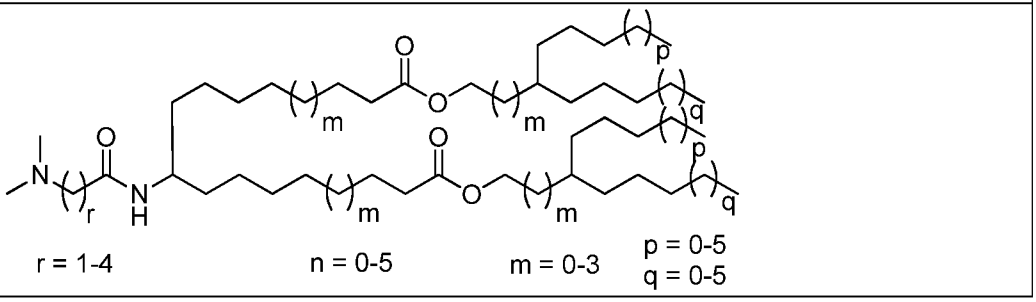

n = 0-5
m = 0-5
p = 0-3

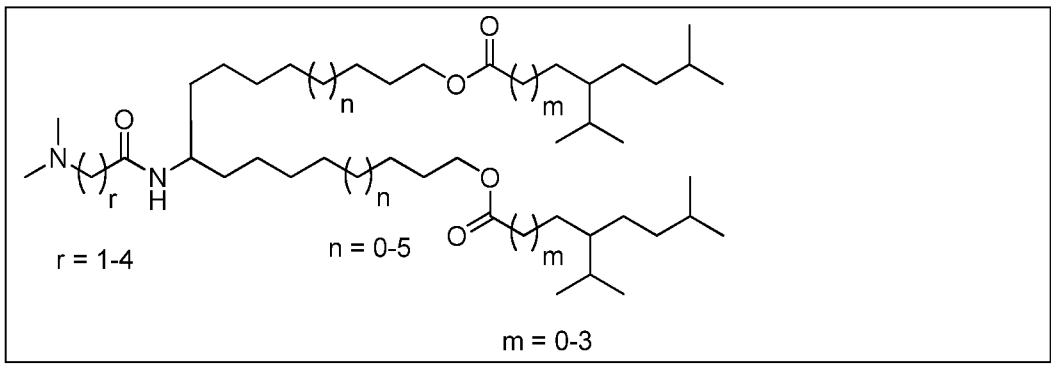

n = 0-5
m = 0-5
p = 0-3


p = 1-3
n = 0-5
m = 0-5
R = alkyl, substituted alkyl, aryl

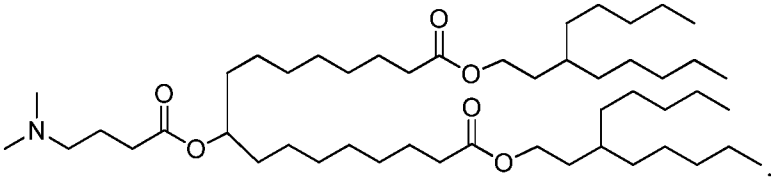

n = 1-5
m = 0-3
= Bodipy, Alexa-647 or other label (e.g., other fluorescent label)


r = 1-4
n = 0-5
m = 0-3
p = 0-5
q = 0-5

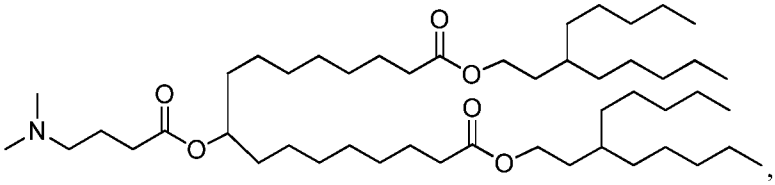

r = 1-4
n = 0-5
m = 0-3
p = 0-5
q = 0-5



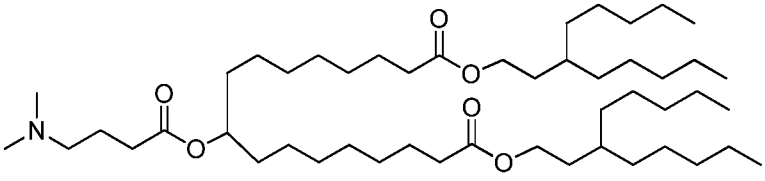

In one preferred embodiment, the cationic lipid is



In certain embodiments, the dsRNA agents of the invention are formulated with a cationic lipid, *e.g.*,



distearoylphosphatidylcholine (DSPC), cholesterol (Chol), and 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol (PEG-DMG). In one embodiment, the ratio of



:DSPC:Chol:PEG-DMG is about 50:12:36:2, respectively.

Included in the present invention is the free form of the cationic lipids described herein, as well as pharmaceutically acceptable salts and stereoisomers thereof. The cationic lipid can be a protonated salt of the amine cationic lipid. The term "free form" refers to the amine cationic lipids in non-salt form. The free form may be regenerated by treating the salt with a suitable dilute aqueous base solution such as dilute aqueous NaOH, potassium carbonate, ammonia and sodium bicarbonate. The pharmaceutically acceptable salts of the instant cationic lipids can be synthesized from the cationic lipids of this invention which contain a basic or acidic moiety by conventional chemical methods. Generally, the salts of the basic cationic lipids are prepared either by ion exchange chromatography or by reacting the free base with stoichiometric amounts or with an excess of the desired salt-forming inorganic or organic acid in a suitable solvent or various combinations of solvents. Similarly, the salts of the acidic compounds are formed by reactions with the appropriate inorganic or organic base.

Thus, pharmaceutically acceptable salts of the cationic lipids of this invention include non-toxic salts of the cationic lipids of this invention as formed by reacting a basic instant cationic lipids with an inorganic or organic acid. For example, non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like, as well as salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxy-benzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and trifluoroacetic (TFA).

When the cationic lipids of the present invention are acidic, suitable "pharmaceutically acceptable salts" refers to salts prepared form pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, and zinc. In one embodiment, the base is selected from ammonium, calcium, magnesium, potassium and sodium. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as arginine, betaine caffeine, choline, N,N$^1$-dibenzylethylenediamine, diethylamin, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine tripropylamine, and tromethamine.

It will also be noted that the cationic lipids of the present invention may potentially be internal salts or zwitterions, since under physiological conditions a deprotonated acidic moiety in the compound, such as a carboxyl group, may be anionic, and this electronic charge might then be balanced off internally against the cationic charge of a protonated or alkylated basic moiety, such as a quaternary nitrogen atom.

*C. 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 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 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 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 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 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 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 a CTNNB13-associated disorder, *e.g.*, a cancer.

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 a CTNNB1-associated disorder, *e.g.*, cancer. 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 CTNNB1 Expression

The present invention also provides methods of inhibiting expression of a CTNNB1 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 CTNNB1 in the cell, thereby inhibiting expression of CTNNB1 in the cell. In some embodiments of the disclosure, expression of a CTNNB1 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 $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

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 a CTNNB1" is intended to refer to inhibition of expression of any CTNNB1 gene (such as, *e.g.*, a mouse CTNNB1 3 gene, a rat CTNNB1 gene, a monkey CTNNB1 gene, or a human CTNNB1 gene) as well as variants or mutants of a CTNNB1 gene. Thus, the CTNNB1 gene may be a wild-type CTNNB1 gene, a mutant CTNNB1 gene, or a transgenic CTNNB1 gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a CTNNB1 gene" includes any level of inhibition of a CTNNB1 gene, *e.g.*, at least partial suppression of the expression of a CTNNB1 gene. The expression of the CTNNB1 gene may be assessed based on the level, or the change in the level, of any variable associated with CTNNB1 gene expression, *e.g.*, CTNNB1 mRNA level or CTNNB1 protein level. It is understood that CTNNB1 is expressed predominantly in the liver.

The expression of a CTNNB1 may also be assessed indirectly based on other variables associated with CTNNB1 gene expression, *e.g.,* level of beta-catenin expression in the cytoplasma, nuclear localization of beta-catenin, or expression of certain target genes such as Jun, c-Myc and CyclinD-1 or other oncogenes under transcription control of beta-catenin.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with CTNNB1 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 as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of a CTNNB1 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 a CTNNB1 gene is inhibited by at least 70%. It is further understood that inhibition of CTNNB1 expression in certain tissues, *e.g.*, in 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 human target gene (*i.e.*, CTNNB1), *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 a CTNNB1 gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example,

in a sample derived from a subject) in which a CTNNB1 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 a CTNNB1 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 a CTNNB1 gene may be assessed in terms of a reduction of a parameter that is functionally linked to CTNNB1 gene expression, *e.g.*, CTNNB1 protein level in blood or serum from a subject. CTNNB1 gene silencing may be determined in any cell expressing CTNNB1, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of a CTNNB1 protein may be manifested by a reduction in the level of the CTNNB1 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 a CTNNB1 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 CTNNB1 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 CTNNB1 in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.,* mRNA of the CTNNB1 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 CTNNB1 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 CTNNB1. 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 CTNNB1 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 CTNNB1 mRNA.

An alternative method for determining the level of expression of CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 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 CTNNB1 may be assessed using measurements of the level or change in the level of CTNNB1 mRNA or CTNNB1 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 CTNNB1, thereby preventing or treating a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.,* hepatocellular carcinoma. 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 a CTNNB1 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-primate cell. In certain embodiments, the cell is a human cell, *e.g.,* a human liver cell. In the methods of the invention, CTNNB1 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 a part of an RNA transcript of the CTNNB1 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, intraocular, anterior or posterior juxtascleral, 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 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 a CTNNB1 gene in a mammal. The methods include administering to the mammal a composition comprising a dsRNA that targets a CTNNB1 gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the CTNNB1 gene, thereby inhibiting expression of the CTNNB1 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 Example 2. Reduction in protein production can be assessed by any methods known it the art, *e.g.* ELISA. In certain embodiments, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the CTNNB1 gene or protein expression. In other embodiments, a blood sample serves as the subject sample for monitoring the reduction in the CTNNB1 protein expression.

The present invention further provides methods of treatment in a subject in need thereof, *e.g.*, a subject diagnosed with a CTNNB1-associated disorder, such as cancer, *e.g.,* hepatocellular carcinoma.

The present invention further provides methods of prophylaxis in a subject in need thereof. 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 CTNNB1 expression, in a prophylactically effective amount of a dsRNA targeting a CTNNB1 gene or a pharmaceutical composition comprising a dsRNA targeting a CTNNB1 gene.

In one aspect, the present invention provides methods of treating a subject having a disorder that would benefit from reduction in CTNNB1 expression, *e.g.,* a CTNNB1-associated disorder, such as cancer, *e.g.,* hepatocellular carcinoma.

Treatment of a subject that would benefit from a reduction and/or inhibition of CTNNB1 gene expression includes therapeutic treatment (*e.g.*, a subject is having a cancer) and prophylactic treatment (*e.g.*, the subject is not having a cancer or a subject may be at risk of developing a cancer).

In some embodiments, the CTNNB1-associated disorder is cancer. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, myeloma and leukemia. In some

embodiments, the cancer comprises a solid tumor cancer. In other embodiments, the cancer comprises a blood based cancer, e..g, leukemia, lymphoma or myeloma. More particular nonlimiting examples of such cancers include squamous cell cancer, small-cell lung cancer, pituitary cancer, esophageal cancer, astrocytoma, soft tissue sarcoma, non-small cell lung cancer (including squamous cell non-small cell lung cancer), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, renal cell carcinoma, hepatocellular carcinoma, hepatoblastomas, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, brain cancer, endometrial cancer, testis cancer, cholangiocarcinoma, gallbladder carcinoma, gastric cancer, melanoma, and various types of head and neck cancer (including squamous cell carcinoma of the head and neck).

In some embodiments, the CTNNB1-associated disorder is hepatocellular carcinoma.

In some embodiments, the RNAi agent is administered to a subject in an amount effective to inhibit CTNNB1 expression in a cell within the subject. The amount effective to inhibit CTNNB1 expression in a cell within a subject may be assessed using methods discussed above, including methods that involve assessment of the inhibition of CTNNB1 mRNA, CTNNB1 protein, or related variables, such as tumor formation.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is 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.

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 CTNNB1 gene expression are subjects susceptible to or diagnosed with a CTNNB1-associated disorder, such as cancer, *e.g.,* hepatocellular carcinoma. In an embodiment, the method includes administering a composition featured herein such that expression of the target a CTNNB1 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 CTNNB1 gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result prevention or treatment of a CTNNB1-associated disorder, *e.g.*, cancer, *e.g.,* hepatocellular carcinoma. Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

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 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 CTNNB1 gene expression, *e.g.*, a subject having a CTNNB1-associated disorder, in combination with 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.

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 CTNNB1 is administered in combination with, *e.g.*, an agent useful in treating a CTNNB1-associated disorder. Exemplary additional therapeutics and treatments for treating a CTNNB1-associated disorder, *e.g.*, cancer, may include surgery, chemotherapy, radiation therapy, or the administration of one or more additional anti-cancer agents, such as a chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent and/or a anti-neoplastic composition. Nonlimiting examples of anti-cancer agents, chemotherapeutic agents, growth inhibitory agents, anti-angiogenesis agents, and anti-neoplastic compositions that can be used in combination with the iRNA of the present invention are as follows.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include, but are not limited to, alkylating agents such as thiotepa and Cytoxan® cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne

antibiotics (e.g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, Adriamycin® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2- ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., Taxol® paclitaxel (Bristol- Myers Squibb Oncology, Princeton, N.J.), Abraxane® Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and Taxotere® doxetaxel (Rhône- Poulenc Rorer, Antony, France); chloranbucil; Gemzar® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11) (including the treatment regimen of irinotecan with 5-FU and leucovorin); topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine; combretastatin; leucovorin (LV); oxaliplatin, including the oxaliplatin treatment regimen (FOLFOX); inhibitors of PKC-alpha, Raf, H-Ras, EGFR (e.g., erlotinib (Tarceva®)) and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Further nonlimiting exemplary chemotherapeutic agents include anti-hormonal agents that act to regulate or inhibit hormone action on cancers such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and Fareston® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, Megase® megestrol acetate, Aromasin® exemestane, formestanie, fadrozole, Rivisor® vorozole, Femara® letrozole, and Arimidex® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Ralf and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., Angiozyme® ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, Allovectin® vaccine, Leuvectin® vaccine, and Vaxid® vaccine; Proleukin® rIL-2; Lurtotecan® topoisomerase 1 inhibitor; Abarelix® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In some embodiments, the iRNA of the invention may be further administered with gemcitabine-based chemotherapy in which one or more chemotherapy agents including gemcitabine or including gemcitabine and nab-paclitaxel are administered. In some such embodiments, the iRNA of the invention may be administered with at least one chemotherapy agent selected from gemcitabine, nab-paclitaxel, leukovorin (folinic acid), 5-fluorouracil (5-FU), irinotecan, and oxaliplatin. FOLFIRINOX is a chemotherapy regime comprising leukovorin, 5-FU, irinotecan (such as liposomal irinotecan injection), and oxaliplatin. In some embodiments, the iRNA of the invention may be further administered with gemcitabine-based chemotherapy. In some embodiments, the iRNA of the invention may be further administered with at least one agent selected from (a) gemcitabine; (b) gemcitabine and nab-paclitaxel; and (c) FOLFIRINOX. In some embodiments, the at least one agent is gemcitabine. In some such embodiments, the cancer to be treated is pancreatic cancer.

An "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide (including, e.g., an inhibitory RNA (RNAi or siRNA)), a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent, e.g., antibodies to VEGF-A (e.g., bevacizumab (Avastin®)) or to the VEGF-A receptor (e.g., KDR receptor or Flt-1 receptor), anti-PDGFR inhibitors such as Gleevec® (Imatinib Mesylate), small molecules that block VEGF receptor signaling (e.g., PTK787/ZK2284, SU6668, Sutent®/SU11248 (sunitinib malate), AMG706, or those described in, e.g., international patent application WO 2004/113304). Anti-angiogensis agents also include native angiogenesis inhibitors , e.g., angiostatin, endostatin, etc. See, e.g., Klagsbrun and D'Amore (1991) Annu. Rev. Physiol. 53:217-39; Streit and Detmar (2003)

Oncogene 22:3172-3179 (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo (1999) Nature Medicine 5(12):1359-1364; Tonini et al. (2003) Oncogene 22:6549-6556 (e.g., Table 2 listing known anti-angiogenic factors); and, Sato (2003) Int. J. Clin. Oncol. 8:200-206 (e.g., Table 1 listing anti-angiogenic agents used in clinical trials).

A "growth inhibitory agent" as used herein refers to a compound or composition that inhibits growth of a cell (such as a cell expressing VEGF) either in vitro or in vivo. Thus, the growth inhibitory agent may be one that significantly reduces the percentage of cells (such as a cell expressing VEGF) in S phase. Examples of growth inhibitory agents include, but are not limited to, agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (Taxotere®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (Taxol®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

The term "anti-neoplastic composition" refers to a composition useful in treating cancer comprising at least one active therapeutic agent. Examples of therapeutic agents include, but are not limited to, e.g., chemotherapeutic agents, growth inhibitory agents, cytotoxic agents, agents used in radiation therapy, anti-angiogenesis agents, cancer immunotherapeutic agents, apoptotic agents, anti-tubulin agents, and other-agents to treat cancer, such as anti-HER-2 antibodies, anti-CD20 antibodies, an epidermal growth factor receptor (EGFR) antagonist (e.g., a tyrosine kinase inhibitor), HER1/EGFR inhibitor (e.g., erlotinib (Tarceva®), platelet derived growth factor inhibitors (e.g., Gleevec® (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferons, cytokines, antagonists (e.g., neutralizing antibodies) that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA, or VEGF receptor(s), and other bioactive and organic chemical agents, etc. Combinations thereof are also included in the invention.

In some embodiments, the iRNA targeting CTNNB1 is administered in combination with, *e.g.*, an agent useful in treating hepatocellular carcinoma (HCC), for example, but not limited to, sorafenib.

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.

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 CTNNB1 (*e.g.,* means for measuring the inhibition of CTNNB1 mRNA, CTNNB1 protein, and/or CTNNB1 activity). Such means for measuring the inhibition of CTNNB1 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. 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 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.

## EXAMPLES

### Example 1. iRNA Synthesis

*Source of reagents*

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

*siRNA Design*

siRNAs targeting the human beta-catenin (CTNNB1) gene (human: NCBI refseqID NM_001904.4, NCBI GeneID: 1499) were designed using custom R and Python scripts. The human NM_001904.4 REFSEQ mRNA, has a length of 3661 bases.

Detailed lists of the unmodified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 2. Detailed lists of the modified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 3.

It is to be understood that, throughout the application, a duplex name without a decimal is equivalent to a duplex name with a decimal which merely references the batch number of the duplex. For example, AD-959917 is equivalent to AD-959917.1.

*siRNA Synthesis*

siRNAs were designed, synthesized, and prepared using methods known in the art.

Briefly, siRNA sequences were synthesized on a 1 μmol scale using a Mermade 192 synthesizer (BioAutomation) with phosphoramidite chemistry on solid supports. The solid support was controlled pore glass (500-1000 Å) loaded with a custom GalNAc ligand (3'-GalNAc conjugates), universal solid support (AM Chemicals), or the first nucleotide of interest. Ancillary synthesis reagents and standard 2-cyanoethyl phosphoramidite monomers (2'-deoxy-2'-fluoro, 2'-O-methyl, RNA, DNA) were obtained from Thermo-Fisher (Milwaukee, WI), Hongene (China), or Chemgenes (Wilmington, MA, USA). Additional phosphoramidite monomers were procured from commercial suppliers, prepared in-house, or procured using custom synthesis from various CMOs. Phosphoramidites were prepared at a concentration of 100 mM in either acetonitrile or 9:1 acetonitrile:DMF and were coupled using 5-Ethylthio-1H-tetrazole (ETT, 0.25 M in acetonitrile) with a reaction time of 400 s. Phosphorothioate linkages were generated using a 100 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, MA, USA)) in anhydrous acetonitrile/pyridine (9:1 v/v). Oxidation time was 5 minutes. All sequences were synthesized with final removal of the DMT group ("DMT-Off").

Upon completion of the solid phase synthesis, solid-supported oligoribonucleotides were treated with 300 μL of Methylamine (40% aqueous) at room temperature in 96 well plates for approximately 2 hours to afford cleavage from the solid support and subsequent removal of all additional base-labile protecting groups. For sequences containing any natural ribonucleotide linkages (2'-OH) protected with a tert-butyl dimethyl silyl (TBDMS) group, a second deprotection step was

performed using TEA.3HF (triethylamine trihydrofluoride). To each oligonucleotide solution in aqueous methylamine was added 200 µL of dimethyl sulfoxide (DMSO) and 300 µL TEA.3HF and the solution was incubated for approximately 30 mins at 60 °C. After incubation, the plate was allowed to come to room temperature and crude oligonucleotides were precipitated by the addition of 1 mL of 9:1 acetontrile:ethanol or 1:1 ethanol:isopropanol. The plates were then centrifuged at 4 °C for 45 mins and the supernatant carefully decanted with the aid of a multichannel pipette. The oligonucleotide pellet was resuspended in 20 mM NaOAc and subsequently desalted using a HiTrap size exclusion column (5 mL, GE Healthcare) on an Agilent LC system equipped with an autosampler, UV detector, conductivity meter, and fraction collector. Desalted samples were collected in 96 well plates and then analyzed by LC-MS and UV spectrometry to confirm identity and quantify the amount of material, respectively.

Duplexing of single strands was performed on a Tecan liquid handling robot. Sense and antisense single strands were combined in an equimolar ratio to a final concentration of 10 µM in 1x PBS in 96 well plates, the plate sealed, incubated at 100 °C for 10 minutes, and subsequently allowed to return slowly to room temperature over a period of 2-3 hours. The concentration and identity of each duplex was confirmed and then subsequently utilized for in vitro screening assays.

**Example 2. *In vitro* screening methods**

*Cell culture and 384-well transfections*

Hep3b cells (ATCC, Manassas, VA) were grown to near confluence at 37°C in an atmosphere of 5% $CO_2$ in Eagle's Minimum Essential Medium (Gibco) supplemented with 10% FBS (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 7.5 µl of Opti-MEM plus 0.1 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 2.5 µl of each siRNA duplex to an individual well in a 384-well plate. The mixture was then incubated at room temperature for 15 minutes. Forty µl of complete growth media without antibiotic containing ~1.5 x$10^4$ 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, 1 nM, and 0.1 nM final duplex concentration.

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

Cells were lysed in 75µl of Lysis/Binding Buffer containing 3 µL of beads per well and mixed for 10 minutes on an electrostatic shaker. The washing steps were automated on a Biotek EL406, using a magnetic plate support. Beads were washed (in 90µL) once in Buffer A, once in Buffer B, and twice in Buffer E, with aspiration steps in between. Following a final aspiration, complete 10µL RT mixture was added to each well, as described below.

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

A master mix of 1µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5µl Reverse Transcriptase, 0.5µl RNase inhibitor and 6.6µl of $H_2O$ per reaction were added per well. Plates were sealed, agitated for 10 minutes on an electrostatic shaker, and then incubated at 37 degrees C for 2 hours. Following this, the plates were agitated at 80 degrees C for 8 minutes.

*Real time PCR*

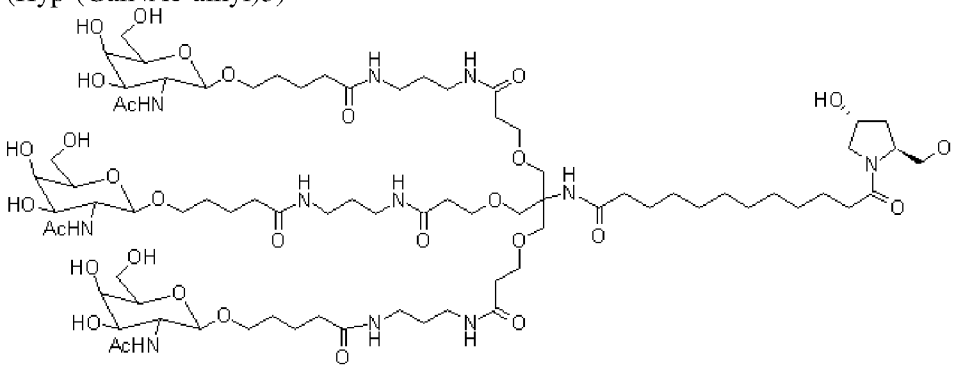
Two microlitre (µl) of cDNA were added to a master mix containing 0.5µl of human GAPDH TaqMan Probe (4326317E), 0.5µl human CTNNB1, 2µl nuclease-free water and 5µl Lightcycler 480 probe master mix (Roche Cat # 04887301001) per well in a 384 well plates (Roche cat # 04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche).

To calculate relative fold change, data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. $IC_{50}$s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 or mock-transfected. The sense and antisense sequences of AD-1955 are: sense: cuuAcGcuGAGuAcuucGAdTsdT and antisense UCGAAGuACUcAGCGuAAGdTsdT.

The results of a single dose screen of the agents in Tables 2 and 3 in Hep3b cells are shown in Table 4.

**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).

| **Abbre*via*tion** | **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 |

| Abbre*via*tion | Nucleotide(s) |
|---|---|
| T | 5’-methyluridine-3’-phosphate |
| Tf | 2’-fluoro-5-methyluridine-3’-phosphate |
| Tfs | 2’-fluoro-5-methyluridine-3’-phosphorothioate |
| Ts | 5-methyluridine-3’-phosphorothioate |
| U | Uridine-3’-phosphate |
| Uf | 2’-fluorouridine-3’-phosphate |
| Ufs | 2’-fluorouridine -3’-phosphorothioate |
| Us | uridine -3’-phosphorothioate |
| N | any nucleotide, modified or unmodified |
| a | 2'-O-methyladenosine-3’-phosphate |
| as | 2'-O-methyladenosine-3’- phosphorothioate |
| c | 2'-O-methylcytidine-3’-phosphate |
| cs | 2'-O-methylcytidine-3’- phosphorothioate |
| g | 2'-O-methylguanosine-3’-phosphate |
| gs | 2'-O-methylguanosine-3’- phosphorothioate |
| 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-tetrahydrofurane-4-methoxy-3-phosphate (abasic 2'-OMe furanose) |
| Y44 | inverted abasic DNA (2-hydroxymethyl-tetrahydrofurane-5-phosphate) |
| (Agn) | Adenosine-glycol nucleic acid (GNA) |
| (Cgn) | Cytidine-glycol nucleic acid (GNA) |
| (Ggn) | Guanosine-glycol nucleic acid (GNA) |
| (Tgn) | Thymidine-glycol nucleic acid (GNA) S-Isomer |
| P | Phosphate |
| VP | Vinyl-phosphonate |
| dA | 2`-deoxyadenosine-3`-phosphate |
| dAs | 2`-deoxyadenosine-3`-phosphorothioate |
| dC | 2`-deoxycytidine-3`-phosphate |
| dCs | 2`-deoxycytidine-3`-phosphorothioate |
| dG | 2`-deoxyguanosine-3`-phosphate |
| dGs | 2`-deoxyguanosine-3`-phosphorothioate |
| dT | 2`-deoxythimidine -3`-phosphate |
| dTs | 2`-deoxythimidine-3`-phosphorothioate |
| dU | 2`-deoxyuridine |
| dUs | 2`-deoxyuridine-3`-phosphorothioate |

| **Abbre*via*tion** | **Nucleotide(s)** |
|---|---|
| (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 CTNNB1 dsRNA Agents**

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548365.1 | GAGGGUAUUUGAAGUAUACCA | | 164-184 | UGGUAUACUUCAAAUACCCUCAG | | 162-184 |
| AD-1548366.1 | GAGGGUAUUUGAAGUAUACCA | | 164-184 | UGGUAUACUUCAAAUACCCUCAG | | 162-184 |
| AD-1548367.1 | GGGUAUUUGAAGUAUACCAUA | | 166-186 | UAUGGUAUACUUCAAAUACCCUC | | 164-186 |
| AD-1548368.1 | GGUAUUUGAAGUAUACCAUAA | | 167-187 | UUAUGGTAUACUUCAAAUACCCU | | 165-187 |
| AD-1548369.1 | UUUGAAGUAUACCAUACAACA | | 171-191 | UGUUGUAUGGUAUACUUCAAAUA | | 169-191 |
| AD-1548370.1 | UUGAAGUAUACCAUACAACUA | | 172-192 | UAGUTGTAUGGUAUACUUCAAAU | | 170-192 |
| AD-1548371.1 | UGGACAAUGGCUACUCAAGCA | | 209-229 | UGCUTGAGUAGCCAUUGUCCACG | | 207-229 |
| AD-1548372.1 | GACAAUGGCUACUCAAGCUGA | | 211-231 | UCAGCUTGAGUAGCCAUUGUCCA | | 209-231 |
| AD-1548373.1 | ACAAUGGCUACUCAAGCUGAA | | 212-232 | UUCAGCTUGAGUAGCCAUUGUCC | | 210-232 |
| AD-1548374.1 | AAGCUGAUUUGAUGGAGUUGA | | 225-245 | UCAACUCCAUCAAAUCAGCUUGA | | 223-245 |
| AD-1548375.1 | AGCUGAUUUGAUGGAGUUGGA | | 226-246 | UCCAACTCCAUCAAAUCAGCUUG | | 224-246 |
| AD-1548376.1 | CCUUCUCUGAGUGGUAAAGGA | | 344-364 | UCCUTUACCACTCAGAGAAGGAG | | 342-364 |
| AD-1548377.1 | CAGUCCUUCACUCAAGAACAA | | 428-448 | UUGUTCTUGAGUGAAGGACUGAG | | 426-448 |
| AD-1548378.1 | CUUCACUCAAGAACAAGUAGA | | 433-453 | UCUACUTGUUCTUGAGUGAAGGA | | 431-453 |
| AD-1548379.1 | CACUCAAGAACAAGUAGCUGA | | 436-456 | UCAGCUACUUGTUCUUGAGUGAA | | 434-456 |
| AD-1548380.1 | GUAGCUGAUAUUGAUGGACAA | | 449-469 | UUGUCCAUCAAUAUCAGCUACUU | | 447-469 |
| AD-1548381.1 | AUUGAUGGACAGUAUGCAAUA | | 458-478 | UAUUGCAUACUGUCCAUCAAUAU | | 456-478 |
| AD-1548382.1 | AGUAUGCAAUGACUCGAGCUA | | 468-488 | UAGCTCGAGUCAUUGCAUACUGU | | 466-488 |
| AD-1548383.1 | GGUACGAGCUGCUAUGUUCCA | | 493-513 | UGGAACAUAGCAGCUCGUACCCU | | 491-513 |
| AD-1548384.1 | ACGAGCUGCUAUGUUCCCUGA | | 496-516 | UCAGGGAACAUAGCAGCUCGUAC | | 494-516 |
| AD-1548385.1 | UGUUCCCUGAGACAUUAGAUA | | 507-527 | UAUCTAAUGUCTCAGGGAACAUA | | 505-527 |
| AD-1548386.1 | UUCCCUGAGACAUUAGAUGAA | | 509-529 | UUCATCTAAUGUCUCAGGGAACA | | 507-529 |
| AD-1548387.1 | AGAUGAGGGCAUGCAGAUCCA | | 523-543 | UGGATCTGCAUGCCCUCAUCUAA | | 521-543 |
| AD-1548388.1 | CUGCUCAUCCCACUAAUGUCA | | 561-581 | UGACAUTAGUGGGAUGAGCAGCA | | 559-581 |
| AD-1548389.1 | ACCAUCACAGAUGCUGAAACA | | 595-615 | UGUUTCAGCAUCUGUGAUGGUUC | | 593-615 |
| AD-1548390.1 | CAUCACAGAUGCUGAAACAUA | | 597-617 | UAUGTUTCAGCAUCUGUGAUGGU | | 595-617 |
| AD-1548391.1 | CAUCACAGAUGCUGAAACAUA | | 597-617 | UAUGTUTCAGCAUCUGUGAUGGU | | 595-617 |
| AD-1548392.1 | AUGCUGAAACAUGCAGUUGUA | | 605-625 | UACAACTGCAUGUUUCAGCAUCU | | 603-625 |
| AD-1548393.1 | AUGCUGAAACAUGCAGUUGUA | | 605-625 | UACAACTGCAUGUUUCAGCAUCU | | 603-625 |
| AD-1548394.1 | ACAUGCAGUUGUAAACUUGAA | | 613-633 | UUCAAGTUUACAACUGCAUGUUU | | 611-633 |
| AD-1548395.1 | AUGCAGUUGUAAACUUGAUUA | | 615-635 | UAAUCAAGUUUACAACUGCAUGU | | 613-635 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548396.1 | AUGCAGUUGUAAACUUGAUUA | | 615-635 | UAAUCAAGUUUACAACUGCAUGU | | 613-635 |
| AD-1548397.1 | ACGUGCAAUCCCUGAACUGAA | | 664-684 | UUCAGUTCAGGGAUUGCACGUGU | | 662-684 |
| AD-1548398.1 | UGCAAUCCCUGAACUGACAAA | | 667-687 | UUUGTCAGUUCAGGGAUUGCACG | | 665-687 |
| AD-1548399.1 | GACCAGGUGGUGGUUAAUAAA | | 704-724 | UUUATUAACCACCACCUGGUCCU | | 702-724 |
| AD-1548400.1 | GACCAGGUGGUGGUUAAUAAA | | 704-724 | UTUATUAACCACCACCUGGUCCU | | 702-724 |
| AD-1548401.1 | CCAGGUGGUGGUUAAUAAGGA | | 706-726 | UCCUTATUAACCACCACCUGGUC | | 704-726 |
| AD-1548402.1 | GUUAAUAAGGCUGCAGUUAUA | | 716-736 | UAUAACTGCAGCCUUAUUAACCA | | 714-736 |
| AD-1548403.1 | CCUCAGAUGGUGUCUGCUAUA | | 788-808 | UAUAGCAGACACCAUCUGAGGAG | | 786-808 |
| AD-1548404.1 | UCAGAUGGUGUCUGCUAUUGA | | 790-810 | UCAATAGCAGACACCAUCUGAGG | | 788-810 |
| AD-1548405.1 | AGAUGGUGUCUGCUAUUGUAA | | 792-812 | UUACAATAGCAGACACCAUCUGA | | 790-812 |
| AD-1548406.1 | AGAUGGUGUCUGCUAUUGUAA | | 792-812 | UTACAATAGCAGACACCAUCUGA | | 790-812 |
| AD-1548407.1 | ACGUACCAUGCAGAAUACAAA | | 811-831 | UUUGTATUCUGCAUGGUACGUAC | | 809-831 |
| AD-1548408.1 | ACGUACCAUGCAGAAUACAAA | | 811-831 | UTUGTATUCUGCAUGGUACGUAC | | 809-831 |
| AD-1548409.1 | CGUACCAUGCAGAAUACAAAA | | 812-832 | UUUUGUAUUCUGCAUGGUACGUA | | 810-832 |
| AD-1548410.1 | GUACCAUGCAGAAUACAAAUA | | 813-833 | UAUUTGTAUUCUGCAUGGUACGU | | 811-833 |
| AD-1548411.1 | UACCAUGCAGAAUACAAAUGA | | 814-834 | UCAUTUGUAUUCUGCAUGGUACG | | 812-834 |
| AD-1548412.1 | ACCAUGCAGAAUACAAAUGAA | | 815-835 | UTCATUTGUAUTCUGCAUGGUAC | | 813-835 |
| AD-1548413.1 | CCAUGCAGAAUACAAAUGAUA | | 816-836 | UAUCAUTUGUATUCUGCAUGGUA | | 814-836 |
| AD-1548414.1 | CAUGCAGAAUACAAAUGAUGA | | 817-837 | UCAUCATUUGUAUUCUGCAUGGU | | 815-837 |
| AD-1548415.1 | AUGCAGAAUACAAAUGAUGUA | | 818-838 | UACATCAUUUGUAUUCUGCAUGG | | 816-838 |
| AD-1548416.1 | GCAGAAUACAAAUGAUGUAGA | | 820-840 | UCUACATCAUUTGUAUUCUGCAU | | 818-840 |
| AD-1548417.1 | CAGAAUACAAAUGAUGUAGAA | | 821-841 | UUCUACAUCAUUUGUAUUCUGCA | | 819-841 |
| AD-1548418.1 | UGAUGUAGAAACAGCUCGUUA | | 832-852 | UAACGAGCUGUTUCUACAUCAUU | | 830-852 |
| AD-1548419.1 | GAUGUAGAAACAGCUCGUUGA | | 833-853 | UCAACGAGCUGTUUCUACAUCAU | | 831-853 |
| AD-1548420.1 | ACUGGCCAUCUUUAAGUCUGA | | 898-918 | UCAGACTUAAAGAUGGCCAGUAA | | 896-918 |
| AD-1548421.1 | CUGGCCAUCUUUAAGUCUGGA | | 899-919 | UCCAGACUUAAAGAUGGCCAGUA | | 897-919 |
| AD-1548422.1 | UGGCCAUCUUUAAGUCUGGAA | | 900-920 | UUCCAGACUUAAAGAUGGCCAGU | | 898-920 |
| AD-1548423.1 | GGCCAUCUUUAAGUCUGGAGA | | 901-921 | UCUCCAGACUUAAAGAUGGCCAG | | 899-921 |
| AD-1548424.1 | GCCAUCUUUAAGUCUGGAGGA | | 902-922 | UCCUCCAGACUTAAAGAUGGCCA | | 900-922 |
| AD-1548425.1 | UGGUUCACCAGUGGAUUCUGA | | 946-966 | UCAGAATCCACTGGUGAACCAAG | | 944-966 |
| AD-1548426.1 | ACCAGUGGAUUCUGUGUUGUA | | 952-972 | UACAACACAGAAUCCACUGGUGA | | 950-972 |
| AD-1548427.1 | AUGGCAGUGCGUUUAGCUGGA | | 1025-1045 | UCCAGCTAAACGCACUGCCAUUU | | 1023-1045 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548428.1 | AUGGCAGUGCGUUUAGCUGGA | | 1025-1045 | UCCAGCTAAACGCACUGCCAUUU | | 1023-1045 |
| AD-1548429.1 | UUGCCUUGCUCAACAAAACAA | | 1062-1082 | UUGUTUTGUUGAGCAAGGCAACC | | 1060-1082 |
| AD-1548430.1 | UGCCUUGCUCAACAAAACAAA | | 1063-1083 | UTUGTUTUGUUGAGCAAGGCAAC | | 1061-1083 |
| AD-1548431.1 | UGCCUUGCUCAACAAAACAAA | | 1063-1083 | UUUGTUTUGUUGAGCAAGGCAAC | | 1061-1083 |
| AD-1548432.1 | GCCUUGCUCAACAAAACAAAA | | 1064-1084 | UUUUGUTUUGUUGAGCAAGGCAA | | 1062-1084 |
| AD-1548433.1 | UUAAAUUCUUGGCUAUUACGA | | 1086-1106 | UCGUAATAGCCAAGAAUUUAACA | | 1084-1106 |
| AD-1548434.1 | AUUCUUGGCUAUUACGACAGA | | 1090-1110 | UCUGTCGUAAUAGCCAAGAAUUU | | 1088-1110 |
| AD-1548435.1 | GGCUAUUACGACAGACUGCCA | | 1096-1116 | UGGCAGTCUGUCGUAAUAGCCAA | | 1094-1116 |
| AD-1548436.1 | UACGACAGACUGCCUUCAAAA | | 1102-1122 | UUUUGAAGGCAGUCUGUCGUAAU | | 1100-1122 |
| AD-1548437.1 | CUUUAGUAAAUAUAAUGAGGA | | 1182-1202 | UCCUCATUAUATUUACUAAAGCU | | 1180-1202 |
| AD-1548438.1 | UAUAAUGAGGACCUAUACUUA | | 1192-1212 | UAAGTATAGGUCCUCAUUAUAUU | | 1190-1212 |
| AD-1548439.1 | AGCUGGUGGAAUGCAAGCUUA | | 1291-1311 | UAAGCUTGCAUUCCACCAGCUUC | | 1289-1311 |
| AD-1548440.1 | CAGAUCCAAGUCAACGUCUUA | | 1326-1346 | UAAGACGUUGACUUGGAUCUGUC | | 1324-1346 |
| AD-1548441.1 | CCAAGUCAACGUCUUGUUCAA | | 1331-1351 | UUGAACAAGACGUUGACUUGGAU | | 1329-1351 |
| AD-1548442.1 | CCAAGUCAACGUCUUGUUCAA | | 1331-1351 | UTGAACAAGACGUUGACUUGGAU | | 1329-1351 |
| AD-1548443.1 | AGAACUGUCUUUGGACUCUCA | | 1350-1370 | UGAGAGTCCAAAGACAGUUCUGA | | 1348-1370 |
| AD-1548444.1 | CUUUCAGAUGCUGCAACUAAA | | 1376-1396 | UUUAGUTGCAGCAUCUGAAAGAU | | 1374-1396 |
| AD-1548445.1 | UCAGAUGCUGCAACUAAACAA | | 1379-1399 | UUGUTUAGUUGCAGCAUCUGAAA | | 1377-1399 |
| AD-1548446.1 | GAUGCUGCAACUAAACAGGAA | | 1382-1402 | UUCCTGTUUAGUUGCAGCAUCUG | | 1380-1402 |
| AD-1548447.1 | CUGCAACUAAACAGGAAGGGA | | 1386-1406 | UCCCTUCCUGUTUAGUUGCAGCA | | 1384-1406 |
| AD-1548448.1 | GAAGGGAUGGAAGGUCUCCUA | | 1400-1420 | UAGGAGACCUUCCAUCCCUUCCU | | 1398-1420 |
| AD-1548449.1 | CUUCUGGGUUCAGAUGAUAUA | | 1436-1456 | UAUATCAUCUGAACCCAGAAGCU | | 1434-1456 |
| AD-1548450.1 | CCUGUGCAGCUGGAAUUCUUA | | 1467-1487 | UAAGAATUCCAGCUGCACAGGUG | | 1465-1487 |
| AD-1548451.1 | UGUGCAGCUGGAAUUCUUUCA | | 1469-1489 | UGAAAGAAUUCCAGCUGCACAGG | | 1467-1489 |
| AD-1548452.1 | AAUUCUUUCUAACCUCACUUA | | 1480-1500 | UAAGTGAGGUUAGAAAGAAUUCC | | 1478-1500 |
| AD-1548453.1 | UUCUUUCUAACCUCACUUGCA | | 1482-1502 | UGCAAGTGAGGTUAGAAAGAAUU | | 1480-1502 |
| AD-1548454.1 | CUUUCUAACCUCACUUGCAAA | | 1484-1504 | UUUGCAAGUGAGGUUAGAAAGAA | | 1482-1504 |
| AD-1548455.1 | UUUCUAACCUCACUUGCAAUA | | 1485-1505 | UAUUGCAAGUGAGGUUAGAAAGA | | 1483-1505 |
| AD-1548456.1 | CUAACCUCACUUGCAAUAAUA | | 1488-1508 | UAUUAUTGCAAGUGAGGUUAGAA | | 1486-1508 |
| AD-1548457.1 | UAACCUCACUUGCAAUAAUUA | | 1489-1509 | UAAUTATUGCAAGUGAGGUUAGA | | 1487-1509 |
| AD-1548458.1 | AACCUCACUUGCAAUAAUUAA | | 1490-1510 | UTAATUAUUGCAAGUGAGGUUAG | | 1488-1510 |
| AD-1548459.1 | ACCUCACUUGCAAUAAUUAUA | | 1491-1511 | UAUAAUTAUUGCAAGUGAGGUUA | | 1489-1511 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548460.1 | CCUCACUUGCAAUAAUUAUAA | | 1492-1512 | UTAUAATUAUUGCAAGUGAGGUU | | 1490-1512 |
| AD-1548461.1 | CUCACUUGCAAUAAUUAUAAA | | 1493-1513 | UUUATAAUUAUUGCAAGUGAGGU | | 1491-1513 |
| AD-1548462.1 | CUCACUUGCAAUAAUUAUAAA | | 1493-1513 | UTUATAAUUAUTGCAAGUGAGGU | | 1491-1513 |
| AD-1548463.1 | UCACUUGCAAUAAUUAUAAGA | | 1494-1514 | UCUUAUAAUUATUGCAAGUGAGG | | 1492-1514 |
| AD-1548464.1 | CACUUGCAAUAAUUAUAAGAA | | 1495-1515 | UTCUTATAAUUAUUGCAAGUGAG | | 1493-1515 |
| AD-1548465.1 | CAAGUGGGUGGUAUAGAGGCA | | 1532-1552 | UGCCTCTAUACCACCCACUUGGC | | 1530-1552 |
| AD-1548466.1 | GCUCUUGUGCGUACUGUCCUA | | 1550-1570 | UAGGACAGUACGCACAAGAGCCU | | 1548-1570 |
| AD-1548467.1 | CCUGCCAUCUGUGCUCUUCGA | | 1601-1621 | UCGAAGAGCACAGAUGGCAGGCU | | 1599-1621 |
| AD-1548468.1 | CUGCCAUCUGUGCUCUUCGUA | | 1602-1622 | UACGAAGAGCACAGAUGGCAGGC | | 1600-1622 |
| AD-1548469.1 | UGCCAUCUGUGCUCUUCGUCA | | 1603-1623 | UGACGAAGAGCACAGAUGGCAGG | | 1601-1623 |
| AD-1548470.1 | GCCAUCUGUGCUCUUCGUCAA | | 1604-1624 | UTGACGAAGAGCACAGAUGGCAG | | 1602-1624 |
| AD-1548471.1 | CCAUCUGUGCUCUUCGUCAUA | | 1605-1625 | UAUGACGAAGAGCACAGAUGGCA | | 1603-1625 |
| AD-1548472.1 | AUCUGUGCUCUUCGUCAUCUA | | 1607-1627 | UAGATGACGAAGAGCACAGAUGG | | 1605-1627 |
| AD-1548473.1 | UCUGUGCUCUUCGUCAUCUGA | | 1608-1628 | UCAGAUGACGAAGAGCACAGAUG | | 1606-1628 |
| AD-1548474.1 | CUGUGCUCUUCGUCAUCUGAA | | 1609-1629 | UUCAGATGACGAAGAGCACAGAU | | 1607-1629 |
| AD-1548475.1 | UGUGCUCUUCGUCAUCUGACA | | 1610-1630 | UGUCAGAUGACGAAGAGCACAGA | | 1608-1630 |
| AD-1548476.1 | UGGACUACCAGUUGUGGUUAA | | 1681-1701 | UUAACCACAACUGGUAGUCCAUA | | 1679-1701 |
| AD-1548477.1 | GACUACCAGUUGUGGUUAAGA | | 1683-1703 | UCUUAACCACAACUGGUAGUCCA | | 1681-1703 |
| AD-1548478.1 | UGUGGUUAAGCUCUUACACCA | | 1693-1713 | UGGUGUAAGAGCUUAACCACAAC | | 1691-1713 |
| AD-1548479.1 | GAUAAAGGCUACUGUUGGAUA | | 1732-1752 | UAUCCAACAGUAGCCUUUAUCAG | | 1730-1752 |
| AD-1548480.1 | AUAAAGGCUACUGUUGGAUUA | | 1733-1753 | UAAUCCAACAGUAGCCUUUAUCA | | 1731-1753 |
| AD-1548481.1 | AUAAAGGCUACUGUUGGAUUA | | 1733-1753 | UAAUCCAACAGTAGCCUUUAUCA | | 1731-1753 |
| AD-1548482.1 | AAGGCUACUGUUGGAUUGAUA | | 1736-1756 | UAUCAATCCAACAGUAGCCUUUA | | 1734-1756 |
| AD-1548483.1 | AAGGCUACUGUUGGAUUGAUA | | 1736-1756 | UAUCAATCCAACAGUAGCCUUUA | | 1734-1756 |
| AD-1548484.1 | AGGCUACUGUUGGAUUGAUUA | | 1737-1757 | UAAUCAAUCCAACAGUAGCCUUU | | 1735-1757 |
| AD-1548485.1 | GGCUACUGUUGGAUUGAUUCA | | 1738-1758 | UGAATCAAUCCAACAGUAGCCUU | | 1736-1758 |
| AD-1548486.1 | GCUACUGUUGGAUUGAUUCGA | | 1739-1759 | UCGAAUCAAUCCAACAGUAGCCU | | 1737-1759 |
| AD-1548487.1 | CUACUGUUGGAUUGAUUCGAA | | 1740-1760 | UTCGAATCAAUCCAACAGUAGCC | | 1738-1760 |
| AD-1548488.1 | UACUGUUGGAUUGAUUCGAAA | | 1741-1761 | UTUCGAAUCAATCCAACAGUAGC | | 1739-1761 |
| AD-1548489.1 | ACUGUUGGAUUGAUUCGAAAA | | 1742-1762 | UTUUCGAAUCAAUCCAACAGUAG | | 1740-1762 |
| AD-1548490.1 | CUGUUGGAUUGAUUCGAAAUA | | 1743-1763 | UAUUTCGAAUCAAUCCAACAGUA | | 1741-1763 |
| AD-1548491.1 | GUUGGAUUGAUUCGAAAUCUA | | 1745-1765 | UAGATUTCGAATCAAUCCAACAG | | 1743-1765 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548492.1 | GUUGGAUUGAUUCGAAAUCUA | | 1745-1765 | UAGATUTCGAAUCAAUCCAACAG | | 1743-1765 |
| AD-1548493.1 | UUGGAUUGAUUCGAAAUCUUA | | 1746-1766 | UAAGAUTUCGAAUCAAUCCAACA | | 1744-1766 |
| AD-1548494.1 | GGAUUGAUUCGAAAUCUUGCA | | 1748-1768 | UGCAAGAUUUCGAAUCAAUCCAA | | 1746-1768 |
| AD-1548495.1 | GGAUUGAUUCGAAAUCUUGCA | | 1748-1768 | UGCAAGAUUUCGAAUCAAUCCAA | | 1746-1768 |
| AD-1548496.1 | UUGAUUCGAAAUCUUGCCCUA | | 1751-1771 | UAGGGCAAGAUUUCGAAUCAAUC | | 1749-1771 |
| AD-1548497.1 | AUUCCACGACUAGUUCAGUUA | | 1811-1831 | UAACTGAACUAGUCGUGGAAUGG | | 1809-1831 |
| AD-1548498.1 | CCACGACUAGUUCAGUUGCUA | | 1814-1834 | UAGCAACUGAACUAGUCGUGGAA | | 1812-1834 |
| AD-1548499.1 | UUCAGUUGCUUGUUCGUGCAA | | 1824-1844 | UTGCACGAACAAGCAACUGAACU | | 1822-1844 |
| AD-1548500.1 | GGACACAGCAGCAAUUUGUGA | | 1878-1898 | UCACAAAUUGCTGCUGUGUCCCA | | 1876-1898 |
| AD-1548501.1 | AUCCUAGCUCGGGAUGUUCAA | | 1949-1969 | UUGAACAUCCCGAGCUAGGAUGU | | 1947-1969 |
| AD-1548502.1 | AGCUCGGGAUGUUCACAACCA | | 1954-1974 | UGGUTGTGAACAUCCCGAGCUAG | | 1952-1974 |
| AD-1548503.1 | GAUGUUCACAACCGAAUUGUA | | 1961-1981 | UACAAUTCGGUTGUGAACAUCCC | | 1959-1981 |
| AD-1548504.1 | UAUCAGAGGACUAAAUACCAA | | 1981-2001 | UUGGTATUUAGUCCUCUGAUAAC | | 1979-2001 |
| AD-1548505.1 | AUCAGAGGACUAAAUACCAUA | | 1982-2002 | UAUGGUAUUUAGUCCUCUGAUAA | | 1980-2002 |
| AD-1548506.1 | UCAGAGGACUAAAUACCAUUA | | 1983-2003 | UAAUGGTAUUUAGUCCUCUGAUA | | 1981-2003 |
| AD-1548507.1 | GAGGACUAAAUACCAUUCCAA | | 1986-2006 | UUGGAATGGUAUUUAGUCCUCUG | | 1984-2006 |
| AD-1548508.1 | GAGGACUAAAUACCAUUCCAA | | 1986-2006 | UTGGAATGGUATUUAGUCCUCUG | | 1984-2006 |
| AD-1548509.1 | GGACUAAAUACCAUUCCAUUA | | 1988-2008 | UAAUGGAAUGGTAUUUAGUCCUC | | 1986-2008 |
| AD-1548510.1 | ACUAAAUACCAUUCCAUUGUA | | 1990-2010 | UACAAUGGAAUGGUAUUUAGUCC | | 1988-2010 |
| AD-1548511.1 | CUAAAUACCAUUCCAUUGUUA | | 1991-2011 | UAACAATGGAATGGUAUUUAGUC | | 1989-2011 |
| AD-1548512.1 | UAAAUACCAUUCCAUUGUUUA | | 1992-2012 | UAAACAAUGGAAUGGUAUUUAGU | | 1990-2012 |
| AD-1548513.1 | AAAUACCAUUCCAUUGUUUGA | | 1993-2013 | UCAAACAAUGGAAUGGUAUUUAG | | 1991-2013 |
| AD-1548514.1 | AAUACCAUUCCAUUGUUUGUA | | 1994-2014 | UACAAACAAUGGAAUGGUAUUUA | | 1992-2014 |
| AD-1548515.1 | AUACCAUUCCAUUGUUUGUGA | | 1995-2015 | UCACAAACAAUGGAAUGGUAUUU | | 1993-2015 |
| AD-1548515.2 | AUACCAUUCCAUUGUUUGUGA | | 1995-2015 | UCACAAACAAUGGAAUGGUAUUU | | 1993-2015 |
| AD-1548516.1 | UACCAUUCCAUUGUUUGUGCA | | 1996-2016 | UGCACAAACAATGGAAUGGUAUU | | 1994-2016 |
| AD-1548517.1 | ACCAUUCCAUUGUUUGUGCAA | | 1997-2017 | UTGCACAAACAAUGGAAUGGUAU | | 1995-2017 |
| AD-1548518.1 | ACCAUUCCAUUGUUUGUGCAA | | 1997-2017 | UUGCACAAACAAUGGAAUGGUAU | | 1995-2017 |
| AD-1548519.1 | CCAUUCCAUUGUUUGUGCAGA | | 1998-2018 | UCUGCACAAACAAUGGAAUGGUA | | 1996-2018 |
| AD-1548520.1 | CAUUCCAUUGUUUGUGCAGCA | | 1999-2019 | UGCUGCACAAACAAUGGAAUGGU | | 1997-2019 |
| AD-1548521.1 | UCCAUUGUUUGUGCAGCUGCA | | 2002-2022 | UGCAGCTGCACAAACAAUGGAAU | | 2000-2022 |
| AD-1548522.1 | GUUUGUGCAGCUGCUUUAUUA | | 2008-2028 | UAAUAAAGCAGCUGCACAAACAA | | 2006-2028 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548523.1 | CUCCUCUGACAGAGUUACUUA | | 2127-2147 | UAAGTAACUCUGUCAGAGGAGCU | | 2125-2147 |
| AD-1548524.1 | GACAGAGUUACUUCACUCUAA | | 2134-2154 | UUAGAGTGAAGUAACUCUGUCAG | | 2132-2154 |
| AD-1548525.1 | AGUUACUUCACUCUAGGAAUA | | 2139-2159 | UAUUCCTAGAGUGAAGUAACUCU | | 2137-2159 |
| AD-1548526.1 | GAGGACAAGCCACAAGAUUAA | | 2204-2224 | UUAATCTUGUGGCUUGUCCUCAG | | 2202-2224 |
| AD-1548527.1 | CACAAGAUUACAAGAAACGGA | | 2214-2234 | UCCGTUTCUUGTAAUCUUGUGGC | | 2212-2234 |
| AD-1548528.1 | ACAAGAUUACAAGAAACGGCA | | 2215-2235 | UGCCGUTUCUUGUAAUCUUGUGG | | 2213-2235 |
| AD-1548529.1 | CAAGAUUACAAGAAACGGCUA | | 2216-2236 | UAGCCGTUUCUTGUAAUCUUGUG | | 2214-2236 |
| AD-1548530.1 | AAGAUUACAAGAAACGGCUUA | | 2217-2237 | UAAGCCGUUUCTUGUAAUCUUGU | | 2215-2237 |
| AD-1548531.1 | UUACAAGAAACGGCUUUCAGA | | 2221-2241 | UCUGAAAGCCGTUUCUUGUAAUC | | 2219-2241 |
| AD-1548532.1 | UACAAGAAACGGCUUUCAGUA | | 2222-2242 | UACUGAAAGCCGUUUCUUGUAAU | | 2220-2242 |
| AD-1548533.1 | AAACGGCUUUCAGUUGAGCUA | | 2228-2248 | UAGCTCAACUGAAAGCCGUUUCU | | 2226-2248 |
| AD-1548534.1 | GCUUUCAGUUGAGCUGACCAA | | 2233-2253 | UUGGTCAGCUCAACUGAAAGCCG | | 2231-2253 |
| AD-1548535.1 | CUUGGACUUGAUAUUGGUGCA | | 2300-2320 | UGCACCAAUAUCAAGUCCAAGAU | | 2298-2320 |
| AD-1548536.1 | UAUCGCCAGGAUGAUCCUAGA | | 2339-2359 | UCUAGGAUCAUCCUGGCGAUAUC | | 2337-2359 |
| AD-1548537.1 | CACCACCCUGGUGCUGACUAA | | 2438-2458 | UUAGTCAGCACCAGGGUGGUGGC | | 2436-2458 |
| AD-1548538.1 | AUCAGCUGGCCUGGUUUGAUA | | 2529-2549 | UAUCAAACCAGGCCAGCUGAUUG | | 2527-2549 |
| AD-1548539.1 | UCAGCUGGCCUGGUUUGAUAA | | 2530-2550 | UUAUCAAACCAGGCCAGCUGAUU | | 2528-2550 |
| AD-1548540.1 | UCAGCUGGCCUGGUUUGAUAA | | 2530-2550 | UTAUCAAACCAGGCCAGCUGAUU | | 2528-2550 |
| AD-1548541.1 | CUGGCCUGGUUUGAUACUGAA | | 2534-2554 | UUCAGUAUCAAACCAGGCCAGCU | | 2532-2554 |
| AD-1548542.1 | CUGUAAAUCAUCCUUUAGGUA | | 2555-2575 | UACCTAAAGGATGAUUUACAGGU | | 2553-2575 |
| AD-1548543.1 | UGUAAAUCAUCCUUUAGGUAA | | 2556-2576 | UTACCUAAAGGAUGAUUUACAGG | | 2554-2576 |
| AD-1548544.1 | AAAGACUUGGUUGGUAGGGUA | | 2634-2654 | UACCCUACCAACCAAGUCUUUCU | | 2632-2654 |
| AD-1548545.1 | ACUUUGAAAGGAGAUGUCUUA | | 2701-2721 | UAAGACAUCUCCUUUCAAAGUAU | | 2699-2721 |
| AD-1548546.1 | ACUUUGAAAGGAGAUGUCUUA | | 2701-2721 | UAAGACAUCUCCUUUCAAAGUAU | | 2699-2721 |
| AD-1548547.1 | UUCUCAGAUUUCUGGUUGUUA | | 2735-2755 | UAACAACCAGAAAUCUGAGAACA | | 2733-2755 |
| AD-1548548.1 | UCUCAGAUUUCUGGUUGUUAA | | 2736-2756 | UUAACAACCAGAAAUCUGAGAAC | | 2734-2756 |
| AD-1548549.1 | UCUCAGAUUUCUGGUUGUUAA | | 2736-2756 | UTAACAACCAGAAAUCUGAGAAC | | 2734-2756 |
| AD-1548550.1 | CUCAGAUUUCUGGUUGUUAUA | | 2737-2757 | UAUAACAACCAGAAAUCUGAGAA | | 2735-2757 |
| AD-1548551.1 | GUUGUUAUGUGAUCAUGUGUA | | 2749-2769 | UACACATGAUCACAUAACAACCA | | 2747-2769 |
| AD-1548552.1 | UUGUUAUGUGAUCAUGUGUGA | | 2750-2770 | UCACACAUGAUCACAUAACAACC | | 2748-2770 |
| AD-1548553.1 | GUGAUCAUGUGUGGAAGUUAA | | 2757-2777 | UUAACUTCCACACAUGAUCACAU | | 2755-2777 |
| AD-1548554.1 | GAUCAUGUGUGGAAGUUAUUA | | 2759-2779 | UAAUAACUUCCACACAUGAUCAC | | 2757-2779 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548555.1 | AUCAUGUGUGGAAGUUAUUAA | | 2760-2780 | UTAATAACUUCCACACAUGAUCA | | 2758-2780 |
| AD-1548556.1 | UGCAACUUAAUACUCAAAUGA | | 2806-2826 | UCAUTUGAGUATUAAGUUGCAAA | | 2804-2826 |
| AD-1548557.1 | CCUUUCUCUCUUUAUACAGCA | | 2859-2879 | UGCUGUAUAAAGAGAGAAAGGCU | | 2857-2879 |
| AD-1548558.1 | UUUAUACAGCUGUAUUGUCUA | | 2869-2889 | UAGACAAUACAGCUGUAUAAAGA | | 2867-2889 |
| AD-1548559.1 | UUUAUACAGCUGUAUUGUCUA | | 2869-2889 | UAGACAAUACAGCUGUAUAAAGA | | 2867-2889 |
| AD-1548560.1 | AUUGUCUGAACUUGCAUUGUA | | 2882-2902 | UACAAUGCAAGTUCAGACAAUAC | | 2880-2902 |
| AD-1548561.1 | UUGGCCUGUAGAGUUGCUGAA | | 2904-2924 | UUCAGCAACUCUACAGGCCAAUC | | 2902-2924 |
| AD-1548562.1 | AGUGCCUGACACACUAACCAA | | 2956-2976 | UUGGTUAGUGUGUCAGGCACUUU | | 2954-2976 |
| AD-1548563.1 | CUGACACACUAACCAAGCUGA | | 2961-2981 | UCAGCUTGGUUAGUGUGUCAGGC | | 2959-2981 |
| AD-1548564.1 | UAACCAAGCUGAGUUUCCUAA | | 2970-2990 | UTAGGAAACUCAGCUUGGUUAGU | | 2968-2990 |
| AD-1548565.1 | AGCUGAGUUUCCUAUGGGAAA | | 2976-2996 | UUUCCCAUAGGAAACUCAGCUUG | | 2974-2996 |
| AD-1548566.1 | UUUCCUAUGGGAACAAUUGAA | | 2983-3003 | UTCAAUTGUUCCCAUAGGAAACU | | 2981-3003 |
| AD-1548567.1 | GUCGAGGAGUAACAAUACAAA | | 3031-3051 | UUUGTATUGUUACUCCUCGACCA | | 3029-3051 |
| AD-1548568.1 | GUCGAGGAGUAACAAUACAAA | | 3031-3051 | UTUGTATUGUUACUCCUCGACCA | | 3029-3051 |
| AD-1548569.1 | AUCAAACCCUAGCCUUGCUUA | | 3114-3134 | UAAGCAAGGCUAGGGUUUGAUAA | | 3112-3134 |
| AD-1548570.1 | UUUGCUUGCUUUGAAGUAGCA | | 3183-3203 | UGCUACTUCAAAGCAAGCAAAGU | | 3181-3203 |
| AD-1548571.1 | UGCUUGCUUUGAAGUAGCUCA | | 3185-3205 | UGAGCUACUUCAAAGCAAGCAAA | | 3183-3205 |
| AD-1548572.1 | GCUUGCUUUGAAGUAGCUCUA | | 3186-3206 | UAGAGCTACUUCAAAGCAAGCAA | | 3184-3206 |
| AD-1548573.1 | AAGUCUCUCGUAGUGUUAAGA | | 3246-3266 | UCUUAACACUACGAGAGACUUAA | | 3244-3266 |
| AD-1548574.1 | UCUCUCGUAGUGUUAAGUUAA | | 3249-3269 | UUAACUTAACACUACGAGAGACU | | 3247-3269 |
| AD-1548575.1 | CUCUCGUAGUGUUAAGUUAUA | | 3250-3270 | UAUAACTUAACACUACGAGAGAC | | 3248-3270 |
| AD-1548576.1 | CUCUCGUAGUGUUAAGUUAUA | | 3250-3270 | UAUAACTUAACACUACGAGAGAC | | 3248-3270 |
| AD-1548577.1 | CUCGUAGUGUUAAGUUAUAGA | | 3252-3272 | UCUATAACUUAACACUACGAGAG | | 3250-3272 |
| AD-1548578.1 | CGUAGUGUUAAGUUAUAGUGA | | 3254-3274 | UCACTATAACUTAACACUACGAG | | 3252-3274 |
| AD-1548579.1 | UAGUGUUAAGUUAUAGUGAAA | | 3256-3276 | UUUCACTAUAACUUAACACUACG | | 3254-3276 |
| AD-1548580.1 | GUGAAUACUGCUACAGCAAUA | | 3271-3291 | UAUUGCTGUAGCAGUAUUCACUA | | 3269-3291 |
| AD-1548581.1 | CUGCUACAGCAAUUUCUAAUA | | 3278-3298 | UAUUAGAAAUUGCUGUAGCAGUA | | 3276-3298 |
| AD-1548582.1 | GAAUUGAGUAAUGGUGUAGAA | | 3304-3324 | UUCUACACCAUUACUCAAUUCUU | | 3302-3324 |
| AD-1548583.1 | UUGAGUAAUGGUGUAGAACAA | | 3307-3327 | UUGUTCTACACCAUUACUCAAUU | | 3305-3327 |
| AD-1548584.1 | GAGUAAUGGUGUAGAACACUA | | 3309-3329 | UAGUGUTCUACACCAUUACUCAA | | 3307-3329 |
| AD-1548585.1 | AGUAAUGGUGUAGAACACUAA | | 3310-3330 | UUAGTGTUCUACACCAUUACUCA | | 3308-3330 |
| AD-1548586.1 | UAAUGGUGUAGAACACUAAUA | | 3312-3332 | UAUUAGTGUUCUACACCAUUACU | | 3310-3332 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548587.1 | AUGGUGUAGAACACUAAUUCA | | 3314-3334 | UGAATUAGUGUTCUACACCAUUA | | 3312-3334 |
| AD-1548588.1 | UGGUGUAGAACACUAAUUCAA | | 3315-3335 | UTGAAUTAGUGTUCUACACCAUU | | 3313-3335 |
| AD-1548589.1 | GGUGUAGAACACUAAUUCAUA | | 3316-3336 | UAUGAATUAGUGUUCUACACCAU | | 3314-3336 |
| AD-1548590.1 | GUGUAGAACACUAAUUCAUAA | | 3317-3337 | UUAUGAAUUAGUGUUCUACACCA | | 3315-3337 |
| AD-1548591.1 | UGUAGAACACUAAUUCAUAAA | | 3318-3338 | UUUATGAAUUAGUGUUCUACACC | | 3316-3338 |
| AD-1548592.1 | GUAGAACACUAAUUCAUAAUA | | 3319-3339 | UAUUAUGAAUUAGUGUUCUACAC | | 3317-3339 |
| AD-1548593.1 | UAGAACACUAAUUCAUAAUCA | | 3320-3340 | UGAUTATGAAUTAGUGUUCUACA | | 3318-3340 |
| AD-1548594.1 | AGAACACUAAUUCAUAAUCAA | | 3321-3341 | UTGATUAUGAATUAGUGUUCUAC | | 3319-3341 |
| AD-1548595.1 | AGAACACUAAUUCAUAAUCAA | | 3321-3341 | UUGATUAUGAAUUAGUGUUCUAC | | 3319-3341 |
| AD-1548596.1 | GAACACUAAUUCAUAAUCACA | | 3322-3342 | UGUGAUTAUGAAUUAGUGUUCUA | | 3320-3342 |
| AD-1548597.1 | AACACUAAUUCAUAAUCACUA | | 3323-3343 | UAGUGATUAUGAAUUAGUGUUCU | | 3321-3343 |
| AD-1548598.1 | AUGGUCCAAUUAGUUUCCUUA | | 3413-3433 | UAAGGAAACUAAUUGGACCAUUU | | 3411-3433 |
| AD-1548599.1 | UUUGGGAUAUGUAUGGGUAGA | | 3489-3509 | UCUACCCAUACAUAUCCCAAAUA | | 3487-3509 |
| AD-1548600.1 | UGUAUGGGUAGGGUAAAUCAA | | 3498-3518 | UUGATUTACCCUACCCAUACAUA | | 3496-3518 |
| AD-1548601.1 | AUGGGUAGGGUAAAUCAGUAA | | 3501-3521 | UUACTGAUUUACCCUACCCAUAC | | 3499-3521 |
| AD-1548602.1 | GUAGGGUAAAUCAGUAAGAGA | | 3505-3525 | UCUCTUACUGATUUACCCUACCC | | 3503-3525 |
| AD-1548603.1 | AGGGUAAAUCAGUAAGAGGUA | | 3507-3527 | UACCTCTUACUGAUUUACCCUAC | | 3505-3527 |
| AD-1548604.1 | GGGUAAAUCAGUAAGAGGUGA | | 3508-3528 | UCACCUCUUACTGAUUUACCCUA | | 3506-3528 |
| AD-1548605.1 | GUAAAUCAGUAAGAGGUGUUA | | 3510-3530 | UAACACCUCUUACUGAUUUACCC | | 3508-3530 |
| AD-1548606.1 | AAAUCAGUAAGAGGUGUUAUA | | 3512-3532 | UAUAACACCUCUUACUGAUUUAC | | 3510-3532 |
| AD-1548607.1 | AAUCAGUAAGAGGUGUUAUUA | | 3513-3533 | UAAUAACACCUCUUACUGAUUUA | | 3511-3533 |
| AD-1548608.1 | CAGUAAGAGGUGUUAUUUGGA | | 3516-3536 | UCCAAATAACACCUCUUACUGAU | | 3514-3536 |
| AD-1548609.1 | UUGGACAUGGCCAUGGAACCA | | 242-262 | UGGUTCCAUGGCCAUGUCCAACU | | 240-262 |
| AD-1548610.1 | UGGACUCUGGAAUCCAUUCUA | | 306-326 | UAGAAUGGAUUCCAGAGUCCAGG | | 304-326 |
| AD-1548611.1 | CUCUGAGUGGUAAAGGCAAUA | | 348-368 | UAUUGCCUUUACCACUCAGAGAA | | 346-368 |
| AD-1548612.1 | UCAAGAACAAGUAGCUGAUAA | | 439-459 | UUAUCAGCUACUUGUUCUUGAGU | | 437-459 |
| AD-1548613.1 | UAGCUGAUAUUGAUGGACAGA | | 450-470 | UCUGTCCAUCAAUAUCAGCUACU | | 448-470 |
| AD-1548614.1 | AGCUGAUAUUGAUGGACAGUA | | 451-471 | UACUGUCCAUCAAUAUCAGCUAC | | 449-471 |
| AD-1548615.1 | CUGAUAUUGAUGGACAGUAUA | | 453-473 | UAUACUGUCCAUCAAUAUCAGCU | | 451-473 |
| AD-1548616.1 | UGAUGGACAGUAUGCAAUGAA | | 460-480 | UUCATUGCAUACUGUCCAUCAAU | | 458-480 |
| AD-1548617.1 | AGUAUGCAAUGACUCGAGCUA | | 468-488 | UAGCTCGAGUCAUUGCAUACUGU | | 466-488 |
| AD-1548618.1 | AGCUCAGAGGGUACGAGCUGA | | 484-504 | UCAGCUCGUACCCUCUGAGCUCG | | 482-504 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548619.1 | CUCAGAGGGUACGAGCUGCUA | | 486-506 | UAGCAGCUCGUACCCUCUGAGCU | | 484-506 |
| AD-1548620.1 | AGAGGGUACGAGCUGCUAUGA | | 489-509 | UCAUAGCAGCUCGUACCCUCUGA | | 487-509 |
| AD-1548621.1 | GCUAUGUUCCCUGAGACAUUA | | 503-523 | UAAUGUCUCAGGGAACAUAGCAG | | 501-523 |
| AD-1548622.1 | AGCGUUUGGCUGAACCAUCAA | | 582-602 | UUGATGGUUCAGCCAAACGCUGG | | 580-602 |
| AD-1548623.1 | UGCUGAAACAUGCAGUUGUAA | | 606-626 | UUACAACUGCAUGUUUCAGCAUC | | 604-626 |
| AD-1548624.1 | CAUGCAGUUGUAAACUUGAUA | | 614-634 | UAUCAAGUUUACAACUGCAUGUU | | 612-634 |
| AD-1548625.1 | GCAGUUGUAAACUUGAUUAAA | | 617-637 | UUUAAUCAAGUUUACAACUGCAU | | 615-637 |
| AD-1548626.1 | UGAUUAACUAUCAAGAUGAUA | | 630-650 | UAUCAUCUUGAUAGUUAAUCAAG | | 628-650 |
| AD-1548627.1 | CAGAACUUGCCACACGUGCAA | | 651-671 | UUGCACGUGUGGCAAGUUCUGCA | | 649-671 |
| AD-1548628.1 | CACACGUGCAAUCCCUGAACA | | 661-681 | UGUUCAGGGAUUGCACGUGUGGC | | 659-681 |
| AD-1548629.1 | GUGCAAUCCCUGAACUGACAA | | 666-686 | UUGUCAGUUCAGGGAUUGCACGU | | 664-686 |
| AD-1548630.1 | GAGGACCAGGUGGUGGUUAAA | | 701-721 | UUUAACCACCACCUGGUCCUCGU | | 699-721 |
| AD-1548631.1 | GUGGUGGUUAAUAAGGCUGCA | | 710-730 | UGCAGCCUUAUUAACCACCACCU | | 708-730 |
| AD-1548632.1 | UGCAGAAUACAAAUGAUGUAA | | 819-839 | UUACAUCAUUUGUAUUCUGCAUG | | 817-839 |
| AD-1548633.1 | AGAAUACAAAUGAUGUAGAAA | | 822-842 | UUUCTACAUCAUUUGUAUUCUGC | | 820-842 |
| AD-1548634.1 | UGAUGUAGAAACAGCUCGUUA | | 832-852 | UAACGAGCUGUUUCUACAUCAUU | | 830-852 |
| AD-1548635.1 | CUUGCAUAACCUUUCCCAUCA | | 865-885 | UGAUGGGAAAGGUUAUGCAAGGU | | 863-885 |
| AD-1548636.1 | UUGCAUAACCUUUCCCAUCAA | | 866-886 | UUGATGGGAAAGGUUAUGCAAGG | | 864-886 |
| AD-1548637.1 | CCAUCUUUAAGUCUGGAGGCA | | 903-923 | UGCCTCCAGACUUAAAGAUGGCC | | 901-923 |
| AD-1548638.1 | GCCAUUACAACUCUCCACAAA | | 977-997 | UUUGTGGAGAGUUGUAAUGGCAU | | 975-997 |
| AD-1548639.1 | GUUGCCUUGCUCAACAAAACA | | 1061-1081 | UGUUTUGUUGAGCAAGGCAACCA | | 1059-1081 |
| AD-1548640.1 | AUUCUUGGCUAUUACGACAGA | | 1090-1110 | UCUGTCGUAAUAGCCAAGAAUUU | | 1088-1110 |
| AD-1548641.1 | AAAUAUAAUGAGGACCUAUAA | | 1189-1209 | UUAUAGGUCCUCAUUAUAUUUAC | | 1187-1209 |
| AD-1548642.1 | AAUAUAAUGAGGACCUAUACA | | 1190-1210 | UGUATAGGUCCUCAUUAUAUUUA | | 1188-1210 |
| AD-1548643.1 | GCAGAGUGCUGAAGGUGCUAA | | 1236-1256 | UUAGCACCUUCAGCACUCUGCUU | | 1234-1256 |
| AD-1548644.1 | UUCUGGGUUCAGAUGAUAUAA | | 1437-1457 | UUAUAUCAUCUGAACCCAGAAGC | | 1435-1457 |
| AD-1548645.1 | GAUGAUAUAAAUGUGGUCACA | | 1448-1468 | UGUGACCACAUUUAUAUCAUCUG | | 1446-1468 |
| AD-1548646.1 | GGAAUUCUUUCUAACCUCACA | | 1478-1498 | UGUGAGGUUAGAAAGAAUUCCAG | | 1476-1498 |
| AD-1548647.1 | UCUUUCUAACCUCACUUGCAA | | 1483-1503 | UUGCAAGUGAGGUUAGAAAGAAU | | 1481-1503 |
| AD-1548648.1 | UUCUAACCUCACUUGCAAUAA | | 1486-1506 | UUAUTGCAAGUGAGGUUAGAAAG | | 1484-1506 |
| AD-1548649.1 | UCUAACCUCACUUGCAAUAAA | | 1487-1507 | UUUATUGCAAGUGAGGUUAGAAA | | 1485-1507 |
| AD-1548650.1 | AAGUGGGUGGUAUAGAGGCUA | | 1533-1553 | UAGCCUCUAUACCACCCACUUGG | | 1531-1553 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548651.1 | UGGGUGGUAUAGAGGCUCUUA | | 1536-1556 | UAAGAGCCUCUAUACCACCCACU | | 1534-1556 |
| AD-1548652.1 | AGCCUGCCAUCUGUGCUCUUA | | 1599-1619 | UAAGAGCACAGAUGGCAGGCUCA | | 1597-1619 |
| AD-1548653.1 | GCCUGCCAUCUGUGCUCUUCA | | 1600-1620 | UGAAGAGCACAGAUGGCAGGCUC | | 1598-1620 |
| AD-1548654.1 | CAUCUGUGCUCUUCGUCAUCA | | 1606-1626 | UGAUGACGAAGAGCACAGAUGGC | | 1604-1626 |
| AD-1548655.1 | GCUCUUCGUCAUCUGACCAGA | | 1613-1633 | UCUGGUCAGAUGACGAAGAGCAC | | 1611-1633 |
| AD-1548656.1 | AUGCAGUUCGCCUUCACUAUA | | 1662-1682 | UAUAGUGAAGGCGAACUGCAUUC | | 1660-1682 |
| AD-1548657.1 | UGGUUAAGCUCUUACACCCAA | | 1695-1715 | UUGGGUGUAAGAGCUUAACCACA | | 1693-1715 |
| AD-1548658.1 | CUGUUGGAUUGAUUCGAAAUA | | 1743-1763 | UAUUTCGAAUCAAUCCAACAGUA | | 1741-1763 |
| AD-1548659.1 | UGUUGGAUUGAUUCGAAAUCA | | 1744-1764 | UGAUTUCGAAUCAAUCCAACAGU | | 1742-1764 |
| AD-1548660.1 | CGUGAGCAGGGUGCCAUUCCA | | 1796-1816 | UGGAAUGGCACCCUGCUCACGCA | | 1794-1816 |
| AD-1548661.1 | CCACGACUAGUUCAGUUGCUA | | 1814-1834 | UAGCAACUGAACUAGUCGUGGAA | | 1812-1834 |
| AD-1548662.1 | UUCAGUUGCUUGUUCGUGCAA | | 1824-1844 | UUGCACGAACAAGCAACUGAACU | | 1822-1844 |
| AD-1548663.1 | UCAGUUGCUUGUUCGUGCACA | | 1825-1845 | UGUGCACGAACAAGCAACUGAAC | | 1823-1845 |
| AD-1548664.1 | AGUUGCUUGUUCGUGCACAUA | | 1827-1847 | UAUGTGCACGAACAAGCAACUGA | | 1825-1847 |
| AD-1548665.1 | CAGAGGACUAAAUACCAUUCA | | 1984-2004 | UGAATGGUAUUUAGUCCUCUGAU | | 1982-2004 |
| AD-1548666.1 | GACUAAAUACCAUUCCAUUGA | | 1989-2009 | UCAATGGAAUGGUAUUUAGUCCU | | 1987-2009 |
| AD-1548667.1 | CCAUUCCAUUGUUUGUGCAGA | | 1998-2018 | UCUGCACAAACAAUGGAAUGGUA | | 1996-2018 |
| AD-1548668.1 | AUUCCAUUGUUUGUGCAGCUA | | 2000-2020 | UAGCTGCACAAACAAUGGAAUGG | | 1998-2020 |
| AD-1548669.1 | UUCCAUUGUUUGUGCAGCUGA | | 2001-2021 | UCAGCUGCACAAACAAUGGAAUG | | 1999-2021 |
| AD-1548670.1 | CCAUUGUUUGUGCAGCUGCUA | | 2003-2023 | UAGCAGCUGCACAAACAAUGGAA | | 2001-2023 |
| AD-1548671.1 | GCCACAGCUCCUCUGACAGAA | | 2120-2140 | UUCUGUCAGAGGAGCUGUGGCUC | | 2118-2140 |
| AD-1548672.1 | AAGCCACAAGAUUACAAGAAA | | 2210-2230 | UUUCTUGUAAUCUUGUGGCUUGU | | 2208-2230 |
| AD-1548673.1 | CCACAAGAUUACAAGAAACGA | | 2213-2233 | UCGUTUCUUGUAAUCUUGUGGCU | | 2211-2233 |
| AD-1548674.1 | AAGAUUACAAGAAACGGCUUA | | 2217-2237 | UAAGCCGUUUCUUGUAAUCUUGU | | 2215-2237 |
| AD-1548675.1 | AUUACAAGAAACGGCUUUCAA | | 2220-2240 | UUGAAAGCCGUUUCUUGUAAUCU | | 2218-2240 |
| AD-1548676.1 | GGCUUUCAGUUGAGCUGACCA | | 2232-2252 | UGGUCAGCUCAACUGAAAGCCGU | | 2230-2252 |
| AD-1548677.1 | UGAGCUGACCAGCUCUCUCUA | | 2242-2262 | UAGAGAGAGCUGGUCAGCUCAAC | | 2240-2262 |
| AD-1548678.1 | GAGCCAAUGGCUUGGAAUGAA | | 2270-2290 | UUCATUCCAAGCCAUUGGCUCUG | | 2268-2290 |
| AD-1548679.1 | CUGAUCUUGGACUUGAUAUUA | | 2295-2315 | UAAUAUCAAGUCCAAGAUCAGCA | | 2293-2315 |
| AD-1548680.1 | UGGUUUGAUACUGACCUGUAA | | 2540-2560 | UUACAGGUCAGUAUCAAACCAGG | | 2538-2560 |
| AD-1548681.1 | UUUGAUACUGACCUGUAAAUA | | 2543-2563 | UAUUTACAGGUCAGUAUCAAACC | | 2541-2563 |
| AD-1548682.1 | GGCUAUUUGUAAAUCUGCCAA | | 2666-2686 | UUGGCAGAUUUACAAAUAGCCUA | | 2664-2686 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1548683.1 | UUUCUGGUUGUUAUGUGAUCA | | 2743-2763 | UGAUCACAUAACAACCAGAAAUC | | 2741-2763 |
| AD-1548684.1 | GAUCAUGUGUGGAAGUUAUUA | | 2759-2779 | UAAUAACUUCCACACAUGAUCAC | | 2757-2779 |
| AD-1548685.1 | UAAUACUCAAAUGAGUAACAA | | 2813-2833 | UUGUTACUCAUUUGAGUAUUAAG | | 2811-2833 |
| AD-1548686.1 | AUUGUCUGAACUUGCAUUGUA | | 2882-2902 | UACAAUGCAAGUUCAGACAAUAC | | 2880-2902 |
| AD-1548687.1 | CAGAAAGUGCCUGACACACUA | | 2951-2971 | UAGUGUGUCAGGCACUUUCUGAG | | 2949-2971 |
| AD-1548688.1 | GAAAGUGCCUGACACACUAAA | | 2953-2973 | UUUAGUGUGUCAGGCACUUUCUG | | 2951-2973 |
| AD-1548689.1 | GCCUGACACACUAACCAAGCA | | 2959-2979 | UGCUTGGUUAGUGUGUCAGGCAC | | 2957-2979 |
| AD-1548690.1 | UGGGAACAAUUGAAGUAAACA | | 2990-3010 | UGUUTACUUCAAUUGUUCCCAUA | | 2988-3010 |
| AD-1548691.1 | AUGGAUCACAAGAUGGAAUUA | | 3093-3113 | UAAUTCCAUCUUGUGAUCCAUUC | | 3091-3113 |
| AD-1548692.1 | UUUAUCAAACCCUAGCCUUGA | | 3111-3131 | UCAAGGCUAGGGUUUGAUAAAUU | | 3109-3131 |
| AD-1548693.1 | UAUCUGUAAUGGUACUGACUA | | 3164-3184 | UAGUCAGUACCAUUACAGAUAUU | | 3162-3184 |
| AD-1548694.1 | UUGCUUGCUUUGAAGUAGCUA | | 3184-3204 | UAGCTACUUCAAAGCAAGCAAAG | | 3182-3204 |
| AD-1548695.1 | AGUGUUAAGUUAUAGUGAAUA | | 3257-3277 | UAUUCACUAUAACUUAACACUAC | | 3255-3277 |
| AD-1548696.1 | UGUUAAGUUAUAGUGAAUACA | | 3259-3279 | UGUATUCACUAUAACUUAACACU | | 3257-3279 |
| AD-1548697.1 | GUAAUGGUGUAGAACACUAAA | | 3311-3331 | UUUAGUGUUCUACACCAUUACUC | | 3309-3331 |
| AD-1548698.1 | AAUGGUGUAGAACACUAAUUA | | 3313-3333 | UAAUTAGUGUUCUACACCAUUAC | | 3311-3333 |
| AD-1548699.1 | CUAAUUCAUAAUCACUCUAAA | | 3327-3347 | UUUAGAGUGAUUAUGAAUUAGUG | | 3325-3347 |
| AD-1548700.1 | UUUGGGAUAUGUAUGGGUAGA | | 3489-3509 | UCUACCCAUACAUAUCCCAAAUA | | 3487-3509 |
| AD-1548701.1 | GGGUAAAUCAGUAAGAGGUGA | | 3508-3528 | UCACCUCUUACUGAUUUACCCUA | | 3506-3528 |
| AD-1548702.1 | GUAAAUCAGUAAGAGGUGUUA | | 3510-3530 | UAACACCUCUUACUGAUUUACCC | | 3508-3530 |
| AD-1548703.1 | UAAAUCAGUAAGAGGUGUUAA | | 3511-3531 | UUAACACCUCUUACUGAUUUACC | | 3509-3531 |
| AD-1548704.1 | AAUCAGUAAGAGGUGUUAUUA | | 3513-3533 | UAAUAACACCUCUUACUGAUUUA | | 3511-3533 |

**Table 3. Modified Sense and Antisense Strand Sequences of CTNNB1 dsRNA Agents**

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548365.1 | gsasggguAfuUfUfGfaaguauacscsa | | VPuGfgudAu(Agn)cuucaaAfuAfcccucsasg | | CUGAGGGUAUUUGAAGUAUACCA | |
| AD-1548366.1 | gsasggguauUfUfGfaaguauacscsa | | VPudGgudAudAcuucdAaAfuacccucsasg | | CUGAGGGUAUUUGAAGUAUACCA | |
| AD- | gsgsguauUfuGfAfAfguauaccasusa | | VPuAfugdGu(Agn)uacuucAfaAfuacccsusc | | GAGGGUAUUUGAAGUAUACCAUA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548367.1 | | | | | | |
| AD-1548368.1 | gsgsuauuUfgAfAfGfuauaccausasa | | VPuUfaudGg(Tgn)auacuuCfaAfauaccscsu | | AGGGUAUUUGAAGUAUACCAUAC | |
| AD-1548369.1 | ususugaaGfuAfUfAfccauacaascsa | | VPuGfuudGu(Agn)ugguauAfcUfucaaasusa | | UAUUUGAAGUAUACCAUACAACU | |
| AD-1548370.1 | ususgaagUfaUfAfCfcauacaacsusa | | VPuAfgudTg(Tgn)augguaUfaCfuucaasasu | | AUUUGAAGUAUACCAUACAACUG | |
| AD-1548371.1 | usgsgacaAfuGfGfCfuacucaagscsa | | VPuGfcudTg(Agn)guagccAfuUfguccascsg | | CGUGGACAAUGGCUACUCAAGCU | |
| AD-1548372.1 | gsascaauggCfUfAfcucaagcusgsa | | VPudCagdCudTgagudAgCfcauugucscsa | | UGGACAAUGGCUACUCAAGCUGA | |
| AD-1548373.1 | ascsaaugGfcUfAfCfucaagcugsasa | | VPuUfcadGc(Tgn)ugaguaGfcCfauuguscsc | | GGACAAUGGCUACUCAAGCUGAU | |
| AD-1548374.1 | asasgcugauUfUfGfauggaguusgsa | | VPudCaadCudCcaucdAaAfucagcuusgsa | | UCAAGCUGAUUUGAUGGAGUUGG | |
| AD-1548375.1 | asgscugauuUfGfAfuggaguugsgsa | | VPudCcadAcdTccaudCaAfaucagcususg | | CAAGCUGAUUUGAUGGAGUUGGA | |
| AD-1548376.1 | cscsuucucuGfAfGfugguaaagsgsa | | VPudCcudTudAccacdTcAfgagaaggsasg | | CUCCUUCUCUGAGUGGUAAAGGC | |
| AD-1548377.1 | csasguccUfuCfAfCfucaagaacsasa | | VPuUfgudTc(Tgn)ugagugAfaGfgacugsasg | | CUCAGUCCUUCACUCAAGAACAA | |
| AD-1548378.1 | csusucacucAfAfGfaacaaguasgsa | | VPudCuadCudTguucdTuGfagugaagsgsa | | UCCUUCACUCAAGAACAAGUAGC | |
| AD-1548379.1 | csascucaagAfAfCfaaguagcusgsa | | VPudCagdCudAcuugdTuCfuugagugsasa | | UUCACUCAAGAACAAGUAGCUGA | |
| AD-1548380.1 | gsusagcuGfaUfAfUfugauggacsasa | | VPuUfgudCc(Agn)ucaauaUfcAfgcuacsusu | | AAGUAGCUGAUAUUGAUGGACAG | |
| AD-1548381.1 | asusugauGfgAfCfAfguaugcaasusa | | VPuAfuudGc(Agn)uacuguCfcAfucaausasu | | AUAUUGAUGGACAGUAUGCAAUG | |
| AD-1548382.1 | asgsuaugcaAfUfGfacucgagcsusa | | VPudAgcdTcdGagucdAuUfgcauacusgsu | | ACAGUAUGCAAUGACUCGAGCUC | |
| AD-1548383.1 | gsgsuacgAfgCfUfGfcuauguucscsa | | VPuGfgadAc(Agn)uagcagCfuCfguaccscsu | | AGGGUACGAGCUGCUAUGUUCCC | |
| AD-1548384.1 | ascsgagcugCfUfAfuguucccusgsa | | VPudCagdGgdAacaudAgCfagcucgusasc | | GUACGAGCUGCUAUGUUCCCUGA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548385.1 | usgsuucccuGfAfGfacauuagasusa | | VPudAucdTadAugucdTcAfgggaacasusa | | UAUGUUCCCUGAGACAUUAGAUG | |
| AD-1548386.1 | ususcccuGfaGfAfCfauuagaugsasa | | VPuUfcadTc(Tgn)aaugucUfcAfgggaascsa | | UGUUCCCUGAGACAUUAGAUGAG | |
| AD-1548387.1 | asgsaugaGfgGfCfAfugcagaucscsa | | VPuGfgadTc(Tgn)gcaugcCfcUfcaucusasa | | UUAGAUGAGGGCAUGCAGAUCCC | |
| AD-1548388.1 | csusgcucauCfCfCfacuaaugusccsa | | VPudGacdAudTagugdGgAfugagcagscsa | | UGCUGCUCAUCCCACUAAUGUCC | |
| AD-1548389.1 | ascscaucAfcAfGfAfugcugaaascsa | | VPuGfuudTc(Agn)gcaucuGfuGfauggususc | | GAACCAUCACAGAUGCUGAAACA | |
| AD-1548390.1 | csasucacAfgAfUfGfcugaaacasusa | | VPuAfugdTu(Tgn)cagcauCfuGfugaugsgsu | | ACCAUCACAGAUGCUGAAACAUG | |
| AD-1548391.1 | csasucacagAfUfGfcugaaacasusa | | VPudAugdTudTcagcdAuCfugugaugsgsu | | ACCAUCACAGAUGCUGAAACAUG | |
| AD-1548392.1 | asusgcugAfaAfCfAfugcaguugsusa | | VPuAfcadAc(Tgn)gcauguUfuCfagcauscsu | | AGAUGCUGAAACAUGCAGUUGUA | |
| AD-1548393.1 | asusgcugaaAfCfAfugcaguugsusa | | VPudAcadAcdTgcaudGuUfucagcauscsu | | AGAUGCUGAAACAUGCAGUUGUA | |
| AD-1548394.1 | ascsaugcAfgUfUfGfuaaacuugsasa | | VPuUfcadAg(Tgn)uuacaaCfuGfcaugususu | | AAACAUGCAGUUGUAAACUUGAU | |
| AD-1548395.1 | asusgcagUfuGfUfAfaacuugaususa | | VPuAfaudCa(Agn)guuuacAfaCfugcausgsu | | ACAUGCAGUUGUAAACUUGAUUA | |
| AD-1548396.1 | asusgcaguuGfUfAfaacuugaususa | | VPudAaudCadAguuudAcAfacugcausgsu | | ACAUGCAGUUGUAAACUUGAUUA | |
| AD-1548397.1 | ascsgugcAfaUfCfCfcugaacugsasa | | VPuUfcadGu(Tgn)cagggaUfuGfcacgusgsu | | ACACGUGCAAUCCCUGAACUGAC | |
| AD-1548398.1 | usgscaauCfcCfUfGfaacugacasasa | | VPuUfugdTc(Agn)guucagGfgAfuugcascsg | | CGUGCAAUCCCUGAACUGACAAA | |
| AD-1548399.1 | gsasccagGfuGfGfUfgguuaauasasa | | VPuUfuadTu(Agn)accaccAfcCfuggucscsu | | AGGACCAGGUGGUGGUUAAUAAG | |
| AD-1548400.1 | gsasccagguGfGfUfgguuaauasasa | | VPudTuadTudAaccadCcAfccuggucscsu | | AGGACCAGGUGGUGGUUAAUAAG | |
| AD-1548401.1 | cscsagguggUfGfGfuuaauaagsgsa | | VPudCcudTadTuaacdCaCfcaccuggsusc | | GACCAGGUGGUGGUUAAUAAGGC | |
| AD- | gsusuaauAfaGfGfCfugcaguuasusa | | VPuAfuadAc(Tgn)gcagccUfuAfuuaacscsa | | UGGUUAAUAAGGCUGCAGUUAUG | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548402.1 | | | | | | |
| AD-1548403.1 | cscsucagAfuGfGfUfgucugcuasusa | | VPuAfuadGc(Agn)gacaccAfuCfugaggsasg | | CUCCUCAGAUGGUGUCUGCUAUU | |
| AD-1548404.1 | uscsagauggUfGfUfcugcuauusgsa | | VPudCaadTadGcagadCaCfcaucugasgsg | | CCUCAGAUGGUGUCUGCUAUUGU | |
| AD-1548405.1 | asgsauggUfgUfCfUfgcuauugusasa | | VPuUfacdAa(Tgn)agcagaCfaCfcaucusgsa | | UCAGAUGGUGUCUGCUAUUGUAC | |
| AD-1548406.1 | asgsauggugUfCfUfgcuauugusasa | | VPudTacdAadTagcadGaCfaccaucusgsa | | UCAGAUGGUGUCUGCUAUUGUAC | |
| AD-1548407.1 | ascsguacCfaUfGfCfagaauacasasa | | VPuUfugdTa(Tgn)ucugcaUfgGfuacgusasc | | GUACGUACCAUGCAGAAUACAAA | |
| AD-1548408.1 | ascsguaccaUfGfCfagaauacasasa | | VPudTugdTadTucugdCaUfgguacgusasc | | GUACGUACCAUGCAGAAUACAAA | |
| AD-1548409.1 | csgsuaccAfuGfCfAfgaauacaasasa | | VPuUfuudGu(Agn)uucugcAfuGfguacgsusa | | UACGUACCAUGCAGAAUACAAAU | |
| AD-1548410.1 | gsusaccaUfgCfAfGfaauacaaasusa | | VPuAfuudTg(Tgn)auucugCfaUfgguacsgsu | | ACGUACCAUGCAGAAUACAAAUG | |
| AD-1548411.1 | usasccaugcAfGfAfauacaaausgsa | | VPudCaudTudGuauudCuGfcaugguascsg | | CGUACCAUGCAGAAUACAAAUGA | |
| AD-1548412.1 | ascscaugcaGfAfAfuacaaaugsasa | | VPudTcadTudTguaudTcUfgcauggusasc | | GUACCAUGCAGAAUACAAAUGAU | |
| AD-1548413.1 | cscsaugcagAfAfUfacaaaugasusa | | VPudAucdAudTuguadTuCfugcauggsusa | | UACCAUGCAGAAUACAAAUGAUG | |
| AD-1548414.1 | csasugcagaAfUfAfcaaaugausgsa | | VPudCaudCadTuugudAuUfcugcaugsgsu | | ACCAUGCAGAAUACAAAUGAUGU | |
| AD-1548415.1 | asusgcagAfaUfAfCfaaaugaugsusa | | VPuAfcadTc(Agn)uuuguaUfuCfugcausgsg | | CCAUGCAGAAUACAAAUGAUGUA | |
| AD-1548416.1 | gscsagaauaCfAfAfaugauguasgsa | | VPudCuadCadTcauudTgUfauucugcsasu | | AUGCAGAAUACAAAUGAUGUAGA | |
| AD-1548417.1 | csasgaauAfcAfAfAfugauguagsasa | | VPuUfcudAc(Agn)ucauuuGfuAfuucugscsa | | UGCAGAAUACAAAUGAUGUAGAA | |
| AD-1548418.1 | usgsauguagAfAfAfcagcucgususa | | VPudAacdGadGcugudTuCfuacaucasusu | | AAUGAUGUAGAAACAGCUCGUUG | |
| AD-1548419.1 | gsasuguagaAfAfCfagcucguusgsa | | VPudCaadCgdAgcugdTuUfcuacaucsasu | | AUGAUGUAGAAACAGCUCGUUGU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548420.1 | ascsuggccaUfCfUfuuaagucusgsa | | VPudCagdAcdTuaaadGaUfggccagusasa | | UUACUGGCCAUCUUUAAGUCUGG | |
| AD-1548421.1 | csusggccauCfUfUfuaagucugsgsa | | VPudCcadGadCuuaadAgAfuggccagsusa | | UACUGGCCAUCUUUAAGUCUGGA | |
| AD-1548422.1 | usgsgccaUfcUfUfUfaagucuggsasa | | VPuUfccdAg(Agn)cuuaaaGfaUfggccasgsu | | ACUGGCCAUCUUUAAGUCUGGAG | |
| AD-1548423.1 | gsgsccaucuUfUfAfagucuggasgsa | | VPudCucdCadGacuudAaAfgauggccsasg | | CUGGCCAUCUUUAAGUCUGGAGG | |
| AD-1548424.1 | gscscaucuuUfAfAfgucuggagsgsa | | VPudCcudCcdAgacudTaAfagauggcscsa | | UGGCCAUCUUUAAGUCUGGAGGC | |
| AD-1548425.1 | usgsguucacCfAfGfuggauucusgsa | | VPudCagdAadTccacdTgGfugaaccasasg | | CUUGGUUCACCAGUGGAUUCUGU | |
| AD-1548426.1 | ascscaguggAfUfUfcuguguugsusa | | VPudAcadAcdAcagadAuCfcacuggusgsa | | UCACCAGUGGAUUCUGUGUUGUU | |
| AD-1548427.1 | asusggcaGfuGfCfGfuuuagcugsgsa | | VPuCfcadGc(Tgn)aaacgcAfcUfgccaususu | | AAAUGGCAGUGCGUUUAGCUGGU | |
| AD-1548428.1 | asusggcaguGfCfGfuuuagcugsgsa | | VPudCcadGcdTaaacdGcAfcugccaususu | | AAAUGGCAGUGCGUUUAGCUGGU | |
| AD-1548429.1 | ususgccuUfgCfUfCfaacaaaacsasa | | VPuUfgudTu(Tgn)guugagCfaAfggcaascsc | | GGUUGCCUUGCUCAACAAAACAA | |
| AD-1548430.1 | usgsccuugcUfCfAfacaaaacasasa | | VPudTugdTudTuguudGaGfcaaggcasasc | | GUUGCCUUGCUCAACAAAACAAA | |
| AD-1548431.1 | usgsccuuGfcUfCfAfacaaaacasasa | | VPuUfugdTu(Tgn)uguugaGfcAfaggcasasc | | GUUGCCUUGCUCAACAAAACAAA | |
| AD-1548432.1 | gscscuugCfuCfAfAfcaaaacaasasa | | VPuUfuudGu(Tgn)uuguugAfgCfaaggcsasa | | UUGCCUUGCUCAACAAAACAAAU | |
| AD-1548433.1 | ususaaauucUfUfGfgcuauuacsgsa | | VPudCgudAadTagccdAaGfaauuuaascsa | | UGUUAAAUUCUUGGCUAUUACGA | |
| AD-1548434.1 | asusucuuggCfUfAfuuacgacasgsa | | VPudCugdTcdGuaaudAgCfcaagaaususu | | AAAUUCUUGGCUAUUACGACAGA | |
| AD-1548435.1 | gsgscuauUfaCfGfAfcagacugcscsa | | VPuGfgcdAg(Tgn)cugucgUfaAfuagccsasa | | UUGGCUAUUACGACAGACUGCCU | |
| AD-1548436.1 | usascgacAfgAfCfUfgccuucaasasa | | VPuUfuudGa(Agn)ggcaguCfuGfucguasasu | | AUUACGACAGACUGCCUUCAAAU | |
| AD- | csusuuaguaAfAfUfauaaugagsgsa | | VPudCcudCadTuauadTuUfacuaaagscsu | | AGCUUUAGUAAAUAUAAUGAGGA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548437.1 | | | | | | |
| AD-1548438.1 | usasuaaugaGfGfAfccuauacususa | | VPudAagdTadTaggudCcUfcauuauasusu | | AAUAUAAUGAGGACCUAUACUUA | |
| AD-1548439.1 | asgscuggUfgGfAfAfugcaagcususa | | VPuAfagdCu(Tgn)gcauucCfaCfcagcususc | | GAAGCUGGUGGAAUGCAAGCUUU | |
| AD-1548440.1 | csasgauccaAfGfUfcaacgucususa | | VPudAagdAcdGuugadCuUfggaucugsusc | | GACAGAUCCAAGUCAACGUCUUG | |
| AD-1548441.1 | cscsaaguCfaAfCfGfucuuguucsasa | | VPuUfgadAc(Agn)agacguUfgAfcuuggsasu | | AUCCAAGUCAACGUCUUGUUCAG | |
| AD-1548442.1 | cscsaagucaAfCfGfucuuguucsasa | | VPudTgadAcdAagacdGuUfgacuuggsasu | | AUCCAAGUCAACGUCUUGUUCAG | |
| AD-1548443.1 | asgsaacuGfuCfUfUfuggacucuscsa | | VPuGfagdAg(Tgn)ccaaagAfcAfguucusgsa | | UCAGAACUGUCUUUGGACUCUCA | |
| AD-1548444.1 | csusuucaGfaUfGfCfugcaacuasasa | | VPuUfuadGu(Tgn)gcagcaUfcUfgaaagsasu | | AUCUUUCAGAUGCUGCAACUAAA | |
| AD-1548445.1 | uscsagauGfcUfGfCfaacuaaacsasa | | VPuUfgudTu(Agn)guugcaGfcAfucugasasa | | UUUCAGAUGCUGCAACUAAACAG | |
| AD-1548446.1 | gsasugcuGfcAfAfCfuaaacaggsasa | | VPuUfccdTg(Tgn)uuaguuGfcAfgcaucsusg | | CAGAUGCUGCAACUAAACAGGAA | |
| AD-1548447.1 | csusgcaacuAfAfAfcaggaaggsgsa | | VPudCccdTudCcugudTuAfguugcagscsa | | UGCUGCAACUAAACAGGAAGGGA | |
| AD-1548448.1 | gsasagggAfuGfGfAfaggucuccsusa | | VPuAfggdAg(Agn)ccuuccAfuCfccuucscsu | | AGGAAGGGAUGGAAGGUCUCCUU | |
| AD-1548449.1 | csusucugGfgUfUfCfagaugauasusa | | VPuAfuadTc(Agn)ucugaaCfcCfagaagscsu | | AGCUUCUGGGUUCAGAUGAUAUA | |
| AD-1548450.1 | cscsugugcaGfCfUfggaauucususa | | VPudAagdAadTuccadGcUfgcacaggsusg | | CACCUGUGCAGCUGGAAUUCUUU | |
| AD-1548451.1 | usgsugcagcUfGfGfaauucuuuscsa | | VPudGaadAgdAauucdCaGfcugcacasgsg | | CCUGUGCAGCUGGAAUUCUUUCU | |
| AD-1548452.1 | asasuucuUfuCfUfAfaccucacususa | | VPuAfagdTg(Agn)gguuagAfaAfgaauuscsc | | GGAAUUCUUUCUAACCUCACUUG | |
| AD-1548453.1 | ususcuuucuAfAfCfcucacuugscsa | | VPudGcadAgdTgaggdTuAfgaaagaasusu | | AAUUCUUUCUAACCUCACUUGCA | |
| AD-1548454.1 | csusuucuAfaCfCfUfcacuugcasasa | | VPuUfugdCa(Agn)gugaggUfuAfgaaagsasa | | UUCUUUCUAACCUCACUUGCAAU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548455.1 | ususucuaAfcCfUfCfacuugcaasusa | | VPuAfuudGc(Agn)agugagGfuUfagaaasgsa | | UCUUUCUAACCUCACUUGCAAUA | |
| AD-1548456.1 | csusaaccucAfCfUfugcaauaasusa | | VPudAuudAudTgcaadGuGfagguuagsasa | | UUCUAACCUCACUUGCAAUAAUU | |
| AD-1548457.1 | usasaccucaCfUfUfgcaauaaususa | | VPudAaudTadTugcadAgUfgagguuasgsa | | UCUAACCUCACUUGCAAUAAUUA | |
| AD-1548458.1 | asasccucacUfUfGfcaauaauusasa | | VPudTaadTudAuugcdAaGfugagguusasg | | CUAACCUCACUUGCAAUAAUUAU | |
| AD-1548459.1 | ascscucacuUfGfCfaauaauuasusa | | VPudAuadAudTauugdCaAfgugaggususa | | UAACCUCACUUGCAAUAAUUAUA | |
| AD-1548460.1 | cscsucacuuGfCfAfauaauuausasa | | VPudTaudAadTuauudGcAfagugaggsusu | | AACCUCACUUGCAAUAAUUAUAA | |
| AD-1548461.1 | csuscacuUfgCfAfAfuaauuauasasa | | VPuUfuadTa(Agn)uuauugCfaAfgugagsgsu | | ACCUCACUUGCAAUAAUUAUAAG | |
| AD-1548462.1 | csuscacuugCfAfAfuaauuauasasa | | VPudTuadTadAuuaudTgCfaagugagsgsu | | ACCUCACUUGCAAUAAUUAUAAG | |
| AD-1548463.1 | uscsacuugcAfAfUfaauuauaasgsa | | VPudCuudAudAauuadTuGfcaagugasgsg | | CCUCACUUGCAAUAAUUAUAAGA | |
| AD-1548464.1 | csascuugcaAfUfAfauuauaagsasa | | VPudTcudTadTaauudAuUfgcaagugsasg | | CUCACUUGCAAUAAUUAUAAGAA | |
| AD-1548465.1 | csasagugGfgUfGfGfuauagaggscsa | | VPuGfccdTc(Tgn)auaccaCfcCfacuugsgsc | | GCCAAGUGGGUGGUAUAGAGGCU | |
| AD-1548466.1 | gscsucuuGfuGfCfGfuacuguccsusa | | VPuAfggdAc(Agn)guacgcAfcAfagagcscsu | | AGGCUCUUGUGCGUACUGUCCUU | |
| AD-1548467.1 | cscsugccauCfUfGfugcucuucsgsa | | VPudCgadAgdAgcacdAgAfuggcaggscsu | | AGCCUGCCAUCUGUGCUCUUCGU | |
| AD-1548468.1 | csusgccaucUfGfUfgcucuucgsusa | | VPudAcgdAadGagcadCaGfauggcagsgsc | | GCCUGCCAUCUGUGCUCUUCGUC | |
| AD-1548469.1 | usgsccaucuGfUfGfcucuucguscsa | | VPudGacdGadAgagcdAcAfgauggcasgsg | | CCUGCCAUCUGUGCUCUUCGUCA | |
| AD-1548470.1 | gscscaucugUfGfCfucuucgucsasa | | VPudTgadCgdAagagdCaCfagauggcsasg | | CUGCCAUCUGUGCUCUUCGUCAU | |
| AD-1548471.1 | cscsaucuguGfCfUfcuucgucasusa | | VPudAugdAcdGaagadGcAfcagauggcsca | | UGCCAUCUGUGCUCUUCGUCAUC | |
| AD- | asuscuguGfcUfCfUfucgucaucsusa | | VPuAfgadTg(Agn)cgaagaGfcAfcagausgsg | | CCAUCUGUGCUCUUCGUCAUCUG | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548472.1 | | | | | | |
| AD-1548473.1 | uscsugugcuCfUfUfcgucaucusgsa | | VPudCagdAudGacgadAgAfgcacagasusg | | CAUCUGUGCUCUUCGUCAUCUGA | |
| AD-1548474.1 | csusgugcUfcUfUfCfgucaucugsasa | | VPuUfcadGa(Tgn)gacgaaGfaGfcacagsasu | | AUCUGUGCUCUUCGUCAUCUGAC | |
| AD-1548475.1 | usgsugcuCfuUfCfGfucaucugascsa | | VPuGfucdAg(Agn)ugacgaAfgAfgcacasgsa | | UCUGUGCUCUUCGUCAUCUGACC | |
| AD-1548476.1 | usgsgacuAfcCfAfGfuugugguusasa | | VPuUfaadCc(Agn)caacugGfuAfguccasusa | | UAUGGACUACCAGUUGUGGUUAA | |
| AD-1548477.1 | gsascuaccaGfUfUfgugguuaasgsa | | VPudCuudAadCcacadAcUfgguagucscsa | | UGGACUACCAGUUGUGGUUAAGC | |
| AD-1548478.1 | usgsugguUfaAfGfCfucuuacacscsa | | VPuGfgudGu(Agn)agagcuUfaAfccacasasc | | GUUGUGGUUAAGCUCUUACACCC | |
| AD-1548479.1 | gsasuaaaggCfUfAfcuguuggasusa | | VPudAucdCadAcagudAgCfcuuuaucsasg | | CUGAUAAAGGCUACUGUUGGAUU | |
| AD-1548480.1 | asusaaagGfcUfAfCfuguuggaususa | | VPuAfaudCc(Agn)acaguaGfcCfuuuauscsa | | UGAUAAAGGCUACUGUUGGAUUG | |
| AD-1548481.1 | asusaaaggcUfAfCfuguuggaususa | | VPudAaudCcdAacagdTaGfccuuuauscsa | | UGAUAAAGGCUACUGUUGGAUUG | |
| AD-1548482.1 | asasggcuAfcUfGfUfuggauugasusa | | VPuAfucdAa(Tgn)ccaacaGfuAfgccuususa | | UAAAGGCUACUGUUGGAUUGAUU | |
| AD-1548483.1 | asasggcuacUfGfUfuggauugasusa | | VPudAucdAadTccaadCaGfuagccuususa | | UAAAGGCUACUGUUGGAUUGAUU | |
| AD-1548484.1 | asgsgcuacuGfUfUfggauugaususa | | VPudAaudCadAuccadAcAfguagccususu | | AAAGGCUACUGUUGGAUUGAUUC | |
| AD-1548485.1 | gsgscuacUfgUfUfGfgauugauuscsa | | VPuGfaadTc(Agn)auccaaCfaGfuagccsusu | | AAGGCUACUGUUGGAUUGAUUCG | |
| AD-1548486.1 | gscsuacuguUfGfGfauugauucsgsa | | VPudCgadAudCaaucdCaAfcaguagcscsu | | AGGCUACUGUUGGAUUGAUUCGA | |
| AD-1548487.1 | csusacuguuGfGfAfuugauucgsasa | | VPudTcgdAadTcaaudCcAfacaguagscsc | | GGCUACUGUUGGAUUGAUUCGAA | |
| AD-1548488.1 | usascuguugGfAfUfugauucgasasa | | VPudTucdGadAucaadTcCfaacaguasgsc | | GCUACUGUUGGAUUGAUUCGAAA | |
| AD-1548489.1 | ascsuguuggAfUfUfgauucgaasasa | | VPudTuudCgdAaucadAuCfcaacagusasg | | CUACUGUUGGAUUGAUUCGAAAU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548490.1 | csusguuggaUfUfGfauucgaaasusa | | VPudAuudTcdGaaucdAaUfccaacagsusa | | UACUGUUGGAUUGAUUCGAAAUC | |
| AD-1548491.1 | gsusuggauuGfAfUfucgaaaucsusa | | VPudAgadTudTcgaadTcAfauccaacsasg | | CUGUUGGAUUGAUUCGAAAUCUU | |
| AD-1548492.1 | gsusuggaUfuGfAfUfucgaaaucsusa | | VPuAfgadTu(Tgn)cgaaucAfaUfccaacsasg | | CUGUUGGAUUGAUUCGAAAUCUU | |
| AD-1548493.1 | ususggauugAfUfUfUfcgaaaucususa | | VPudAagdAudTucgadAuCfaauccaascsa | | UGUUGGAUUGAUUCGAAAUCUUG | |
| AD-1548494.1 | gsgsauugAfuUfCfGfaaaucuugscsa | | VPuGfcadAg(Agn)uuucgaAfuCfaauccsasa | | UUGGAUUGAUUCGAAAUCUUGCC | |
| AD-1548495.1 | gsgsauugauUfCfGfaaaucuugscsa | | VPudGcadAgdAuuucdGaAfucaauccsasa | | UUGGAUUGAUUCGAAAUCUUGCC | |
| AD-1548496.1 | ususgauuCfgAfAfAfucuugcccsusa | | VPuAfggdGc(Agn)agauuuCfgAfaucaasusc | | GAUUGAUUCGAAAUCUUGCCCUU | |
| AD-1548497.1 | asususccaCfgAfCfUfaguucagususa | | VPuAfacdTg(Agn)acuaguCfgUfggaausgsg | | CCAUUCCACGACUAGUUCAGUUG | |
| AD-1548498.1 | cscsacgacuAfGfUfucaguugcsusa | | VPudAgcdAadCugaadCuAfgucguggsasa | | UUCCACGACUAGUUCAGUUGCUU | |
| AD-1548499.1 | ususcaguugCfUfUfguucgugcsasa | | VPudTgcdAcdGaacadAgCfaacugaascsu | | AGUUCAGUUGCUUGUUCGUGCAC | |
| AD-1548500.1 | gsgsacacagCfAfGfcaauuugusgsa | | VPudCacdAadAuugcdTgCfuguguccscsa | | UGGGACACAGCAGCAAUUUGUGG | |
| AD-1548501.1 | asusccuaGfcUfCfGfggauguucsasa | | VPuUfgadAc(Agn)ucccgaGfcUfaggausgsu | | ACAUCCUAGCUCGGGAUGUUCAC | |
| AD-1548502.1 | asgscucgGfgAfUfGfuucacaacscsa | | VPuGfgudTg(Tgn)gaacauCfcCfgagcusasg | | CUAGCUCGGGAUGUUCACAACCG | |
| AD-1548503.1 | gsasuguucaCfAfAfccgaauugsusa | | VPudAcadAudTcggudTgUfgaacaucscsc | | GGGAUGUUCACAACCGAAUUGUU | |
| AD-1548504.1 | usasucagAfgGfAfCfuaaauaccsasa | | VPuUfggdTa(Tgn)uuagucCfuCfugauasasc | | GUUAUCAGAGGACUAAAUACCAU | |
| AD-1548505.1 | asuscagaGfgAfCfUfaaauaccasusa | | VPuAfugdGu(Agn)uuuaguCfcUfcugausasa | | UUAUCAGAGGACUAAAUACCAUU | |
| AD-1548506.1 | uscsagagGfaCfUfAfaauaccaususa | | VPuAfaudGg(Tgn)auuuagUfcCfucugasusa | | UAUCAGAGGACUAAAUACCAUUC | |
| AD- | gsasggacUfaAfAfUfaccauuccsasa | | VPuUfggdAa(Tgn)gguauuUfaGfuccucsusg | | CAGAGGACUAAAUACCAUUCCAU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548507.1 | | | | | | |
| AD-1548508.1 | gsasggacuaAfAfUfaccauuccsasa | | VPudTggdAadTgguadTuUfaguccucsusg | | CAGAGGACUAAAUACCAUUCCAU | |
| AD-1548509.1 | gsgsacuaaaUfAfCfcauuccaususa | | VPudAaudGgdAauggdTaUfuuaguccsusc | | GAGGACUAAAUACCAUUCCAUUG | |
| AD-1548510.1 | ascsuaaauaCfCfAfuuccauugsusa | | VPudAcadAudGgaaudGgUfauuuaguscsc | | GGACUAAAUACCAUUCCAUUGUU | |
| AD-1548511.1 | csusaaauacCfAfUfuccauugususa | | VPudAacdAadTggaadTgGfuauuuagsusc | | GACUAAAUACCAUUCCAUUGUUU | |
| AD-1548512.1 | usasaauaccAfUfUfccauuguususa | | VPudAaadCadAuggadAuGfguauuuasgsu | | ACUAAAUACCAUUCCAUUGUUUG | |
| AD-1548513.1 | asasauaccaUfUfCfcauuguuusgsa | | VPudCaadAcdAauggdAaUfgguauuusasg | | CUAAAUACCAUUCCAUUGUUUGU | |
| AD-1548514.1 | asasuaccauUfCfCfauuguuugsusa | | VPudAcadAadCaaugdGaAfugguauususa | | UAAAUACCAUUCCAUUGUUUGUG | |
| AD-1548515.1 | asusaccauuCfCfAfuuguuugusgsa | | VPudCacdAadAcaaudGgAfaugguaususu | | AAAUACCAUUCCAUUGUUUGUGC | |
| AD-1548515.2 | asusaccauuCfCfAfuuguuugusgsa | | VPudCacdAadAcaaudGgAfaugguaususu | | AAAUACCAUUCCAUUGUUUGUGC | |
| AD-1548516.1 | usasccauucCfAfUfuguuugugscsa | | VPudGcadCadAacaadTgGfaaugguasusu | | AAUACCAUUCCAUUGUUUGUGCA | |
| AD-1548517.1 | ascscauuccAfUfUfguuugugcsasa | | VPudTgcdAcdAaacadAuGfgaauggusasu | | AUACCAUUCCAUUGUUUGUGCAG | |
| AD-1548518.1 | ascscauuCfcAfUfUfguuugugcsasa | | VPuUfgcdAc(Agn)aacaauGfgAfauggusasu | | AUACCAUUCCAUUGUUUGUGCAG | |
| AD-1548519.1 | cscsauuccaUfUfGfuuugugcasgsa | | VPudCugdCadCaaacdAaUfggaauggsusa | | UACCAUUCCAUUGUUUGUGCAGC | |
| AD-1548520.1 | csasuuccAfuUfGfUfuugugcagscsa | | VPuGfcudGc(Agn)caaacaAfuGfgaaugsgsu | | ACCAUUCCAUUGUUUGUGCAGCU | |
| AD-1548521.1 | uscscauuGfuUfUfGfugcagcugscsa | | VPuGfcadGc(Tgn)gcacaaAfcAfauggasasu | | AUUCCAUUGUUUGUGCAGCUGCU | |
| AD-1548522.1 | gsusuugugcAfGfCfugcuuuaususa | | VPudAaudAadAgcagdCuGfcacaaacsasa | | UUGUUUGUGCAGCUGCUUUAUUC | |
| AD-1548523.1 | csusccucugAfCfAfgaguuacususa | | VPudAagdTadAcucudGuCfagaggagscsu | | AGCUCCUCUGACAGAGUUACUUC | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548524.1 | gsascagaGfuUfAfCfuucacucusasa | | VPuUfagdAg(Tgn)gaaguaAfcUfcugucsasg | | CUGACAGAGUUACUUCACUCUAG | |
| AD-1548525.1 | asgsuuacUfuCfAfCfucuaggaasusa | | VPuAfuudCc(Tgn)agagugAfaGfuaacuscsu | | AGAGUUACUUCACUCUAGGAAUG | |
| AD-1548526.1 | gsasggacAfaGfCfCfacaagauusasa | | VPuUfaadTc(Tgn)uguggcUfuGfuccucsasg | | CUGAGGACAAGCCACAAGAUUAC | |
| AD-1548527.1 | csascaagauUfAfCfaagaaacgsgsa | | VPudCcgdTudTcuugdTaAfucuugugsgsc | | GCCACAAGAUUACAAGAAACGGC | |
| AD-1548528.1 | ascsaagauuAfCfAfagaaacggscsa | | VPudGccdGudTucuudGuAfaucuugusgsg | | CCACAAGAUUACAAGAAACGGCU | |
| AD-1548529.1 | csasagauuaCfAfAfgaaacggcsusa | | VPudAgcdCgdTuucudTgUfaaucuugsusg | | CACAAGAUUACAAGAAACGGCUU | |
| AD-1548530.1 | asasgauuacAfAfGfaaacggcususa | | VPudAagdCcdGuuucdTuGfuaaucuusgsu | | ACAAGAUUACAAGAAACGGCUUU | |
| AD-1548531.1 | ususacaagaAfAfCfggcuuucasgsa | | VPudCugdAadAgccgdTuUfcuuguaasusc | | GAUUACAAGAAACGGCUUUCAGU | |
| AD-1548532.1 | usascaagaaAfCfGfgcuuucagsusa | | VPudAcudGadAagccdGuUfucuuguasasu | | AUUACAAGAAACGGCUUUCAGUU | |
| AD-1548533.1 | asasacggCfuUfUfCfaguugagcsusa | | VPuAfgcdTc(Agn)acugaaAfgCfcguuuscsu | | AGAAACGGCUUUCAGUUGAGCUG | |
| AD-1548534.1 | gscsuuucAfgUfUfGfagcugaccsasa | | VPuUfggdTc(Agn)gcucaaCfuGfaaagcscsg | | CGGCUUUCAGUUGAGCUGACCAG | |
| AD-1548535.1 | csusuggacuUfGfAfuauuggugscsa | | VPudGcadCcdAauaudCaAfguccaagsasu | | AUCUUGGACUUGAUAUUGGUGCC | |
| AD-1548536.1 | usasucgcCfaGfGfAfugauccuasgsa | | VPuCfuadGg(Agn)ucauccUfgGfcgauasusc | | GAUAUCGCCAGGAUGAUCCUAGC | |
| AD-1548537.1 | csasccacCfcUfGfGfugcugacusasa | | VPuUfagdTc(Agn)gcaccaGfgGfuggugsgsc | | GCCACCACCCUGGUGCUGACUAU | |
| AD-1548538.1 | asuscagcugGfCfCfcfugguuugasusa | | VPudAucdAadAccagdGcCfagcugaususg | | CAAUCAGCUGGCCUGGUUUGAUA | |
| AD-1548539.1 | uscsagcuGfgCfCfUfgguuugausasa | | VPuUfaudCa(Agn)accaggCfcAfgcugasusu | | AAUCAGCUGGCCUGGUUUGAUAC | |
| AD-1548540.1 | uscsagcuggCfCfUfgguuugausasa | | VPudTaudCadAaccadGgCfcagcugasusu | | AAUCAGCUGGCCUGGUUUGAUAC | |
| AD- | csusggccUfgGfUfUfugauacugsasa | | VPuUfcadGu(Agn)ucaaacCfaGfgccagscsu | | AGCUGGCCUGGUUUGAUACUGAC | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548541.1 | | | | | | |
| AD-1548542.1 | csusguaaauCfAfUfccuuuaggsusa | | VPudAccdTadAaggadTgAfuuuacagsgsu | | ACCUGUAAAUCAUCCUUUAGGUA | |
| AD-1548543.1 | usgsuaaaucAfUfCfcuuuaggusasa | | VPudTacdCudAaaggdAuGfauuuacasgsg | | CCUGUAAAUCAUCCUUUAGGUAA | |
| AD-1548544.1 | asasagacuuGfGfUfugguagggsusa | | VPudAccdCudAccaadCcAfagucuuuscsu | | AGAAAGACUUGGUUGGUAGGGUG | |
| AD-1548545.1 | ascsuuugAfaAfGfGfagaugucususa | | VPuAfagdAc(Agn)ucuccuUfuCfaaagusasu | | AUACUUUGAAAGGAGAUGUCUUG | |
| AD-1548546.1 | ascsuuugaaAfGfGfagaugucususa | | VPudAagdAcdAucucdCuUfucaaagusasu | | AUACUUUGAAAGGAGAUGUCUUG | |
| AD-1548547.1 | ususcucagaUfUfUfcugguugususa | | VPudAacdAadCcagadAaUfcugagaascsa | | UGUUCUCAGAUUUCUGGUUGUUA | |
| AD-1548548.1 | uscsucagAfuUfUfCfugguuguusasa | | VPuUfaadCa(Agn)ccagaaAfuCfugagasasc | | GUUCUCAGAUUUCUGGUUGUUAU | |
| AD-1548549.1 | uscsucagauUfUfUfCfugguuguusasa | | VPudTaadCadAccagdAaAfucugagasasc | | GUUCUCAGAUUUCUGGUUGUUAU | |
| AD-1548550.1 | csuscagauuUfCfUfgguuguuasusa | | VPudAuadAcdAaccadGaAfaucugagsasa | | UUCUCAGAUUUCUGGUUGUUAUG | |
| AD-1548551.1 | gsusuguuauGfUfGfaucaugugsusa | | VPudAcadCadTgaucdAcAfuaacaacscsa | | UGGUUGUUAUGUGAUCAUGUGUG | |
| AD-1548552.1 | ususguuaugUfGfAfucaugugusgsa | | VPudCacdAcdAugaudCaCfauaacaascsc | | GGUUGUUAUGUGAUCAUGUGUGG | |
| AD-1548553.1 | gsusgaucAfuGfUfGfuggaaguusasa | | VPuUfaadCu(Tgn)ccacacAfuGfaucacsasu | | AUGUGAUCAUGUGUGGAAGUUAU | |
| AD-1548554.1 | gsasucauguGfUfGfgaaguuaususa | | VPudAaudAadCuuccdAcAfcaugaucsasc | | GUGAUCAUGUGUGGAAGUUAUUA | |
| AD-1548555.1 | asuscaugugUfGfGfaaguuauusasa | | VPudTaadTadAcuucdCaCfacaugauscsa | | UGAUCAUGUGUGGAAGUUAUUAA | |
| AD-1548556.1 | usgscaacuuAfAfUfacucaaausgsa | | VPudCaudTudGaguadTuAfaguugcasasa | | UUUGCAACUUAAUACUCAAAUGA | |
| AD-1548557.1 | cscsuuucUfcUfCfUfuuauacagscsa | | VPuGfcudGu(Agn)uaaagaGfaGfaaaggscsu | | AGCCUUUCUCUCUUUAUACAGCU | |
| AD-1548558.1 | ususuauaCfaGfCfUfguauugucsusa | | VPuAfgadCa(Agn)uacagcUfgUfauaaasgsa | | UCUUUAUACAGCUGUAUUGUCUG | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548559.1 | ususuauacaGfCfUfguauugucsusa | | VPudAgadCadAuacadGcUfguauaaasgsa | | UCUUUAUACAGCUGUAUUGUCUG | |
| AD-1548560.1 | asusugucugAfAfCfuugcauugsusa | | VPudAcadAudGcaagdTuCfagacaausasc | | GUAUUGUCUGAACUUGCAUUGUG | |
| AD-1548561.1 | ususggccUfgUfAfGfaguugcugsasa | | VPuUfcadGc(Agn)acucuaCfaGfgccaasusc | | GAUUGGCCUGUAGAGUUGCUGAG | |
| AD-1548562.1 | asgsugccUfgAfCfAfcacuaaccsasa | | VPuUfggdTu(Agn)gugugugCfaGfgcacususu | | AAAGUGCCUGACACACUAACCAA | |
| AD-1548563.1 | csusgacacaCfUfAfaccaagcusgsa | | VPudCagdCudTgguudAgUfgugucagsgsc | | GCCUGACACACUAACCAAGCUGA | |
| AD-1548564.1 | usasaccaagCfUfGfaguuuccusasa | | VPudTagdGadAacucdAgCfuugguuasgsu | | ACUAACCAAGCUGAGUUUCCUAU | |
| AD-1548565.1 | asgscugaGfuUfUfCfcuaugggasasa | | VPuUfucdCc(Agn)uaggaaAfcUfcagcususg | | CAAGCUGAGUUUCCUAUGGGAAC | |
| AD-1548566.1 | ususuccuauGfGfGfaacaauugsasa | | VPudTcadAudTguucdCcAfuaggaaascsu | | AGUUUCCUAUGGGAACAAUUGAA | |
| AD-1548567.1 | gsuscgagGfaGfUfAfacaauacasasa | | VPuUfugdTa(Tgn)uguuacUfcCfucgacscsa | | UGGUCGAGGAGUAACAAUACAAA | |
| AD-1548568.1 | gsuscgaggaGfUfAfacaauacasasa | | VPudTugdTadTuguudAcUfccucgacscsa | | UGGUCGAGGAGUAACAAUACAAA | |
| AD-1548569.1 | asuscaaaCfcCfUfAfgccuugcususa | | VPuAfagdCa(Agn)ggcuagGfgUfuugausasa | | UUAUCAAACCCUAGCCUUGCUUG | |
| AD-1548570.1 | ususugcuUfgCfUfUfugaaguagscsa | | VPuGfcudAc(Tgn)ucaaagCfaAfgcaaasgsu | | ACUUUGCUUGCUUUGAAGUAGCU | |
| AD-1548571.1 | usgscuugCfuUfUfGfaaguagcuscsa | | VPuGfagdCu(Agn)cuucaaAfgCfaagcasasa | | UUUGCUUGCUUUGAAGUAGCUCU | |
| AD-1548572.1 | gscsuugcUfuUfGfAfaguagcucsusa | | VPuAfgadGc(Tgn)acuucaAfaGfcaagcsasa | | UUGCUUGCUUUGAAGUAGCUCUU | |
| AD-1548573.1 | asasgucucuCfGfUfaguguuaasgsa | | VPudCuudAadCacuadCgAfgagacuusasa | | UUAAGUCUCUCGUAGUGUUAAGU | |
| AD-1548574.1 | uscsucucGfuAfGfUfguuaaguusasa | | VPuUfaadCu(Tgn)aacacuAfcGfagagascsu | | AGUCUCUCGUAGUGUUAAGUUAU | |
| AD-1548575.1 | csuscucgUfaGfUfGfuuaaguuasusa | | VPuAfuadAc(Tgn)uaacacUfaCfgagagsasc | | GUCUCUCGUAGUGUUAAGUUAUA | |
| AD- | csuscucguaGfUfGfuuaaguuasusa | | VPudAuadAcdTuaacdAcUfacgagagsasc | | GUCUCUCGUAGUGUUAAGUUAUA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548576.1 | | | | | | |
| AD-1548577.1 | csuscguaguGfUfUfaaguuauasgsa | | VPudCuadTadAcuuadAcAfcuacgagsasg | | CUCUCGUAGUGUUAAGUUAUAGU | |
| AD-1548578.1 | csgsuaguguUfAfAfguuauagusgsa | | VPudCacdTadTaacudTaAfcacuacgsasg | | CUCGUAGUGUUAAGUUAUAGUGA | |
| AD-1548579.1 | usasguguUfaAfGfUfuauagugasasa | | VPuUfucdAc(Tgn)auaacuUfaAfcacuascsg | | CGUAGUGUUAAGUUAUAGUGAAU | |
| AD-1548580.1 | gsusgaauAfcUfGfCfuacagcaasusa | | VPuAfuudGc(Tgn)guagcaGfuAfuucacsusa | | UAGUGAAUACUGCUACAGCAAUU | |
| AD-1548581.1 | csusgcuacaGfCfAfauuucuaasusa | | VPudAuudAgdAaauudGcUfguagcagsusa | | UACUGCUACAGCAAUUUCUAAUU | |
| AD-1548582.1 | gsasauugAfgUfAfAfugguguagsasa | | VPuUfcudAc(Agn)ccauuaCfuCfaauucsusu | | AAGAAUUGAGUAAUGGUGUAGAA | |
| AD-1548583.1 | ususgaguAfaUfGfGfuguagaacsasa | | VPuUfgudTc(Tgn)acaccaUfuAfcucaasusu | | AAUUGAGUAAUGGUGUAGAACAC | |
| AD-1548584.1 | gsasguaaUfgGfUfGfuagaacacsusa | | VPuAfgudGu(Tgn)cuacacCfaUfuacucsasa | | UUGAGUAAUGGUGUAGAACACUA | |
| AD-1548585.1 | asgsuaauGfgUfGfUfagaacacusasa | | VPuUfagdTg(Tgn)ucuacaCfcAfuuacuscsa | | UGAGUAAUGGUGUAGAACACUAA | |
| AD-1548586.1 | usasauggUfgUfAfGfaacacuaasusa | | VPuAfuudAg(Tgn)guucuaCfaCfcauuascsu | | AGUAAUGGUGUAGAACACUAAUU | |
| AD-1548587.1 | asusgguguaGfAfAfcacuaauuscsa | | VPudGaadTudAgugudTcUfacaccaususa | | UAAUGGUGUAGAACACUAAUUCA | |
| AD-1548588.1 | usgsguguagAfAfCfacuaauucsasa | | VPudTgadAudTagugdTuCfuacaccasusu | | AAUGGUGUAGAACACUAAUUCAU | |
| AD-1548589.1 | gsgsuguagaAfCfAfcuaauucasusa | | VPudAugdAadTuagudGuUfcuacaccsasu | | AUGGUGUAGAACACUAAUUCAUA | |
| AD-1548590.1 | gsusguagAfaCfAfCfuaauucausasa | | VPuUfaudGa(Agn)uuagugUfuCfuacacscsa | | UGGUGUAGAACACUAAUUCAUAA | |
| AD-1548591.1 | usgsuagaAfcAfCfUfaauucauasasa | | VPuUfuadTg(Agn)auuaguGfuUfcuacascsc | | GGUGUAGAACACUAAUUCAUAAU | |
| AD-1548592.1 | gsusagaacaCfUfAfauucauaasusa | | VPudAuudAudGaauudAgUfguucuacsasc | | GUGUAGAACACUAAUUCAUAAUC | |
| AD-1548593.1 | usasgaacacUfAfAfuucauaauscsa | | VPudGaudTadTgaaudTaGfuguucuascsa | | UGUAGAACACUAAUUCAUAAUCA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548594.1 | asgsaacacuAfAfUfucauaaucsasa | | VPudTgadTudAugaadTuAfguguucusasc | | GUAGAACACUAAUUCAUAAUCAC | |
| AD-1548595.1 | asgsaacaCfuAfAfUfucauaaucsasa | | VPuUfgadTu(Agn)ugaauuAfgUfguucusasc | | GUAGAACACUAAUUCAUAAUCAC | |
| AD-1548596.1 | gsasacacuaAfUfUfcauaaucascsa | | VPudGugdAudTaugadAuUfaguguucsusa | | UAGAACACUAAUUCAUAAUCACU | |
| AD-1548597.1 | asascacuAfaUfUfCfauaaucacsusa | | VPuAfgudGa(Tgn)uaugaaUfuAfguguuscsu | | AGAACACUAAUUCAUAAUCACUC | |
| AD-1548598.1 | asusgguccaAfUfUfaguuuccususa | | VPudAagdGadAacuadAuUfggaccaususu | | AAAUGGUCCAAUUAGUUUCCUUU | |
| AD-1548599.1 | ususugggauAfUfGfuauggguasgsa | | VPudCuadCcdCauacdAuAfucccaaasusa | | UAUUUGGGAUAUGUAUGGGUAGG | |
| AD-1548600.1 | usgsuaugGfgUfAfGfgguaaaucsasa | | VPuUfgadTu(Tgn)acccuaCfcCfauacasusa | | UAUGUAUGGGUAGGGUAAAUCAG | |
| AD-1548601.1 | asusggguAfgGfGfUfaaaucagusasa | | VPuUfacdTg(Agn)uuuaccCfuAfcccausasc | | GUAUGGGUAGGGUAAAUCAGUAA | |
| AD-1548602.1 | gsusaggguaAfAfUfcaguaagasgsa | | VPudCucdTudAcugadTuUfacccuacscsc | | GGGUAGGGUAAAUCAGUAAGAGG | |
| AD-1548603.1 | asgsgguaAfaUfCfAfguaagaggsusa | | VPuAfccdTc(Tgn)uacugaUfuUfacccusasc | | GUAGGGUAAAUCAGUAAGAGGUG | |
| AD-1548604.1 | gsgsguaaauCfAfGfuaagaggusgsa | | VPudCacdCudCuuacdTgAfuuuacccsusa | | UAGGGUAAAUCAGUAAGAGGUGU | |
| AD-1548605.1 | gsusaaaucaGfUfAfagaggugususa | | VPudAacdAcdCucuudAcUfgauuuacscsc | | GGGUAAAUCAGUAAGAGGUGUUA | |
| AD-1548606.1 | asasaucaGfuAfAfGfagguguuasusa | | VPuAfuadAc(Agn)ccucuuAfcUfgauuusasc | | GUAAAUCAGUAAGAGGUGUUAUU | |
| AD-1548607.1 | asasucaguaAfGfAfgguguuaususa | | VPudAaudAadCaccudCuUfacugauususa | | UAAAUCAGUAAGAGGUGUUAUUU | |
| AD-1548608.1 | csasguaagaGfGfUfguuauuugsgsa | | VPudCcadAadTaacadCcUfcuuacugsasu | | AUCAGUAAGAGGUGUUAUUUGGA | |
| AD-1548609.1 | ususggacAfuGfGfCfcauggaacscsa | | VPuGfgudTc(C2p)auggccAfuGfuccaascsu | | AGUUGGACAUGGCCAUGGAACCA | |
| AD-1548610.1 | usgsgacuCfuGfGfAfauccauucsusa | | VPuAfgadAu(G2p)gauuccAfgAfguccasgsg | | CCUGGACUCUGGAAUCCAUUCUG | |
| AD- | csuscugaGfuGfGfUfaaaggcaasusa | | VPuAfuudGc(C2p)uuuaccAfcUfcagagsasa | | UUCUCUGAGUGGUAAAGGCAAUC | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548611.1 | | | | | | |
| AD-1548612.1 | uscsaagaAfcAfAfGfuagcugausasa | | VPuUfaudCa(G2p)cuacuuGfuUfcuugasgsu | | ACUCAAGAACAAGUAGCUGAUAU | |
| AD-1548613.1 | usasgcugAfuAfUfUfgauggacasgsa | | VPuCfugdTc(C2p)aucaauAfuCfagcuascsu | | AGUAGCUGAUAUUGAUGGACAGU | |
| AD-1548614.1 | asgscugaUfaUfUfGfauggacagsusa | | VPuAfcudGu(C2p)caucaaUfaUfcagcusasc | | GUAGCUGAUAUUGAUGGACAGUA | |
| AD-1548615.1 | csusgauaUfuGfAfUfggacaguasusa | | VPuAfuadCu(G2p)uccaucAfaUfaucagscsu | | AGCUGAUAUUGAUGGACAGUAUG | |
| AD-1548616.1 | usgsauggAfcAfGfUfaugcaaugsasa | | VPuUfcadTu(G2p)cauacuGfuCfcaucasasu | | AUUGAUGGACAGUAUGCAAUGAC | |
| AD-1548617.1 | asgsuaugCfaAfUfGfacucgagcsusa | | VPuAfgcdTc(G2p)agucauUfgCfauacusgsu | | ACAGUAUGCAAUGACUCGAGCUC | |
| AD-1548618.1 | asgscucaGfaGfGfGfuacgagcusgsa | | VPuCfagdCu(C2p)guacccUfcUfgagcuscsg | | CGAGCUCAGAGGGUACGAGCUGC | |
| AD-1548619.1 | csuscagaGfgGfUfAfcgagcugcsusa | | VPuAfgcdAg(C2p)ucguacCfcUfcugagscsu | | AGCUCAGAGGGUACGAGCUGCUA | |
| AD-1548620.1 | asgsagggUfaCfGfAfgcugcuausgsa | | VPuCfaudAg(C2p)agcucgUfaCfccucusgsa | | UCAGAGGGUACGAGCUGCUAUGU | |
| AD-1548621.1 | gscsuaugUfuCfCfCfugagacaususa | | VPuAfaudGu(C2p)ucagggAfaCfauagcsasg | | CUGCUAUGUUCCCUGAGACAUUA | |
| AD-1548622.1 | asgscguuUfgGfCfUfgaaccaucsasa | | VPuUfgadTg(G2p)uucagcCfaAfacgcusgsg | | CCAGCGUUUGGCUGAACCAUCAC | |
| AD-1548623.1 | usgscugaAfaCfAfUfgcaguugusasa | | VPuUfacdAa(C2p)ugcaugUfuUfcagcasusc | | GAUGCUGAAACAUGCAGUUGUAA | |
| AD-1548624.1 | csasugcaGfuUfGfUfaaacuugasusa | | VPuAfucdAa(G2p)uuuacaAfcUfgcaugsusu | | AACAUGCAGUUGUAAACUUGAUU | |
| AD-1548625.1 | gscsaguuGfuAfAfAfcuugauuasasa | | VPuUfuadAu(C2p)aaguuuAfcAfacugcsasu | | AUGCAGUUGUAAACUUGAUUAAC | |
| AD-1548626.1 | usgsauuaAfcUfAfUfcaagaugasusa | | VPuAfucdAu(C2p)uugauaGfuUfaaucasasg | | CUUGAUUAACUAUCAAGAUGAUG | |
| AD-1548627.1 | csasgaacUfuGfCfCfacacgugcsasa | | VPuUfgcdAc(G2p)uguggcAfaGfuucugscsa | | UGCAGAACUUGCCACACGUGCAA | |
| AD-1548628.1 | csascacgUfgCfAfAfucccugaascsa | | VPuGfuudCa(G2p)ggauugCfaCfgugugsgsc | | GCCACACGUGCAAUCCCUGAACU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548629.1 | gsusgcaaUfcCfCfCfUfgaacugacsasa | | VPuUfgudCa(G2p)uucaggGfaUfugcacsgsu | | ACGUGCAAUCCCUGAACUGACAA | |
| AD-1548630.1 | gsasggacCfaGfGfUfggugguuasasa | | VPuUfuadAc(C2p)accaccUfgGfuccucsgsu | | ACGAGGACCAGGUGGUGGUUAAU | |
| AD-1548631.1 | gsusggugGfuUfAfAfuaaggcugscsa | | VPuGfcadGc(C2p)uuauuaAfcCfaccacscsu | | AGGUGGUGGUUAAUAAGGCUGCA | |
| AD-1548632.1 | usgscagaAfuAfCfAfaaugaugusasa | | VPuUfacdAu(C2p)auuuguAfuUfcugcasusg | | CAUGCAGAAUACAAAUGAUGUAG | |
| AD-1548633.1 | asgsaauaCfaAfAfUfgauguagasasa | | VPuUfucdTa(C2p)aucauuUfgUfauucusgsc | | GCAGAAUACAAAUGAUGUAGAAA | |
| AD-1548634.1 | usgsauguAfgAfAfAfcagcucgususa | | VPuAfacdGa(G2p)cuguuuCfuAfcaucasusu | | AAUGAUGUAGAAACAGCUCGUUG | |
| AD-1548635.1 | csusugcaUfaAfCfCfuuucccauscsa | | VPuGfaudGg(G2p)aaagguUfaUfgcaagsgsu | | ACCUUGCAUAACCUUUCCCAUCA | |
| AD-1548636.1 | ususgcauAfaCfCfUfuucccaucsasa | | VPuUfgadTg(G2p)gaaaggUfuAfugcaasgsg | | CCUUGCAUAACCUUUCCCAUCAU | |
| AD-1548637.1 | cscsaucuUfuAfAfGfucuggaggscsa | | VPuGfccdTc(C2p)agacuuAfaAfgauggcsc | | GGCCAUCUUUAAGUCUGGAGGCA | |
| AD-1548638.1 | gscscauuAfcAfAfCfucuccacasasa | | VPuUfugdTg(G2p)agaguuGfuAfauggcsasu | | AUGCCAUUACAACUCUCCACAAC | |
| AD-1548639.1 | gsusugccUfuGfCfUfcaacaaaascsa | | VPuGfuudTu(G2p)uugagcAfaGfgcaacscsa | | UGGUUGCCUUGCUCAACAAAACA | |
| AD-1548640.1 | asusucuuGfgCfUfAfuuacgacasgsa | | VPuCfugdTc(G2p)uaauagCfcAfagaaususu | | AAAUUCUUGGCUAUUACGACAGA | |
| AD-1548641.1 | asasauauAfaUfGfAfggaccuausasa | | VPuUfaudAg(G2p)uccucaUfuAfuauuusasc | | GUAAAUAUAAUGAGGACCUAUAC | |
| AD-1548642.1 | asasuauaAfuGfAfGfgaccuauascsa | | VPuGfuadTa(G2p)guccucAfuUfauauususa | | UAAAUAUAAUGAGGACCUAUACU | |
| AD-1548643.1 | gscsagagUfgCfUfGfaaggugcusasa | | VPuUfagdCa(C2p)cuucagCfaCfucugcsusu | | AAGCAGAGUGCUGAAGGUGCUAU | |
| AD-1548644.1 | ususcuggGfuUfCfAfgaugauausasa | | VPuUfaudAu(C2p)aucugaAfcCfcagaasgsc | | GCUUCUGGGUUCAGAUGAUAUAA | |
| AD-1548645.1 | gsasugauAfuAfAfAfuguggucascsa | | VPuGfugdAc(C2p)acauuuAfuAfucaucsusg | | CAGAUGAUAUAAAUGUGGUCACC | |
| AD- | gsgsaauuCfuUfUfCfuaaccucascsa | | VPuGfugdAg(G2p)uuagaaAfgAfauuccsasg | | CUGGAAUUCUUUCUAACCUCACU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548646.1 | | | | | | |
| AD-1548647.1 | uscsuuucUfaAfCfCfucacuugcsasa | | VPuUfgcdAa(G2p)ugagguUfaGfaaagasasu | | AUUCUUUCUAACCUCACUUGCAA | |
| AD-1548648.1 | ususcuaaCfcUfCfAfcuugcaausasa | | VPuUfaudTg(C2p)aagugaGfgUfuagaasasg | | CUUUCUAACCUCACUUGCAAUAA | |
| AD-1548649.1 | uscsuaacCfuCfAfCfuugcaauasasa | | VPuUfuadTu(G2p)caagugAfgGfuuagasasa | | UUUCUAACCUCACUUGCAAUAAU | |
| AD-1548650.1 | asasguggGfuGfGfUfauagaggcsusa | | VPuAfgcdCu(C2p)uauaccAfcCfcacuusgsg | | CCAAGUGGGUGGUAUAGAGGCUC | |
| AD-1548651.1 | usgsggugGfuAfUfAfgaggcucususa | | VPuAfagdAg(C2p)cucuauAfcCfacccascsu | | AGUGGGUGGUAUAGAGGCUCUUG | |
| AD-1548652.1 | asgsccugCfcAfUfCfugugcucususa | | VPuAfagdAg(C2p)acagauGfgCfaggcuscsa | | UGAGCCUGCCAUCUGUGCUCUUC | |
| AD-1548653.1 | gscscugcCfaUfCfUfgugcucuuscsa | | VPuGfaadGa(G2p)cacagaUfgGfcaggcsusc | | GAGCCUGCCAUCUGUGCUCUUCG | |
| AD-1548654.1 | csasucugUfgCfUfCfuucgucauscsa | | VPuGfaudGa(C2p)gaagagCfaCfagaugsgsc | | GCCAUCUGUGCUCUUCGUCAUCU | |
| AD-1548655.1 | gscsucuuCfgUfCfAfucugaccasgsa | | VPuCfugdGu(C2p)agaugaCfgAfagagcsasc | | GUGCUCUUCGUCAUCUGACCAGC | |
| AD-1548656.1 | asusgcagUfuCfGfCfcuucacuasusa | | VPuAfuadGu(G2p)aaggcgAfaCfugcaususc | | GAAUGCAGUUCGCCUUCACUAUG | |
| AD-1548657.1 | usgsguuaAfgCfUfCfuuacacccsasa | | VPuUfggdGu(G2p)uaagagCfuUfaaccascsa | | UGUGGUUAAGCUCUUACACCCAC | |
| AD-1548658.1 | csusguugGfaUfUfGfauucgaaasusa | | VPuAfuudTc(G2p)aaucaaUfcCfaacagsusa | | UACUGUUGGAUUGAUUCGAAAUC | |
| AD-1548659.1 | usgsuuggAfuUfGfAfuucgaaauscsa | | VPuGfaudTu(C2p)gaaucaAfuCfcaacasgsu | | ACUGUUGGAUUGAUUCGAAAUCU | |
| AD-1548660.1 | csgsugagCfaGfGfGfugccauucscsa | | VPuGfgadAu(G2p)gcacccUfgCfucacgscsa | | UGCGUGAGCAGGGUGCCAUUCCA | |
| AD-1548661.1 | cscsacgaCfuAfGfUfucaguugcsusa | | VPuAfgcdAa(C2p)ugaacuAfgUfcguggsasa | | UUCCACGACUAGUUCAGUUGCUU | |
| AD-1548662.1 | ususcaguUfgCfUfUfguucgugcsasa | | VPuUfgcdAc(G2p)aacaagCfaAfcugaascsu | | AGUUCAGUUGCUUGUUCGUGCAC | |
| AD-1548663.1 | uscsaguuGfcUfUfGfuucgugcascsa | | VPuGfugdCa(C2p)gaacaaGfcAfacugasasc | | GUUCAGUUGCUUGUUCGUGCACA | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548664.1 | asgsuugcUfuGfUfUfcgugcacasusa | | VPuAfugdTg(C2p)acgaacAfaGfcaacusgsa | | UCAGUUGCUUGUUCGUGCACAUC | |
| AD-1548665.1 | csasgaggAfcUfAfAfauaccauuscsa | | VPuGfaadTg(G2p)uauuuaGfuCfcucugsasu | | AUCAGAGGACUAAAUACCAUUCC | |
| AD-1548666.1 | gsascuaaAfuAfCfCfauuccauusgsa | | VPuCfaadTg(G2p)aaugguAfuUfuagucscsu | | AGGACUAAAUACCAUUCCAUUGU | |
| AD-1548667.1 | cscsauucCfaUfUfGfuuugugcasgsa | | VPuCfugdCa(C2p)aaacaaUfgGfaauggsusa | | UACCAUUCCAUUGUUUGUGCAGC | |
| AD-1548668.1 | asusuccaUfuGfUfUfugugcagcsusa | | VPuAfgcdTg(C2p)acaaacAfaUfggaausgsg | | CCAUUCCAUUGUUUGUGCAGCUG | |
| AD-1548669.1 | ususccauUfgUfUfUfgugcagcusgsa | | VPuCfagdCu(G2p)cacaaaCfaAfuggaasusg | | CAUUCCAUUGUUUGUGCAGCUGC | |
| AD-1548670.1 | cscsauugUfuUfGfUfgcagcugcsusa | | VPuAfgcdAg(C2p)ugcacaAfaCfaauggsasa | | UUCCAUUGUUUGUGCAGCUGCUU | |
| AD-1548671.1 | gscscacaGfcUfCfCfucugacagsasa | | VPuUfcudGu(C2p)agaggaGfcUfguggcsusc | | GAGCCACAGCUCCUCUGACAGAG | |
| AD-1548672.1 | asasgccaCfaAfGfAfuuacaagasasa | | VPuUfucdTu(G2p)uaaucuUfgUfggcuusgsu | | ACAAGCCACAAGAUUACAAGAAA | |
| AD-1548673.1 | cscsacaaGfaUfUfAfcaagaaacsgsa | | VPuCfgudTu(C2p)uuguaaUfcUfuguggscsu | | AGCCACAAGAUUACAAGAAACGG | |
| AD-1548674.1 | asasgauuAfcAfAfGfaaacggcususa | | VPuAfagdCc(G2p)uuucuuGfuAfaucuusgsu | | ACAAGAUUACAAGAAACGGCUUU | |
| AD-1548675.1 | asusuacaAfgAfAfAfcggcuuucsasa | | VPuUfgadAa(G2p)ccguuuCfuUfguaauscsu | | AGAUUACAAGAAACGGCUUUCAG | |
| AD-1548676.1 | gsgscuuuCfaGfUfUfgagcugacscsa | | VPuGfgudCa(G2p)cucaacUfgAfaagccsgsu | | ACGGCUUUCAGUUGAGCUGACCA | |
| AD-1548677.1 | usgsagcuGfaCfCfAfgcucucucsusa | | VPuAfgadGa(G2p)agcuggUfcAfgcucasasc | | GUUGAGCUGACCAGCUCUCUCUU | |
| AD-1548678.1 | gsasgccaAfuGfGfCfuuggaaugsasa | | VPuUfcadTu(C2p)caagccAfuUfggcucsusg | | CAGAGCCAAUGGCUUGGAAUGAG | |
| AD-1548679.1 | csusgaucUfuGfGfAfcuugauaususa | | VPuAfaudAu(C2p)aaguccAfaGfaucagscsa | | UGCUGAUCUUGGACUUGAUAUUG | |
| AD-1548680.1 | usgsguuuGfaUfAfCfugaccugusasa | | VPuUfacdAg(G2p)ucaguaUfcAfaaccasgsg | | CCUGGUUUGAUACUGACCUGUAA | |
| AD- | ususugauAfcUfGfAfccuguaaasusa | | VPuAfuudTa(C2p)aggucaGfuAfucaaascsc | | GGUUUGAUACUGACCUGUAAAUC | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1548681.1 | | | | | | |
| AD-1548682.1 | gsgscuauUfuGfUfAfaaucugccsasa | | VPuUfggdCa(G2p)auuuacAfaAfuagccsusa | | UAGGCUAUUUGUAAAUCUGCCAC | |
| AD-1548683.1 | ususucugGfuUfGfUfuaugugauscsa | | VPuGfaudCa(C2p)auaacaAfcCfagaaasusc | | GAUUUCUGGUUGUUAUGUGAUCA | |
| AD-1548684.1 | gsasucauGfuGfUfGfgaaguuaususa | | VPuAfaudAa(C2p)uuccacAfcAfugaucsasc | | GUGAUCAUGUGUGGAAGUUAUUA | |
| AD-1548685.1 | usasauacUfcAfAfAfugaguaacsasa | | VPuUfgudTa(C2p)ucauuuGfaGfuauuasasg | | CUUAAUACUCAAAUGAGUAACAU | |
| AD-1548686.1 | asusugucUfgAfAfCfuugcauugsusa | | VPuAfcadAu(G2p)caaguuCfaGfacaausasc | | GUAUUGUCUGAACUUGCAUUGUG | |
| AD-1548687.1 | csasgaaaGfuGfCfCfugacacacsusa | | VPuAfgudGu(G2p)ucaggcAfcUfuucugsasg | | CUCAGAAAGUGCCUGACACACUA | |
| AD-1548688.1 | gsasaaguGfcCfUfGfacacacuasasa | | VPuUfuadGu(G2p)ugucagGfcAfcuuucsusg | | CAGAAAGUGCCUGACACACUAAC | |
| AD-1548689.1 | gscscugaCfaCfAfCfuaaccaagscsa | | VPuGfcudTg(G2p)uuagugUfgUfcaggcsasc | | GUGCCUGACACACUAACCAAGCU | |
| AD-1548690.1 | usgsggaaCfaAfUfUfgaaguaaascsa | | VPuGfuudTa(C2p)uucaauUfgUfucccasusa | | UAUGGGAACAAUUGAAGUAAACU | |
| AD-1548691.1 | asusggauCfaCfAfAfgauggaaususa | | VPuAfaudTc(C2p)aucuugUfgAfuccaususc | | GAAUGGAUCACAAGAUGGAAUUU | |
| AD-1548692.1 | ususuaucAfaAfCfCfcuagccuusgsa | | VPuCfaadGg(C2p)uaggguUfuGfauaaasusu | | AAUUUAUCAAACCCUAGCCUUGC | |
| AD-1548693.1 | usasucugUfaAfUfGfguacugacsusa | | VPuAfgudCa(G2p)uaccauUfaCfagauasusu | | AAUAUCUGUAAUGGUACUGACUU | |
| AD-1548694.1 | ususgcuuGfcUfUfUfgaaguagcsusa | | VPuAfgcdTa(C2p)uucaaaGfcAfagcaasasg | | CUUUGCUUGCUUUGAAGUAGCUC | |
| AD-1548695.1 | asgsuguuAfaGfUfUfauagugaasusa | | VPuAfuudCa(C2p)uauaacUfuAfacacusasc | | GUAGUGUUAAGUUAUAGUGAAUA | |
| AD-1548696.1 | usgsuuaaGfuUfAfUfagugaauascsa | | VPuGfuadTu(C2p)acuauaAfcUfuaacascsu | | AGUGUUAAGUUAUAGUGAAUACU | |
| AD-1548697.1 | gsusaaugGfuGfUfAfgaacacuasasa | | VPuUfuadGu(G2p)uucuacAfcCfauuacsusc | | GAGUAAUGGUGUAGAACACUAAU | |
| AD-1548698.1 | asasugguGfuAfGfAfacacuaaususa | | VPuAfaudTa(G2p)uguucuAfcAfccauusasc | | GUAAUGGUGUAGAACACUAAUUC | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence 5' to 3' | SEQ ID NO: |
|---|---|---|---|---|---|---|
| AD-1548699.1 | csusaauuCfaUfAfAfucacucuasasa | | VPuUfuadGa(G2p)ugauuaUfgAfauuagsusg | | CACUAAUUCAUAAUCACUCUAAU | |
| AD-1548700.1 | ususugggAfuAfUfGfuauggguasgsa | | VPuCfuadCc(C2p)auacauAfuCfccaaasusa | | UAUUUGGGAUAUGUAUGGGUAGG | |
| AD-1548701.1 | gsgsguaaAfuCfAfGfuaagaggusgsa | | VPuCfacdCu(C2p)uuacugAfuUfuaccccsusa | | UAGGGUAAAUCAGUAAGAGGUGU | |
| AD-1548702.1 | gsusaaauCfaGfUfAfagaggugususa | | VPuAfacdAc(C2p)ucuuacUfgAfuuuacscsc | | GGGUAAAUCAGUAAGAGGUGUUA | |
| AD-1548703.1 | usasaaucAfgUfAfAfgagguguusasa | | VPuUfaadCa(C2p)cucuuaCfuGfauuuascsc | | GGUAAAUCAGUAAGAGGUGUUAU | |
| AD-1548704.1 | asasucagUfaAfGfAfgguguaususa | | VPuAfaudAa(C2p)accucuUfaCfugauususa | | UAAAUCAGUAAGAGGUGUUAUUU | |

**Table 4. Single Dose Screens in Hep3b Cells**

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| **Duplex** | **% of Message Remaining** | **ST DEV** | **% of Message Remaining** | **ST DEV** | **% of Message Remaining** | **ST DEV** |
| AD-1548396.1 | 6.38 | 1.03 | 8.14 | 0.85 | 7.67 | 0.28 |
| AD-1548453.1 | 7.45 | 0.60 | 8.67 | 0.85 | 8.67 | 1.01 |
| AD-1548515.1 | 6.52 | 0.20 | 8.04 | 1.05 | 13.41 | 1.52 |
| AD-1548392.1 | 7.49 | 0.99 | 8.81 | 0.97 | 8.79 | 1.23 |
| AD-1548503.1 | 5.63 | 0.33 | 6.74 | 0.42 | 9.09 | 1.34 |
| AD-1548426.1 | 6.57 | 0.29 | 9.69 | 1.48 | 9.30 | 0.62 |
| AD-1548440.1 | 9.93 | 1.75 | 10.55 | 0.90 | 9.50 | 0.33 |
| AD-1548487.1 | 3.41 | 0.36 | 5.58 | 0.39 | 9.73 | 0.65 |
| AD-1548513.1 | 6.68 | 0.55 | 10.00 | 1.36 | 13.85 | 1.46 |
| AD-1548516.1 | 7.28 | 0.80 | 10.11 | 0.56 | 19.31 | 1.98 |
| AD-1548404.1 | 10.39 | 0.55 | 11.08 | 1.12 | 10.02 | 0.97 |
| AD-1548471.1 | 6.96 | 0.44 | 11.22 | 1.12 | 14.67 | 1.32 |
| AD-1548512.1 | 8.02 | 0.18 | 10.54 | 1.63 | 16.98 | 1.85 |
| AD-1548511.1 | 6.66 | 0.68 | 8.59 | 0.09 | 15.71 | 2.44 |
| AD-1548414.1 | 14.20 | 1.19 | 14.68 | 1.56 | 13.32 | 1.68 |
| AD-1548393.1 | 7.67 | 0.71 | 9.02 | 0.41 | 10.19 | 0.68 |
| AD-1548672.1 | 6.39 | 0.31 | 7.62 | 0.80 | 10.46 | 1.71 |
| AD-1548408.1 | 9.44 | 1.32 | 11.92 | 0.84 | 10.66 | 0.65 |
| AD-1548372.1 | 9.55 | 1.53 | 12.01 | 0.64 | 10.04 | 1.96 |
| AD-1548459.1 | 5.18 | 0.60 | 9.67 | 1.51 | 12.19 | 0.56 |
| AD-1548488.1 | 3.62 | 0.46 | 8.29 | 1.49 | 10.67 | 1.06 |
| AD-1548450.1 | 7.29 | 1.92 | 9.29 | 1.12 | 10.88 | 1.02 |
| AD-1548419.1 | 9.67 | 1.28 | 10.25 | 1.16 | 10.88 | 1.07 |
| AD-1548442.1 | 8.85 | 1.52 | 12.38 | 1.81 | 10.94 | 2.21 |
| AD-1548397.1 | 7.99 | 0.70 | 11.90 | 1.06 | 10.98 | 0.95 |
| AD-1548371.1 | 13.85 | 1.90 | 12.69 | 0.88 | 11.73 | 2.51 |
| AD-1548416.1 | 10.39 | 0.46 | 10.76 | 0.71 | 13.19 | 1.85 |
| AD-1548413.1 | 10.47 | 0.91 | 12.09 | 0.88 | 10.85 | 0.79 |
| AD-1548458.1 | 6.58 | 0.54 | 9.68 | 0.55 | 12.90 | 2.99 |
| AD-1548375.1 | 7.69 | 0.87 | 11.20 | 1.02 | 11.89 | 1.60 |
| AD-1548612.1 | 11.49 | 1.89 | 15.86 | 0.60 | 11.90 | 4.52 |
| AD-1548378.1 | 9.35 | 1.53 | 9.44 | 0.76 | 11.98 | 1.88 |
| AD-1548438.1 | 11.23 | 0.85 | 12.12 | 1.18 | 12.01 | 1.26 |
| AD-1548407.1 | 9.40 | 1.08 | 11.16 | 0.68 | 12.06 | 0.22 |
| AD-1548627.1 | 9.40 | 1.33 | 12.63 | 2.23 | 12.18 | 4.01 |
| AD-1548374.1 | 11.24 | 1.10 | 12.55 | 0.65 | 12.19 | 1.35 |
| AD-1548384.1 | 12.70 | 1.74 | 13.02 | 0.75 | 12.21 | 1.36 |
| AD-1548452.1 | 12.80 | 1.34 | 11.34 | 0.47 | 12.28 | 0.89 |
| AD-1548456.1 | 5.17 | 0.70 | 7.42 | 2.27 | 11.28 | 2.45 |
| AD-1548441.1 | 8.35 | 1.39 | 11.01 | 1.49 | 12.35 | 1.24 |
| AD-1548457.1 | 5.68 | 0.27 | 6.23 | 1.71 | 11.24 | 3.32 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548510.1 | 6.38 | 0.21 | 7.57 | 0.58 | 11.50 | 0.23 |
| AD-1548486.1 | 5.65 | 0.50 | 9.61 | 2.34 | 20.32 | 0.74 |
| AD-1548379.1 | 15.88 | 2.14 | 14.66 | 1.39 | 12.56 | 1.61 |
| AD-1548460.1 | 4.93 | 0.41 | 7.78 | 0.95 | 12.74 | 1.35 |
| AD-1548420.1 | 10.96 | 1.03 | 12.19 | 0.77 | 12.82 | 0.92 |
| AD-1548367.1 | 9.70 | 1.48 | 9.48 | 1.53 | 12.87 | 2.76 |
| AD-1548385.1 | 13.75 | 0.39 | 12.75 | 1.83 | 12.88 | 2.32 |
| AD-1548444.1 | 9.02 | 2.60 | 11.81 | 0.97 | 12.99 | 2.01 |
| AD-1548649.1 | 4.50 | 0.55 | 8.05 | 1.03 | 13.24 | 1.43 |
| AD-1548530.1 | 4.98 | 0.39 | 6.79 | 0.69 | 13.34 | 1.48 |
| AD-1548412.1 | 11.99 | 2.06 | 12.72 | 0.64 | 13.36 | 0.53 |
| AD-1548634.1 | 7.14 | 1.01 | 8.32 | 1.00 | 13.42 | 2.35 |
| AD-1548489.1 | 4.83 | 0.68 | 8.46 | 1.23 | 13.56 | 2.12 |
| AD-1548515.2 | 6.37 | 0.93 | 6.60 | 0.40 | 13.65 | 1.03 |
| AD-1548647.1 | 4.83 | 0.46 | 5.58 | 0.60 | 13.72 | 1.92 |
| AD-1548434.1 | 8.56 | 1.46 | 10.73 | 0.82 | 13.80 | 1.17 |
| AD-1548636.1 | 8.56 | 0.90 | 7.90 | 0.63 | 14.00 | 2.14 |
| AD-1548373.1 | 9.78 | 1.54 | 12.36 | 1.29 | 14.09 | 2.93 |
| AD-1548462.1 | 6.93 | 0.64 | 7.82 | 0.54 | 14.19 | 1.42 |
| AD-1548493.1 | 6.44 | 0.54 | 7.75 | 1.04 | 14.36 | 2.94 |
| AD-1548380.1 | 12.76 | 2.64 | 12.82 | 3.47 | 14.70 | 1.39 |
| AD-1548661.1 | 5.76 | 0.49 | 8.06 | 1.18 | 14.74 | 5.30 |
| AD-1548646.1 | 6.74 | 0.66 | 8.04 | 0.56 | 15.03 | 2.10 |
| AD-1548628.1 | 11.04 | 0.92 | 13.58 | 1.76 | 15.18 | 0.54 |
| AD-1548568.1 | 9.90 | 0.86 | 13.13 | 0.54 | 15.29 | 1.19 |
| AD-1548656.1 | 7.95 | 0.91 | 11.71 | 2.24 | 15.67 | 2.39 |
| AD-1548389.1 | 11.48 | 0.95 | 13.50 | 2.69 | 15.77 | 1.34 |
| AD-1548498.1 | 4.87 | 0.76 | 10.24 | 0.99 | 15.86 | 2.71 |
| AD-1548654.1 | 14.94 | 0.85 | 14.24 | 1.57 | 16.04 | 3.97 |
| AD-1548508.1 | 7.63 | 0.60 | 9.04 | 1.56 | 16.33 | 3.64 |
| AD-1548479.1 | 5.68 | 0.36 | 13.48 | 1.31 | 16.35 | 0.88 |
| AD-1548406.1 | 12.96 | 1.18 | 14.48 | 2.52 | 16.56 | 2.24 |
| AD-1548623.1 | 9.27 | 0.86 | 15.72 | 2.21 | 16.81 | 2.08 |
| AD-1548524.1 | 7.63 | 0.39 | 9.67 | 0.70 | 17.00 | 1.22 |
| AD-1548667.1 | 5.29 | 0.39 | 6.76 | 1.52 | 17.09 | 1.86 |
| AD-1548417.1 | 13.99 | 0.91 | 15.81 | 1.89 | 17.18 | 1.89 |
| AD-1548463.1 | 6.24 | 0.58 | 10.25 | 1.35 | 17.21 | 0.78 |
| AD-1548395.1 | 13.88 | 2.73 | 18.28 | 2.39 | 17.40 | 0.89 |
| AD-1548531.1 | 6.98 | 0.83 | 9.32 | 0.72 | 17.42 | 2.09 |
| AD-1548451.1 | 15.00 | 1.09 | 16.43 | 1.70 | 17.68 | 1.08 |
| AD-1548472.1 | 10.59 | 1.11 | 11.66 | 0.63 | 17.75 | 1.96 |
| AD-1548509.1 | 6.57 | 0.52 | 10.31 | 1.27 | 17.82 | 2.07 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548418.1 | 21.86 | 2.87 | 20.91 | 2.73 | 17.93 | 1.86 |
| AD-1548673.1 | 6.19 | 1.10 | 9.52 | 1.04 | 17.97 | 4.26 |
| AD-1548529.1 | 5.73 | 0.22 | 8.13 | 1.82 | 17.99 | 2.17 |
| AD-1548491.1 | 7.40 | 0.70 | 10.89 | 0.54 | 18.26 | 1.46 |
| AD-1548477.1 | 8.26 | 1.06 | 12.06 | 0.51 | 18.43 | 1.18 |
| AD-1548522.1 | 6.47 | 0.54 | 9.15 | 0.28 | 18.48 | 1.71 |
| AD-1548484.1 | 9.90 | 0.92 | 12.49 | 1.00 | 18.56 | 0.30 |
| AD-1548644.1 | 6.13 | 1.28 | 8.70 | 0.18 | 19.07 | 3.28 |
| AD-1548523.1 | 7.43 | 0.63 | 10.91 | 0.55 | 19.17 | 1.07 |
| AD-1548492.1 | 5.55 | 0.40 | 10.03 | 1.65 | 19.35 | 1.98 |
| AD-1548633.1 | 9.62 | 1.25 | 16.60 | 1.14 | 19.74 | 2.02 |
| AD-1548635.1 | 9.48 | 1.36 | 11.04 | 1.83 | 19.75 | 4.14 |
| AD-1548481.1 | 7.80 | 1.00 | 13.18 | 1.50 | 20.01 | 1.84 |
| AD-1548443.1 | 19.56 | 1.55 | 21.60 | 0.56 | 20.22 | 2.30 |
| AD-1548631.1 | 12.52 | 1.44 | 17.76 | 2.09 | 20.26 | 0.37 |
| AD-1548526.1 | 7.39 | 0.42 | 10.58 | 0.86 | 20.33 | 3.06 |
| AD-1548657.1 | 12.13 | 0.57 | 18.77 | 2.33 | 20.56 | 2.80 |
| AD-1548587.1 | 9.33 | 1.31 | 17.28 | 0.88 | 20.68 | 1.12 |
| AD-1548467.1 | 8.27 | 0.74 | 10.99 | 1.09 | 20.77 | 1.28 |
| AD-1548629.1 | 15.55 | 0.86 | 16.64 | 2.21 | 20.78 | 1.95 |
| AD-1548525.1 | 6.95 | 0.52 | 12.95 | 1.92 | 20.96 | 1.38 |
| AD-1548376.1 | 11.36 | 2.27 | 14.99 | 2.74 | 21.14 | 2.87 |
| AD-1548577.1 | 10.56 | 1.16 | 17.41 | 2.87 | 21.30 | 1.21 |
| AD-1548527.1 | 6.72 | 0.85 | 11.13 | 1.04 | 21.31 | 2.80 |
| AD-1548391.1 | 13.61 | 1.66 | 13.79 | 1.99 | 21.35 | 3.20 |
| AD-1548598.1 | 15.53 | 1.28 | 18.39 | 1.40 | 21.47 | 2.29 |
| AD-1548464.1 | 7.48 | 0.76 | 13.54 | 1.25 | 21.74 | 3.29 |
| AD-1548495.1 | 5.64 | 0.78 | 12.72 | 1.19 | 21.85 | 0.68 |
| AD-1548519.1 | 6.82 | 0.99 | 11.39 | 1.46 | 21.94 | 1.05 |
| AD-1548674.1 | 10.13 | 0.44 | 11.33 | 0.76 | 22.04 | 2.25 |
| AD-1548645.1 | 6.81 | 0.91 | 8.44 | 0.75 | 22.08 | 3.60 |
| AD-1548368.1 | 19.56 | 1.04 | 14.07 | 6.45 | 22.43 | 4.40 |
| AD-1548639.1 | 9.21 | 0.99 | 8.58 | 1.79 | 22.58 | 2.32 |
| AD-1548500.1 | 9.39 | 1.45 | 13.35 | 1.85 | 22.70 | 4.33 |
| AD-1548592.1 | 9.53 | 0.72 | 16.19 | 1.92 | 22.86 | 2.98 |
| AD-1548663.1 | 11.04 | 1.16 | 15.07 | 2.45 | 23.09 | 7.78 |
| AD-1548664.1 | 9.26 | 0.56 | 10.71 | 1.03 | 23.13 | 5.77 |
| AD-1548437.1 | 10.58 | 0.73 | 15.25 | 2.29 | 23.28 | 4.19 |
| AD-1548650.1 | 16.55 | 1.64 | 19.93 | 0.53 | 23.31 | 2.49 |
| AD-1548470.1 | 8.86 | 0.30 | 11.95 | 1.73 | 23.40 | 1.85 |
| AD-1548400.1 | 24.27 | 3.49 | 24.69 | 1.81 | 23.50 | 0.87 |
| AD-1548447.1 | 15.34 | 1.63 | 17.24 | 2.43 | 23.54 | 1.51 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548490.1 | 6.08 | 0.39 | 10.68 | 1.64 | 23.58 | 3.59 |
| AD-1548411.1 | 16.12 | 0.98 | 17.98 | 1.22 | 23.62 | 2.94 |
| AD-1548478.1 | 10.55 | 0.36 | 16.18 | 1.05 | 23.64 | 1.08 |
| AD-1548439.1 | 20.22 | 1.13 | 17.45 | 2.57 | 23.82 | 4.15 |
| AD-1548528.1 | 8.01 | 0.84 | 12.14 | 1.29 | 24.05 | 1.11 |
| AD-1548625.1 | 10.53 | 1.11 | 16.61 | 2.55 | 24.30 | 4.54 |
| AD-1548651.1 | 10.00 | 1.04 | 14.27 | 0.50 | 24.40 | 2.83 |
| AD-1548666.1 | 8.37 | 0.75 | 11.28 | 1.30 | 24.54 | 3.77 |
| AD-1548473.1 | 8.07 | 1.74 | 16.17 | 1.87 | 24.56 | 1.26 |
| AD-1548475.1 | 12.55 | 1.55 | 18.97 | 3.74 | 24.83 | 4.38 |
| AD-1548507.1 | 9.05 | 0.82 | 11.94 | 0.61 | 24.86 | 1.23 |
| AD-1548415.1 | 18.37 | 1.98 | 23.35 | 2.11 | 24.93 | 0.83 |
| AD-1548432.1 | 15.29 | 1.47 | 17.18 | 3.00 | 24.98 | 2.37 |
| AD-1548621.1 | 11.78 | 1.95 | 16.47 | 1.38 | 25.01 | 2.42 |
| AD-1548421.1 | 14.29 | 1.36 | 20.69 | 1.98 | 25.33 | 2.94 |
| AD-1548589.1 | 12.69 | 0.62 | 20.37 | 1.13 | 25.39 | 1.96 |
| AD-1548542.1 | 8.92 | 0.54 | 14.23 | 1.21 | 25.50 | 1.82 |
| AD-1548584.1 | 17.11 | 1.13 | 21.38 | 3.49 | 25.53 | 3.97 |
| AD-1548662.1 | 14.97 | 2.02 | 18.28 | 1.63 | 25.80 | 6.01 |
| AD-1548581.1 | 12.26 | 0.55 | 21.88 | 2.80 | 25.84 | 3.91 |
| AD-1548640.1 | 8.81 | 1.67 | 8.58 | 2.43 | 25.87 | 5.07 |
| AD-1548402.1 | 14.54 | 0.69 | 17.32 | 2.59 | 26.09 | 2.01 |
| AD-1548632.1 | 13.51 | 1.05 | 21.62 | 1.40 | 26.72 | 6.06 |
| AD-1548578.1 | 10.78 | 1.04 | 20.42 | 1.28 | 26.73 | 3.01 |
| AD-1548593.1 | 11.74 | 1.09 | 19.67 | 1.47 | 26.78 | 3.58 |
| AD-1548377.1 | 12.46 | 2.91 | 19.46 | 2.23 | 26.81 | 2.92 |
| AD-1548496.1 | 11.31 | 1.64 | 14.83 | 1.74 | 26.95 | 3.35 |
| AD-1548388.1 | 21.97 | 1.07 | 26.08 | 2.29 | 27.05 | 2.96 |
| AD-1548590.1 | 12.25 | 1.59 | 21.74 | 1.53 | 27.21 | 2.34 |
| AD-1548658.1 | 5.22 | 0.39 | 10.51 | 0.81 | 27.25 | 6.98 |
| AD-1548605.1 | 20.18 | 0.67 | 29.82 | 1.60 | 27.41 | 3.01 |
| AD-1548622.1 | 16.42 | 2.02 | 23.12 | 1.89 | 27.48 | 2.31 |
| AD-1548538.1 | 8.58 | 1.48 | 12.92 | 1.65 | 27.62 | 3.04 |
| AD-1548638.1 | 7.55 | 1.28 | 8.47 | 1.01 | 27.71 | 7.94 |
| AD-1548585.1 | 15.28 | 1.54 | 21.00 | 3.11 | 27.97 | 3.47 |
| AD-1548648.1 | 8.61 | 0.86 | 12.71 | 2.60 | 28.14 | 5.02 |
| AD-1548571.1 | 17.57 | 2.16 | 23.78 | 3.76 | 28.29 | 3.82 |
| AD-1548543.1 | 11.67 | 1.08 | 18.29 | 2.71 | 28.79 | 2.01 |
| AD-1548680.1 | 7.08 | 0.83 | 7.68 | 0.62 | 28.83 | 3.59 |
| AD-1548449.1 | 14.11 | 0.95 | 20.05 | 2.50 | 28.85 | 4.29 |
| AD-1548660.1 | 13.84 | 1.14 | 20.22 | 0.80 | 28.94 | 7.75 |
| AD-1548390.1 | 19.25 | 2.84 | 24.73 | 1.74 | 28.95 | 1.41 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548570.1 | 17.42 | 2.96 | 24.87 | 1.44 | 28.96 | 3.94 |
| AD-1548588.1 | 14.55 | 1.40 | 26.35 | 2.36 | 28.97 | 3.87 |
| AD-1548573.1 | 21.45 | 0.94 | 24.36 | 3.10 | 29.06 | 3.55 |
| AD-1548483.1 | 9.85 | 0.27 | 16.80 | 1.43 | 29.46 | 3.36 |
| AD-1548596.1 | 11.62 | 0.67 | 22.62 | 3.55 | 30.17 | 4.83 |
| AD-1548607.1 | 17.82 | 0.85 | 26.01 | 3.58 | 30.36 | 5.10 |
| AD-1548659.1 | 11.61 | 1.07 | 19.23 | 0.82 | 30.44 | 2.75 |
| AD-1548668.1 | 9.82 | 1.11 | 13.03 | 1.92 | 30.49 | 4.08 |
| AD-1548608.1 | 14.54 | 1.63 | 24.25 | 0.79 | 30.72 | 3.79 |
| AD-1548675.1 | 7.84 | 1.33 | 10.33 | 0.68 | 30.92 | 12.04 |
| AD-1548610.1 | 14.80 | 1.79 | 23.80 | 3.02 | 31.45 | 4.54 |
| AD-1548366.1 | 26.87 | 5.12 | 21.19 | 5.66 | 31.64 | 4.05 |
| AD-1548381.1 | 21.26 | 3.33 | 25.24 | 2.13 | 31.66 | 3.31 |
| AD-1548572.1 | 13.12 | 1.54 | 22.46 | 0.38 | 31.93 | 5.76 |
| AD-1548583.1 | 10.91 | 1.01 | 21.08 | 1.62 | 31.99 | 6.79 |
| AD-1548536.1 | 12.87 | 0.64 | 15.24 | 2.42 | 32.44 | 2.87 |
| AD-1548466.1 | 13.51 | 1.91 | 22.00 | 4.72 | 32.45 | 6.26 |
| AD-1548403.1 | 16.94 | 2.15 | 22.97 | 1.86 | 32.70 | 2.72 |
| AD-1548430.1 | 15.62 | 0.93 | 26.92 | 2.40 | 32.91 | 3.08 |
| AD-1548665.1 | 11.39 | 1.00 | 16.60 | 0.86 | 32.98 | 3.84 |
| AD-1548617.1 | 14.63 | 0.73 | 23.69 | 1.99 | 33.31 | 4.86 |
| AD-1548695.1 | 10.55 | 1.10 | 15.38 | 1.12 | 33.33 | 3.42 |
| AD-1548435.1 | 18.39 | 2.95 | 24.84 | 2.05 | 33.51 | 5.08 |
| AD-1548678.1 | 11.29 | 1.58 | 20.13 | 1.17 | 33.88 | 12.74 |
| AD-1548576.1 | 16.46 | 0.92 | 21.96 | 2.80 | 34.05 | 2.61 |
| AD-1548405.1 | 43.97 | 2.86 | 30.82 | 1.28 | 34.28 | 5.50 |
| AD-1548567.1 | 19.94 | 0.70 | 33.92 | 6.25 | 34.39 | 6.91 |
| AD-1548398.1 | 40.94 | 2.30 | 34.52 | 3.56 | 34.48 | 4.35 |
| AD-1548676.1 | 11.28 | 1.95 | 18.41 | 3.54 | 34.77 | 7.34 |
| AD-1548703.1 | 13.68 | 1.10 | 16.41 | 1.30 | 35.02 | 3.94 |
| AD-1548679.1 | 7.46 | 2.31 | 9.27 | 1.49 | 35.07 | 3.99 |
| AD-1548653.1 | 11.97 | 1.36 | 19.94 | 1.39 | 35.27 | 11.85 |
| AD-1548468.1 | 14.09 | 2.45 | 20.35 | 2.02 | 35.30 | 4.07 |
| AD-1548424.1 | 21.68 | 0.33 | 25.41 | 1.54 | 35.35 | 1.98 |
| AD-1548469.1 | 26.40 | 1.17 | 32.38 | 4.08 | 35.43 | 1.11 |
| AD-1548559.1 | 27.67 | 2.64 | 34.28 | 4.55 | 35.58 | 4.63 |
| AD-1548423.1 | 16.10 | 2.29 | 24.32 | 4.06 | 35.94 | 3.82 |
| AD-1548582.1 | 17.06 | 1.23 | 28.34 | 3.52 | 36.25 | 7.23 |
| AD-1548382.1 | 45.21 | 8.23 | 42.04 | 6.06 | 36.54 | 3.61 |
| AD-1548580.1 | 14.06 | 0.70 | 26.71 | 0.83 | 36.58 | 3.69 |
| AD-1548641.1 | 9.23 | 0.46 | 10.30 | 2.23 | 36.88 | 1.98 |
| AD-1548575.1 | 18.56 | 1.19 | 26.60 | 2.59 | 37.22 | 5.34 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548604.1 | 19.01 | 1.57 | 27.01 | 1.26 | 37.24 | 1.15 |
| AD-1548602.1 | 21.31 | 1.77 | 29.32 | 1.72 | 37.49 | 3.21 |
| AD-1548698.1 | 14.96 | 0.60 | 17.71 | 1.02 | 37.57 | 7.68 |
| AD-1548537.1 | 14.60 | 2.53 | 18.74 | 1.58 | 37.59 | 7.88 |
| AD-1548532.1 | 9.71 | 0.88 | 18.89 | 1.45 | 37.64 | 0.60 |
| AD-1548701.1 | 17.62 | 1.10 | 24.05 | 1.50 | 37.68 | 5.03 |
| AD-1548560.1 | 27.12 | 2.64 | 30.64 | 3.75 | 37.86 | 2.07 |
| AD-1548521.1 | 12.87 | 1.02 | 18.94 | 2.25 | 37.97 | 5.59 |
| AD-1548586.1 | 28.93 | 3.53 | 38.43 | 4.62 | 37.99 | 3.03 |
| AD-1548614.1 | 16.21 | 1.85 | 28.99 | 1.01 | 38.16 | 5.60 |
| AD-1548579.1 | 20.14 | 3.26 | 30.17 | 5.02 | 39.14 | 4.64 |
| AD-1548574.1 | 23.15 | 1.12 | 35.42 | 7.88 | 40.18 | 4.60 |
| AD-1548594.1 | 12.07 | 1.69 | 26.96 | 1.21 | 40.43 | 2.06 |
| AD-1548566.1 | 35.69 | 2.42 | 40.44 | 1.83 | 40.48 | 3.69 |
| AD-1548669.1 | 12.29 | 0.81 | 23.07 | 2.86 | 41.45 | 4.39 |
| AD-1548599.1 | 19.00 | 0.96 | 28.68 | 4.15 | 41.61 | 2.77 |
| AD-1548702.1 | 13.33 | 3.44 | 19.36 | 1.43 | 41.68 | 7.97 |
| AD-1548670.1 | 13.24 | 2.23 | 22.00 | 3.12 | 41.75 | 4.24 |
| AD-1548603.1 | 21.91 | 1.14 | 31.21 | 0.39 | 42.02 | 4.96 |
| AD-1548618.1 | 17.70 | 1.78 | 32.19 | 4.96 | 42.04 | 5.88 |
| AD-1548454.1 | 48.70 | 7.62 | 35.11 | 8.17 | 42.08 | 3.08 |
| AD-1548476.1 | 22.22 | 1.36 | 25.88 | 4.20 | 42.27 | 2.65 |
| AD-1548642.1 | 13.28 | 2.72 | 20.28 | 0.72 | 42.79 | 3.65 |
| AD-1548428.1 | 19.26 | 1.52 | 25.84 | 2.04 | 42.99 | 3.37 |
| AD-1548591.1 | 28.95 | 1.33 | 41.38 | 6.83 | 43.15 | 2.82 |
| AD-1548535.1 | 10.42 | 1.59 | 15.98 | 1.43 | 43.15 | 5.71 |
| AD-1548497.1 | 21.94 | 1.09 | 31.64 | 6.08 | 43.54 | 3.70 |
| AD-1548499.1 | 12.19 | 1.55 | 23.86 | 3.65 | 43.57 | 6.17 |
| AD-1548485.1 | 20.98 | 1.67 | 25.49 | 1.00 | 43.78 | 7.35 |
| AD-1548696.1 | 13.02 | 0.52 | 17.61 | 2.48 | 43.84 | 9.07 |
| AD-1548595.1 | 18.63 | 1.33 | 28.11 | 2.17 | 43.93 | 5.75 |
| AD-1548600.1 | 37.25 | 4.42 | 40.05 | 1.14 | 44.22 | 4.66 |
| AD-1548562.1 | 30.58 | 4.74 | 38.90 | 6.24 | 44.29 | 4.01 |
| AD-1548637.1 | 19.40 | 3.39 | 16.72 | 2.47 | 44.45 | 5.63 |
| AD-1548461.1 | 14.88 | 2.05 | 23.14 | 5.89 | 44.89 | 6.85 |
| AD-1548569.1 | 31.37 | 3.13 | 40.14 | 2.61 | 45.05 | 4.67 |
| AD-1548643.1 | 12.27 | 2.88 | 20.13 | 2.05 | 45.16 | 6.86 |
| AD-1548626.1 | 26.76 | 1.77 | 30.61 | 3.05 | 45.63 | 4.42 |
| AD-1548564.1 | 39.03 | 6.65 | 48.94 | 6.30 | 46.42 | 5.03 |
| AD-1548502.1 | 23.79 | 2.17 | 29.80 | 1.46 | 46.46 | 2.17 |
| AD-1548624.1 | 17.45 | 1.21 | 27.91 | 3.87 | 46.60 | 4.51 |
| AD-1548692.1 | 6.41 | 0.55 | 7.69 | 0.71 | 47.08 | 4.32 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548436.1 | 24.81 | 2.43 | 30.84 | 3.14 | 47.16 | 4.16 |
| AD-1548704.1 | 23.91 | 3.34 | 24.84 | 1.62 | 47.72 | 11.64 |
| AD-1548399.1 | 39.84 | 3.46 | 46.40 | 1.89 | 48.24 | 4.95 |
| AD-1548480.1 | 16.30 | 1.74 | 30.98 | 4.33 | 48.56 | 4.19 |
| AD-1548677.1 | 29.54 | 1.97 | 38.96 | 3.32 | 48.98 | 12.50 |
| AD-1548700.1 | 28.81 | 3.39 | 35.89 | 1.73 | 49.29 | 8.86 |
| AD-1548563.1 | 36.84 | 2.84 | 57.51 | 4.48 | 50.14 | 5.07 |
| AD-1548409.1 | 36.18 | 2.63 | 36.45 | 4.31 | 50.63 | 4.52 |
| AD-1548615.1 | 17.26 | 1.11 | 31.22 | 3.63 | 51.34 | 2.28 |
| AD-1548655.1 | 21.30 | 1.03 | 36.46 | 4.30 | 52.54 | 6.51 |
| AD-1548620.1 | 43.39 | 4.46 | 46.13 | 4.44 | 53.63 | 2.80 |
| AD-1548561.1 | 44.16 | 3.11 | 51.68 | 11.02 | 54.08 | 7.09 |
| AD-1548691.1 | 11.51 | 1.24 | 11.86 | 0.86 | 54.62 | 5.09 |
| AD-1548630.1 | 33.06 | 4.42 | 48.73 | 4.25 | 54.71 | 1.58 |
| AD-1548699.1 | 19.52 | 2.63 | 31.18 | 2.77 | 55.12 | 4.78 |
| AD-1548565.1 | 50.21 | 1.79 | 56.31 | 4.17 | 55.24 | 4.41 |
| AD-1548540.1 | 14.99 | 0.71 | 26.91 | 2.95 | 55.62 | 7.48 |
| AD-1548547.1 | 42.53 | 6.12 | 43.33 | 9.30 | 55.94 | 8.82 |
| AD-1548494.1 | 16.04 | 1.62 | 23.42 | 2.87 | 56.02 | 4.26 |
| AD-1548427.1 | 20.30 | 1.80 | 39.62 | 2.79 | 56.31 | 5.48 |
| AD-1548533.1 | 22.83 | 2.60 | 32.51 | 3.96 | 56.94 | 4.72 |
| AD-1548551.1 | 64.67 | 5.99 | 70.86 | 8.34 | 59.94 | 3.46 |
| AD-1548431.1 | 23.57 | 2.41 | 38.70 | 3.03 | 60.07 | 8.69 |
| AD-1548546.1 | 58.04 | 4.95 | 55.65 | 14.18 | 61.06 | 4.60 |
| AD-1548652.1 | 26.94 | 7.85 | 36.75 | 1.18 | 61.12 | 9.75 |
| AD-1548365.1 | 42.82 | 5.73 | 47.35 | 8.81 | 61.23 | 6.99 |
| AD-1548550.1 | 69.47 | 3.49 | 66.66 | 9.04 | 61.73 | 11.15 |
| AD-1548597.1 | 77.13 | 7.94 | 85.82 | 6.84 | 62.23 | 1.92 |
| AD-1548609.1 | 38.53 | 2.81 | 46.04 | 4.21 | 62.29 | 3.83 |
| AD-1548611.1 | 27.84 | 4.41 | 44.97 | 3.59 | 62.77 | 8.22 |
| AD-1548697.1 | 22.27 | 2.84 | 39.72 | 4.24 | 62.83 | 10.09 |
| AD-1548448.1 | 32.75 | 11.47 | 41.01 | 2.67 | 62.97 | 11.53 |
| AD-1548517.1 | 14.80 | 2.09 | 27.45 | 0.75 | 63.45 | 5.81 |
| AD-1548554.1 | 45.81 | 3.39 | 61.81 | 12.03 | 64.22 | 3.38 |
| AD-1548383.1 | 84.65 | 14.88 | 61.70 | 8.95 | 64.61 | 6.46 |
| AD-1548401.1 | 25.14 | 2.36 | 40.57 | 4.28 | 64.77 | 4.53 |
| AD-1548514.1 | 37.42 | 3.56 | 44.60 | 5.56 | 64.95 | 5.36 |
| AD-1548606.1 | 60.53 | 5.55 | 72.68 | 7.18 | 64.98 | 12.46 |
| AD-1548465.1 | 38.63 | 3.94 | 57.55 | 14.57 | 66.40 | 7.64 |
| AD-1548394.1 | 33.78 | 4.96 | 41.43 | 5.79 | 66.49 | 9.12 |
| AD-1548557.1 | 64.98 | 4.25 | 67.64 | 4.73 | 66.52 | 7.88 |
| AD-1548558.1 | 75.14 | 6.26 | 81.46 | 9.27 | 66.78 | 8.61 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| Duplex | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV | % of Message Remaining | ST DEV |
| AD-1548548.1 | 52.05 | 10.64 | 55.97 | 11.61 | 67.41 | 3.48 |
| AD-1548387.1 | 67.89 | 8.73 | 52.43 | 3.19 | 67.67 | 5.18 |
| AD-1548474.1 | 50.99 | 6.78 | 65.89 | 7.44 | 67.88 | 7.82 |
| AD-1548619.1 | 36.48 | 0.37 | 52.65 | 10.68 | 68.49 | 5.56 |
| AD-1548601.1 | 51.77 | 0.64 | 56.93 | 4.47 | 68.83 | 5.62 |
| AD-1548369.1 | 55.87 | 16.62 | 51.63 | 8.97 | 68.84 | 11.40 |
| AD-1548613.1 | 35.52 | 1.70 | 41.91 | 2.62 | 69.40 | 12.19 |
| AD-1548553.1 | 54.07 | 4.53 | 57.10 | 2.69 | 69.80 | 3.75 |
| AD-1548693.1 | 15.16 | 0.34 | 15.48 | 1.04 | 69.92 | 5.84 |
| AD-1548555.1 | 47.63 | 7.43 | 57.73 | 9.47 | 70.52 | 5.40 |
| AD-1548520.1 | 16.86 | 0.88 | 32.98 | 2.06 | 70.81 | 3.77 |
| AD-1548552.1 | 52.43 | 7.14 | 59.80 | 1.96 | 72.33 | 6.73 |
| AD-1548370.1 | 46.33 | 12.84 | 53.84 | 6.50 | 72.52 | 8.10 |
| AD-1548505.1 | 45.53 | 6.49 | 56.21 | 5.63 | 72.91 | 3.56 |
| AD-1548545.1 | 58.24 | 11.98 | 58.99 | 8.49 | 74.12 | 16.72 |
| AD-1548539.1 | 51.88 | 1.66 | 59.78 | 4.26 | 75.56 | 2.24 |
| AD-1548549.1 | 61.61 | 2.48 | 64.92 | 10.07 | 75.70 | 5.95 |
| AD-1548556.1 | 55.49 | 4.88 | 62.86 | 3.83 | 75.83 | 3.59 |
| AD-1548616.1 | 45.80 | 7.83 | 55.40 | 7.94 | 79.15 | 2.99 |
| AD-1548482.1 | 69.01 | 11.78 | 78.29 | 6.48 | 79.33 | 5.40 |
| AD-1548501.1 | 60.77 | 2.61 | 73.05 | 6.25 | 79.67 | 7.90 |
| AD-1548681.1 | 12.07 | 1.95 | 20.36 | 2.91 | 80.58 | 18.49 |
| AD-1548504.1 | 77.48 | 6.09 | 84.06 | 5.28 | 81.64 | 7.97 |
| AD-1548425.1 | 45.11 | 2.53 | 49.58 | 8.06 | 82.13 | 10.13 |
| AD-1548671.1 | 51.86 | 8.58 | 72.37 | 5.01 | 84.11 | 14.72 |
| AD-1548544.1 | 66.15 | 19.91 | 82.59 | 13.59 | 85.56 | 10.49 |
| AD-1548534.1 | 41.26 | 0.92 | 57.38 | 2.99 | 86.89 | 4.85 |
| AD-1548445.1 | 97.62 | 8.98 | 81.83 | 6.71 | 87.11 | 5.86 |
| AD-1548433.1 | 94.39 | 5.60 | 83.20 | 3.63 | 88.16 | 2.91 |
| AD-1548455.1 | 74.25 | 2.00 | 80.48 | 9.46 | 88.69 | 4.55 |
| AD-1548429.1 | 72.90 | 5.96 | 74.01 | 4.26 | 89.02 | 9.93 |
| AD-1548386.1 | 77.39 | 5.39 | 69.06 | 7.07 | 91.10 | 2.33 |
| AD-1548690.1 | 28.19 | 5.69 | 30.38 | 4.70 | 91.93 | 10.63 |
| AD-1548541.1 | 68.53 | 7.69 | 91.69 | 19.67 | 97.83 | 10.51 |
| AD-1548506.1 | 68.16 | 9.18 | 78.19 | 5.42 | 102.21 | 9.47 |
| AD-1548518.1 | 75.11 | 6.07 | 84.41 | 8.00 | 102.82 | 6.29 |
| AD-1548410.1 | 87.37 | 18.16 | 95.77 | 9.50 | 102.99 | 5.91 |
| AD-1548686.1 | 24.37 | 2.14 | 38.09 | 8.32 | 105.02 | 8.13 |
| AD-1548694.1 | 14.47 | 0.55 | 21.59 | 1.41 | 106.25 | 12.84 |
| AD-1548422.1 | 108.87 | 9.66 | 106.72 | 12.95 | 109.92 | 8.24 |
| AD-1548689.1 | 35.02 | 4.55 | 32.82 | 2.77 | 120.89 | 23.72 |
| AD-1548446.1 | 115.57 | 9.32 | 103.06 | 10.57 | 122.37 | 8.71 |

| | 10 nM | | 1 nM | | 0.1 nM | |
|---|---|---|---|---|---|---|
| **Duplex** | **% of Message Remaining** | **ST DEV** | **% of Message Remaining** | **ST DEV** | **% of Message Remaining** | **ST DEV** |
| AD-1548688.1 | 44.04 | 2.29 | 54.21 | 2.64 | 141.92 | 15.39 |
| AD-1548687.1 | 42.98 | 7.70 | 50.98 | 3.19 | 142.40 | 24.95 |
| AD-1548683.1 | 55.32 | 5.04 | 59.79 | 5.28 | 156.44 | 36.70 |
| AD-1548685.1 | 33.95 | 6.03 | 45.16 | 2.86 | 157.19 | 13.12 |
| AD-1548682.1 | 69.60 | 5.53 | 56.71 | 2.73 | 161.28 | 8.92 |
| AD-1548684.1 | 59.65 | 4.71 | 67.76 | 2.58 | 161.94 | 28.86 |

**Example 3. Additonal Duplexes Targeting Beta-Catenin (CTNNB1)**

Additional siRNAs targeting the human beta-catenin (CTNNB1) gene (human: NCBI refseqID NM_001904.4, NCBI GeneID: 1499) were designed and synthesized as described above. Single dose screens at 10 nM, 1 nM, and 01. nM were performed in Hep3B cells as described above.

Detailed lists of the additional unmodified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 5. Detailed lists of the additional modified CTNNB1 sense and antisense strand nucleotide sequences are shown in Table 6.

The results of a single dose screen of the agents in Tables 5 and 6 in Hep3B cells are shown in Table 7.

**Table 5. Unmodified Sense and Antisense Strand Sequences of CTNNB1 dsRNA Agents**

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1210479 | ACUCUGGAAUCCAUUCUGGUA | | 309-329 | UACCAGAAUGGAUUCCAGAGUCC | | 307-329 |
| AD-1210480 | CUCUGGAAUCCAUUCUGGUGA | | 310-330 | UCACCAGAAUGGAUUCCAGAGUC | | 308-330 |
| AD-1210732 | UGCUCAUCCCACUAAUGUCCA | | 562-582 | UGGACAUUAGUGGGAUGAGCAGC | | 560-582 |
| AD-1210734 | CUCAUCCCACUAAUGUCCAGA | | 564-584 | UCUGGACAUUAGUGGGAUGAGCA | | 562-584 |
| AD-1211064 | CCAUGCAGAAUACAAAUGAUA | | 816-836 | UAUCAUUUGUAUUCUGCAUGGUA | | 814-836 |
| AD-1211065 | CAUGCAGAAUACAAAUGAUGA | | 817-837 | UCAUCAUUUGUAUUCUGCAUGGU | | 815-837 |
| AD-1211578 | AGAAGGAGCUAAAAUGGCAGA | | 1012-1032 | UCUGCCAUUUUAGCUCCUUCUUG | | 1010-1032 |
| AD-1211582 | GGAGCUAAAAUGGCAGUGCGA | | 1016-1036 | UCGCACUGCCAUUUUAGCUCCUU | | 1014-1036 |
| AD-1211675 | UACUGGCCAUCUUUAAGUCUA | | 897-917 | UAGACUUAAAGAUGGCCAGUAAG | | 895-917 |
| AD-1211676 | ACUGGCCAUCUUUAAGUCUGA | | 898-918 | UCAGACUUAAAGAUGGCCAGUAA | | 896-918 |
| AD-1211677 | CUGGCCAUCUUUAAGUCUGGA | | 899-919 | UCCAGACUUAAAGAUGGCCAGUA | | 897-919 |
| AD-1212720 | UAACCUCACUUGCAAUAAUUA | | 1489-1509 | UAAUUAUUGCAAGUGAGGUUAGA | | 1487-1509 |
| AD-1212721 | AACCUCACUUGCAAUAAUUAA | | 1490-1510 | UUAAUUAUUGCAAGUGAGGUUAG | | 1488-1510 |
| AD-1213112 | GCCAUCUGUGCUCUUCGUCAA | | 1604-1624 | UUGACGAAGAGCACAGAUGGCAG | | 1602-1624 |
| AD-1213113 | CCAUCUGUGCUCUUCGUCAUA | | 1605-1625 | UAUGACGAAGAGCACAGAUGGCA | | 1603-1625 |
| AD-1213115 | AUCUGUGCUCUUCGUCAUCUA | | 1607-1627 | UAGAUGACGAAGAGCACAGAUGG | | 1605-1627 |
| AD-1213116 | UCUGUGCUCUUCGUCAUCUGA | | 1608-1628 | UCAGAUGACGAAGAGCACAGAUG | | 1606-1628 |
| AD-1213117 | CUGUGCUCUUCGUCAUCUGAA | | 1609-1629 | UUCAGAUGACGAAGAGCACAGAU | | 1607-1629 |
| AD-1213118 | UGUGCUCUUCGUCAUCUGACA | | 1610-1630 | UGUCAGAUGACGAAGAGCACAGA | | 1608-1630 |
| AD-1213119 | GUGCUCUUCGUCAUCUGACCA | | 1611-1631 | UGGUCAGAUGACGAAGAGCACAG | | 1609-1631 |
| AD-1213749 | ACUGUUGGAUUGAUUCGAAAA | | 1742-1762 | UUUUCGAAUCAAUCCAACAGUAG | | 1740-1762 |
| AD-1214765 | AAAUACCAUUCCAUUGUUUGA | | 1993-2013 | UCAAACAAUGGAAUGGUAUUUAG | | 1991-2013 |
| AD-1214768 | UACCAUUCCAUUGUUUGUGCA | | 1996-2016 | UGCACAAACAAUGGAAUGGUAUU | | 1994-2016 |
| AD-1214770 | CCAUUCCAUUGUUUGUGCAGA | | 1998-2018 | UCUGCACAAACAAUGGAAUGGUA | | 1996-2018 |
| AD-1215017 | GUCCUCUGUGAACUUGCUCAA | | 2063-2083 | UUGAGCAAGUUCACAGAGGACCC | | 2061-2083 |
| AD-1215018 | UCCUCUGUGAACUUGCUCAGA | | 2064-2084 | UCUGAGCAAGUUCACAGAGGACC | | 2062-2084 |
| AD-1216131 | AUCGUUCUUUUCACUCUGGUA | | 2361-2381 | UACCAGAGUGAAAAGAACGAUAG | | 2359-2381 |
| AD-1216132 | UCGUUCUUUUCACUCUGGUGA | | 2362-2382 | UCACCAGAGUGAAAAGAACGAUA | | 2360-2382 |
| AD-1216345 | UGGUGCUGACUAUCCAGUUGA | | 2446-2466 | UCAACUGGAUAGUCAGCACCAGG | | 2444-2466 |
| AD-1216349 | GCUGACUAUCCAGUUGAUGGA | | 2450-2470 | UCCAUCAACUGGAUAGUCAGCAC | | 2448-2470 |
| AD-1216350 | CUGACUAUCCAGUUGAUGGGA | | 2451-2471 | UCCCAUCAACUGGAUAGUCAGCA | | 2449-2471 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1216352 | GACUAUCCAGUUGAUGGGCUA | | 2453-2473 | UAGCCCAUCAACUGGAUAGUCAG | | 2451-2473 |
| AD-1216506 | AUUGGCCUGUAGAGUUGCUGA | | 2903-2923 | UCAGCAACUCUACAGGCCAAUCA | | 2901-2923 |
| AD-1216525 | GGCUGGUAUCUCAGAAAGUGA | | 2940-2960 | UCACUUUCUGAGAUACCAGCCCA | | 2938-2960 |
| AD-1216526 | GCUGGUAUCUCAGAAAGUGCA | | 2941-2961 | UGCACUUUCUGAGAUACCAGCCC | | 2939-2961 |
| AD-1216528 | UGGUAUCUCAGAAAGUGCCUA | | 2943-2963 | UAGGCACUUUCUGAGAUACCAGC | | 2941-2963 |
| AD-1216546 | CUGACACACUAACCAAGCUGA | | 2961-2981 | UCAGCUUGGUUAGUGUGUCAGGC | | 2959-2981 |
| AD-1216556 | AACCAAGCUGAGUUUCCUAUA | | 2971-2991 | UAUAGGAAACUCAGCUUGGUUAG | | 2969-2991 |
| AD-1216558 | CCAAGCUGAGUUUCCUAUGGA | | 2973-2993 | UCCAUAGGAAACUCAGCUUGGUU | | 2971-2993 |
| AD-1216559 | CAAGCUGAGUUUCCUAUGGGA | | 2974-2994 | UCCCAUAGGAAACUCAGCUUGGU | | 2972-2994 |
| AD-1216560 | AAGCUGAGUUUCCUAUGGGAA | | 2975-2995 | UUCCCAUAGGAAACUCAGCUUGG | | 2973-2995 |
| AD-1216561 | AGCUGAGUUUCCUAUGGGAAA | | 2976-2996 | UUUCCCAUAGGAAACUCAGCUUG | | 2974-2996 |
| AD-1216592 | UUGUUCUGGUCCUUUUUGGUA | | 3013-3033 | UACCAAAAAGGACCAGAACAAAA | | 3011-3033 |
| AD-1216593 | UGUUCUGGUCCUUUUUGGUCA | | 3014-3034 | UGACCAAAAAGGACCAGAACAAA | | 3012-3034 |
| AD-1216594 | GUUCUGGUCCUUUUUGGUCGA | | 3015-3035 | UCGACCAAAAAGGACCAGAACAA | | 3013-3035 |
| AD-1216595 | UUCUGGUCCUUUUUGGUCGAA | | 3016-3036 | UUCGACCAAAAAGGACCAGAACA | | 3014-3036 |
| AD-1216596 | UCUGGUCCUUUUUGGUCGAGA | | 3017-3037 | UCUCGACCAAAAAGGACCAGAAC | | 3015-3037 |
| AD-1216597 | CUGGUCCUUUUUGGUCGAGGA | | 3018-3038 | UCCUCGACCAAAAAGGACCAGAA | | 3016-3038 |
| AD-1216613 | GAGGAGUAACAAUACAAAUGA | | 3034-3054 | UCAUUUGUAUUGUUACUCCUCGA | | 3032-3054 |
| AD-1216784 | AUGGUGUAGAACACUAAUUCA | | 3314-3334 | UGAAUUAGUGUUCUACACCAUUA | | 3312-3334 |
| AD-1216786 | GGUGUAGAACACUAAUUCAUA | | 3316-3336 | UAUGAAUUAGUGUUCUACACCAU | | 3314-3336 |
| AD-1216787 | GUGUAGAACACUAAUUCAUAA | | 3317-3337 | UUAUGAAUUAGUGUUCUACACCA | | 3315-3337 |
| AD-1216790 | UAGAACACUAAUUCAUAAUCA | | 3320-3340 | UGAUUAUGAAUUAGUGUUCUACA | | 3318-3340 |
| AD-1216792 | GAACACUAAUUCAUAAUCACA | | 3322-3342 | UGUGAUUAUGAAUUAGUGUUCUA | | 3320-3342 |
| AD-1216895 | UGCUUAAAAUAAGCAGGUGGA | | 3440-3460 | UCCACCUGCUUAUUUUAAGCAUA | | 3438-3460 |
| AD-1216904 | UAAGCAGGUGGAUCUAUUUCA | | 3449-3469 | UGAAAUAGAUCCACCUGCUUAUU | | 3447-3469 |
| AD-1216906 | AGCAGGUGGAUCUAUUUCAUA | | 3451-3471 | UAUGAAAUAGAUCCACCUGCUUA | | 3449-3471 |
| AD-1216907 | GCAGGUGGAUCUAUUUCAUGA | | 3452-3472 | UCAUGAAAUAGAUCCACCUGCUU | | 3450-3472 |
| AD-1216910 | GGUGGAUCUAUUUCAUGUUUA | | 3455-3475 | UAAACAUGAAAUAGAUCCACCUG | | 3453-3475 |
| AD-1216911 | GUGGAUCUAUUUCAUGUUUUA | | 3456-3476 | UAAAACAUGAAAUAGAUCCACCU | | 3454-3476 |
| AD-1216913 | GGAUCUAUUUCAUGUUUUUGA | | 3458-3478 | UCAAAAACAUGAAAUAGAUCCAC | | 3456-3478 |
| AD-1216938 | GGGUAGGGUAAAUCAGUAAGA | | 3503-3523 | UCUUACUGAUUUACCCUACCCAU | | 3501-3523 |
| AD-1216940 | GUAGGGUAAAUCAGUAAGAGA | | 3505-3525 | UCUCUUACUGAUUUACCCUACCC | | 3503-3525 |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 | Antisense Sequence 5' to 3' | SEQ ID NO: | Range in NM_001904.4 |
|---|---|---|---|---|---|---|
| AD-1216941 | UAGGGUAAAUCAGUAAGAGGA | | 3506-3526 | UCCUCUUACUGAUUUACCCUACC | | 3504-3526 |
| AD-1216942 | AGGGUAAAUCAGUAAGAGGUA | | 3507-3527 | UACCUCUUACUGAUUUACCCUAC | | 3505-3527 |
| AD-1216944 | GGUAAAUCAGUAAGAGGUGUA | | 3509-3529 | UACACCUCUUACUGAUUUACCCU | | 3507-3529 |
| AD-1216945 | GUAAAUCAGUAAGAGGUGUUA | | 3510-3530 | UAACACCUCUUACUGAUUUACCC | | 3508-3530 |
| AD-1216989 | UUACCAGUUGCCUUUUAUCCA | | 3554-3574 | UGGAUAAAAGGCAACUGGUAAAC | | 3552-3574 |
| AD-1216990 | UACCAGUUGCCUUUUAUCCCA | | 3555-3575 | UGGGAUAAAAGGCAACUGGUAAA | | 3553-3575 |
| AD-1217004 | UAUCCCAAAGUUGUUGUAACA | | 3569-3589 | UGUUACAACAACUUUGGGAUAAA | | 3567-3589 |
| AD-1217007 | CCCAAAGUUGUUGUAACCUGA | | 3572-3592 | UCAGGUUACAACAACUUUGGGAU | | 3570-3592 |
| AD-70947 | UACUGUUGGAUUGAUUCGAAA | | 1741-1761 | UUUCGAAUCAAUCCAACAGUAGC | | 1739-1761 |

**Table 6. Modified Sense and Antisense Strand Sequences of CTNNB1 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-1210479 | ascsucugGfaAfUfCfcauucugguaL96 | | VPusAfsccaGfaAfUfggauUfcCfagaguscsc | | GGACUCUGGAAUCCAUUCUGGUG | |
| AD-1210480 | csuscuggAfaUfCfCfauucuggugaL96 | | VPusCfsaccAfgAfAfuggaUfuCfcagagsusc | | GACUCUGGAAUCCAUUCUGGUGC | |
| AD-1210732 | usgscucaUfcCfCfAfcuaauguccaL96 | | VPusGfsgacAfuUfAfguggGfaUfgagcasgsc | | GCUGCUCAUCCCACUAAUGUCCA | |
| AD-1210734 | csuscaucCfcAfCfUfaauguccagaL96 | | VPusCfsuggAfcAfUfuaguGfgGfaugagscsa | | UGCUCAUCCCACUAAUGUCCAGC | |
| AD-1211064 | cscsaugcAfgAfAfUfacaaaugauaL96 | | VPusAfsucaUfuUfGfuauuCfuGfcauggsusa | | UACCAUGCAGAAUACAAAUGAUG | |
| AD-1211065 | csasugcaGfaAfUfAfcaaaugaugaL96 | | VPusCfsaucAfuUfUfguauUfcUfgcaugsgsu | | ACCAUGCAGAAUACAAAUGAUGU | |
| AD-1211578 | asgsaaggAfgCfUfAfaaauggcagaL96 | | VPusCfsugcCfaUfUfuuagCfuCfcuucususg | | CAAGAAGGAGCUAAAAUGGCAGU | |
| AD-1211582 | gsgsagcuAfaAfAfUfggcagugcgaL96 | | VPusCfsgcaCfuGfCfcauuUfuAfgcuccsusu | | AAGGAGCUAAAAUGGCAGUGCGU | |
| AD-1211675 | usascuggCfcAfUfCfuuuaagucuaL96 | | VPusAfsgacUfuAfAfagauGfgCfcaguasasg | | CUUACUGGCCAUCUUUAAGUCUG | |

| 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-1211676 | ascsuggcCfaUfCfUfuuaagucugaL96 | | VPusCfsagaCfuUfAfaagaUfgGfccagusasa | | UUACUGGCCAUCUUUAAGUCUGG | |
| AD-1211677 | csusggccAfuCfUfUfuaagucuggaL96 | | VPusCfscagAfcUfUfaaagAfuGfgccagsusa | | UACUGGCCAUCUUUAAGUCUGGA | |
| AD-1212720 | usasaccuCfaCfUfUfgcaauaauuaL96 | | VPusAfsauuAfuUfGfcaagUfgAfgguuasgsa | | UCUAACCUCACUUGCAAUAAUUA | |
| AD-1212721 | asasccucAfcUfUfGfcaauaauuaaL96 | | VPusUfsaauUfaUfUfgcaaGfuGfagguusasg | | CUAACCUCACUUGCAAUAAUUAU | |
| AD-1213112 | gscscaucUfgUfGfCfucuucgucaaL96 | | VPusUfsgacGfaAfGfagcaCfaGfauggcsasg | | CUGCCAUCUGUGCUCUUCGUCAU | |
| AD-1213113 | cscsaucuGfuGfCfUfcuucgucauaL96 | | VPusAfsugaCfgAfAfgagcAfcAfgauggscsa | | UGCCAUCUGUGCUCUUCGUCAUC | |
| AD-1213115 | asuscuguGfcUfCfUfucgucaucuaL96 | | VPusAfsgauGfaCfGfaagaGfcAfcagausgsg | | CCAUCUGUGCUCUUCGUCAUCUG | |
| AD-1213116 | uscsugugCfuCfUfUfcgucaucugaL96 | | VPusCfsagaUfgAfCfgaagAfgCfacagasusg | | CAUCUGUGCUCUUCGUCAUCUGA | |
| AD-1213117 | csusgugcUfcUfUfCfgucaucugaaL96 | | VPusUfscagAfuGfAfcgaaGfaGfcacagsasu | | AUCUGUGCUCUUCGUCAUCUGAC | |
| AD-1213118 | usgsugcuCfuUfCfGfucaucugacaL96 | | VPusGfsucaGfaUfGfacgaAfgAfgcacasgsa | | UCUGUGCUCUUCGUCAUCUGACC | |
| AD-1213119 | gsusgcucUfuCfGfUfcaucugaccaL96 | | VPusGfsgucAfgAfUfgacgAfaGfagcacsasg | | CUGUGCUCUUCGUCAUCUGACCA | |
| AD-1213749 | ascsuguuGfgAfUfUfgauucgaaaaL96 | | VPusUfsuucGfaAfUfcaauCfcAfacagusasg | | CUACUGUUGGAUUGAUUCGAAAU | |
| AD-1214765 | asasauacCfaUfUfCfcauuguuugaL96 | | VPusCfsaaaCfaAfUfggaaUfgGfuauuusasg | | CUAAAUACCAUUCCAUUGUUUGU | |
| AD-1214768 | usasccauUfcCfAfUfuguuugugcaL96 | | VPusGfscacAfaAfCfaaugGfaAfugguasusu | | AAUACCAUUCCAUUGUUUGUGCA | |
| AD-1214770 | cscsauucCfaUfUfGfuuugugcagaL96 | | VPusCfsugcAfcAfAfacaaUfgGfaauggsusa | | UACCAUUCCAUUGUUUGUGCAGC | |
| AD-1215017 | gsusccucUfgUfGfAfacuugcucaaL96 | | VPusUfsgagCfaAfGfuucaCfaGfaggacscsc | | GGGUCCUCUGUGAACUUGCUCAG | |
| AD- | uscscucuGfuGfAfAfcuugcucagaL96 | | VPusCfsugaGfcAfAfguucAfcAfgaggascsc | | GGUCCUCUGUGAACUUGCUCAGG | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1215018 | | | | | | |
| AD-1216131 | asuscguuCfuUfUfUfcacucugguaL96 | | VPusAfsccaGfaGfUfgaaaAfgAfacgausasg | | CUAUCGUUCUUUUCACUCUGGUG | |
| AD-1216132 | uscsguucUfuUfUfCfacucuggugaL96 | | VPusCfsaccAfgAfGfugaaAfaGfaacgasusa | | UAUCGUUCUUUUCACUCUGGUGG | |
| AD-1216345 | usgsgugcUfgAfCfUfauccaguugaL96 | | VPusCfsaacUfgGfAfuaguCfaGfcaccasgsg | | CCUGGUGCUGACUAUCCAGUUGA | |
| AD-1216349 | gscsugacUfaUfCfCfaguugauggaL96 | | VPusCfscauCfaAfCfuggaUfaGfucagcsasc | | GUGCUGACUAUCCAGUUGAUGGG | |
| AD-1216350 | csusgacuAfuCfCfAfguugaugggaL96 | | VPusCfsccaUfcAfAfcuggAfuAfgucagscsa | | UGCUGACUAUCCAGUUGAUGGGC | |
| AD-1216352 | gsascuauCfcAfGfUfugaugggcuaL96 | | VPusAfsgccCfaUfCfaacuGfgAfuagucsasg | | CUGACUAUCCAGUUGAUGGGCUG | |
| AD-1216506 | asusuggcCfuGfUfAfgaguugcugaL96 | | VPusCfsagcAfaCfUfcuacAfgGfccaauscsa | | UGAUUGGCCUGUAGAGUUGCUGA | |
| AD-1216525 | gsgscuggUfaUfCfUfcagaaagugaL96 | | VPusCfsacuUfuCfUfgagaUfaCfcagccscsa | | UGGGCUGGUAUCUCAGAAAGUGC | |
| AD-1216526 | gscsugguAfuCfUfCfagaaagugcaL96 | | VPusGfscacUfuUfCfugagAfuAfccagcscsc | | GGGCUGGUAUCUCAGAAAGUGCC | |
| AD-1216528 | usgsguauCfuCfAfGfaaagugccuaL96 | | VPusAfsggcAfcUfUfucugAfgAfuaccasgsc | | GCUGGUAUCUCAGAAAGUGCCUG | |
| AD-1216546 | csusgacaCfaCfUfAfaccaagcugaL96 | | VPusCfsagcUfuGfGfuuagUfgUfgucagsgsc | | GCCUGACACACUAACCAAGCUGA | |
| AD-1216556 | asasccaaGfcUfGfAfguuuccuauaL96 | | VPusAfsuagGfaAfAfcucaGfcUfugguusasg | | CUAACCAAGCUGAGUUUCCUAUG | |
| AD-1216558 | cscsaagcUfgAfGfUfuuccuauggaL96 | | VPusCfscauAfgGfAfaacuCfaGfcuuggsusu | | AACCAAGCUGAGUUUCCUAUGGG | |
| AD-1216559 | csasagcuGfaGfUfUfuccuaugggaL96 | | VPusCfsccaUfaGfGfaaacUfcAfgcuugsgsu | | ACCAAGCUGAGUUUCCUAUGGGA | |
| AD-1216560 | asasgcugAfgUfUfUfccuaugggaaL96 | | VPusUfscccAfuAfGfgaaaCfuCfagcuusgsg | | CCAAGCUGAGUUUCCUAUGGGAA | |
| AD-1216561 | asgscugaGfuUfUfCfcuaugggaaaL96 | | VPusUfsuccCfaUfAfggaaAfcUfcagcususg | | CAAGCUGAGUUUCCUAUGGGAAC | |

| 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-1216592 | ususguucUfgGfUfCfcuuuuugguaL96 | | VPusAfsccaAfaAfAfggacCfaGfaacaasasa | | UUUUGUUCUGGUCCUUUUUGGUC | |
| AD-1216593 | usgsuucuGfgUfCfCfuuuuuggucaL96 | | VPusGfsaccAfaAfAfaggaCfcAfgaacasasa | | UUUGUUCUGGUCCUUUUUGGUCG | |
| AD-1216594 | gsusucugGfuCfCfUfuuuuggucgaL96 | | VPusCfsgacCfaAfAfaaggAfcCfagaacsasa | | UUGUUCUGGUCCUUUUUGGUCGA | |
| AD-1216595 | ususcuggUfcCfUfUfuuuggucgaaL96 | | VPusUfscgaCfcAfAfaaagGfaCfcagaascsa | | UGUUCUGGUCCUUUUUGGUCGAG | |
| AD-1216596 | uscsugguCfcUfUfUfuuggucgagaL96 | | VPusCfsucgAfcCfAfaaaaGfgAfccagasasc | | GUUCUGGUCCUUUUUGGUCGAGG | |
| AD-1216597 | csusggucCfuUfUfUfuggucgaggaL96 | | VPusCfscucGfaCfCfaaaaAfgGfaccagsasa | | UUCUGGUCCUUUUUGGUCGAGGA | |
| AD-1216613 | gsasggagUfaAfCfAfauacaaaugaL96 | | VPusCfsauuUfgUfAfuuguUfaCfuccucsgsa | | UCGAGGAGUAACAAUACAAAUGG | |
| AD-1216784 | asusggugUfaGfAfAfcacuaauucaL96 | | VPusGfsaauUfaGfUfguucUfaCfaccaususa | | UAAUGGUGUAGAACACUAAUUCA | |
| AD-1216786 | gsgsuguaGfaAfCfAfcuaauucauaL96 | | VPusAfsugaAfuUfAfguguUfcUfacaccsasu | | AUGGUGUAGAACACUAAUUCAUA | |
| AD-1216787 | gsusguagAfaCfAfCfuaauucauaaL96 | | VPusUfsaugAfaUfUfagugUfuCfuacacscsa | | UGGUGUAGAACACUAAUUCAUAA | |
| AD-1216790 | usasgaacAfcUfAfAfuucauaaucaL96 | | VPusGfsauuAfuGfAfauuaGfuGfuucuascsa | | UGUAGAACACUAAUUCAUAAUCA | |
| AD-1216792 | gsasacacUfaAfUfUfcauaaucacaL96 | | VPusGfsugaUfuAfUfgaauUfaGfuguucsusa | | UAGAACACUAAUUCAUAAUCACU | |
| AD-1216895 | usgscuuaAfaAfUfAfagcagguggaL96 | | VPusCfscacCfuGfCfuuauUfuUfaagcasusa | | UAUGCUUAAAAUAAGCAGGUGGA | |
| AD-1216904 | usasagcaGfgUfGfGfaucuauuucaL96 | | VPusGfsaaaUfaGfAfuccaCfcUfgcuuasusu | | AAUAAGCAGGUGGAUCUAUUUCA | |
| AD-1216906 | asgscaggUfgGfAfUfcuauuucauaL96 | | VPusAfsugaAfaUfAfgaucCfaCfcugcususa | | UAAGCAGGUGGAUCUAUUUCAUG | |
| AD-1216907 | gscsagguGfgAfUfCfuauuucaugaL96 | | VPusCfsaugAfaAfUfagauCfcAfccugcsusu | | AAGCAGGUGGAUCUAUUUCAUGU | |
| AD- | gsgsuggaUfcUfAfUfuucauguuuaL96 | | VPusAfsaacAfuGfAfaauaGfaUfccaccsusg | | CAGGUGGAUCUAUUUCAUGUUUU | |

| Duplex Name | Sense Sequence 5' to 3' | SEQ ID NO: | Antisense Sequence 5' to 3' | SEQ ID NO: | mRNA Target Sequence | SEQ ID NO: |
|---|---|---|---|---|---|---|
| 1216910 | | | | | | |
| AD-1216911 | gsusggauCfuAfUfUfucauguuuuaL96 | | VPusAfsaaaCfaUfGfaaauAfgAfuccacscsu | | AGGUGGAUCUAUUUCAUGUUUUU | |
| AD-1216913 | gsgsaucuAfuUfUfCfauguuuuugaL96 | | VPusCfsaaaAfaCfAfugaaAfuAfgauccsasc | | GUGGAUCUAUUUCAUGUUUUUGA | |
| AD-1216938 | gsgsguagGfgUfAfAfaucaguaagaL96 | | VPusCfsuuaCfuGfAfuuuaCfcCfuacccsasu | | AUGGGUAGGGUAAAUCAGUAAGA | |
| AD-1216940 | gsusagggUfaAfAfUfcaguaagagaL96 | | VPusCfsucuUfaCfUfgauuUfaCfccuacscsc | | GGGUAGGGUAAAUCAGUAAGAGG | |
| AD-1216941 | usasggguAfaAfUfCfaguaagaggaL96 | | VPusCfscucUfuAfCfugauUfuAfcccuascsc | | GGUAGGGUAAAUCAGUAAGAGGU | |
| AD-1216942 | asgsgguaAfaUfCfAfguaagagguaL96 | | VPusAfsccuCfuUfAfcugaUfuUfacccusasc | | GUAGGGUAAAUCAGUAAGAGGUG | |
| AD-1216944 | gsgsuaaaUfcAfGfUfaagagguguaL96 | | VPusAfscacCfuCfUfuacuGfaUfuuaccscsu | | AGGGUAAAUCAGUAAGAGGUGUU | |
| AD-1216945 | gsusaaauCfaGfUfAfagagguguuaL96 | | VPusAfsacaCfcUfCfuuacUfgAfuuuacscsc | | GGGUAAAUCAGUAAGAGGUGUUA | |
| AD-1216989 | ususaccaGfuUfGfCfcuuuuauccaL96 | | VPusGfsgauAfaAfAfggcaAfcUfgguaasasc | | GUUUACCAGUUGCCUUUUAUCCC | |
| AD-1216990 | usasccagUfuGfCfCfuuuuaucccaL96 | | VPusGfsggaUfaAfAfaggcAfaCfugguasasa | | UUUACCAGUUGCCUUUUAUCCCA | |
| AD-1217004 | usasucccAfaAfGfUfuguuguaacaL96 | | VPusGfsuuaCfaAfCfaacuUfuGfggauasasa | | UUUAUCCCAAAGUUGUUGUAACC | |
| AD-1217007 | cscscaaaGfuUfGfUfuguaaccugaL96 | | VPusCfsaggUfuAfCfaacaAfcUfuugggsasu | | AUCCCAAAGUUGUUGUAACCUGC | |
| AD-70947 | usascuguUfgGfAfUfugauucgaaaL96 | | VPusUfsucgAfaUfCfaaucCfaAfcaguasgsc | | GCUACUGUUGGAUUGAUUCGAAA | |

**Table 7. Single Dose Screens in Hep3b Cells**

| Duplex | Avg 10 nM | SD | Avg 1 nM | SD | Avg 0.1 nM | SD |
|---|---|---|---|---|---|---|
| AD-70947 | 11.5 | 1.8 | 22.32 | 1.27 | 44.29 | 2.41 |
| AD-1216613 | 16.7 | 3.2 | 22.86 | 1.17 | 45.84 | 1.90 |
| AD-1214765 | 16.0 | 2.7 | 26.93 | 7.66 | 46.32 | 4.90 |
| AD-70947 | 9.5 | 2.0 | 22.89 | 2.32 | 46.86 | 5.57 |
| AD-1216904 | 19.6 | 1.7 | 33.00 | 4.75 | 55.35 | 1.63 |
| AD-1211676 | 16.3 | 1.8 | 28.31 | 2.58 | 55.61 | 3.66 |
| AD-1214770 | 14.7 | 3.3 | 27.87 | 2.51 | 55.97 | 3.06 |
| AD-1216784 | 11.8 | 0.9 | 28.35 | 2.48 | 57.01 | 2.10 |
| AD-1211065 | 25.9 | 12.7 | 37.93 | 15.20 | 57.44 | 2.20 |
| AD-1216910 | 20.9 | 2.5 | 33.63 | 2.51 | 57.90 | 6.75 |
| AD-1211064 | 16.1 | 2.9 | 32.53 | 3.90 | 58.39 | 1.17 |
| AD-1213113 | 16.3 | 2.9 | 30.68 | 1.07 | 60.99 | 6.32 |
| AD-1216790 | 17.9 | 4.1 | 35.54 | 2.50 | 61.19 | 1.44 |
| AD-1216911 | 23.9 | 2.2 | 41.70 | 5.24 | 64.07 | 3.50 |
| AD-1216792 | 21.3 | 1.9 | 39.55 | 4.48 | 65.87 | 3.48 |
| AD-1216787 | 20.8 | 1.9 | 45.87 | 1.90 | 67.18 | 8.76 |
| AD-1212720 | 20.0 | 5.4 | 42.28 | 5.20 | 67.21 | 2.28 |
| AD-1216790 | 23.2 | 2.2 | 36.54 | 4.30 | 67.25 | 5.88 |
| AD-1216913 | 20.9 | 1.6 | 39.18 | 6.07 | 68.42 | 5.84 |
| AD-1212720 | 19.2 | 2.1 | 46.19 | 3.61 | 68.98 | 8.77 |
| AD-1216792 | 20.8 | 3.0 | 36.61 | 6.33 | 69.00 | 7.37 |
| AD-1213116 | 25.2 | 2.1 | 42.65 | 5.17 | 69.14 | 3.63 |
| AD-1214768 | 22.3 | 1.5 | 34.43 | 3.82 | 69.90 | 4.53 |
| AD-1213112 | 25.8 | 6.7 | 37.46 | 5.52 | 70.27 | 6.74 |
| AD-1216597 | 41.0 | 2.9 | 57.74 | 5.54 | 70.35 | 3.58 |
| AD-1216945 | 24.4 | 1.4 | 40.98 | 3.24 | 71.68 | 7.48 |
| AD-1216556 | 39.6 | 3.8 | 51.67 | 4.94 | 72.05 | 3.10 |
| AD-1216593 | 46.8 | 1.6 | 53.51 | 1.69 | 73.22 | 4.02 |
| AD-1211675 | 21.8 | 2.6 | 46.93 | 5.04 | 73.29 | 5.65 |
| AD-1216546 | 48.5 | 3.8 | 50.31 | 6.67 | 73.33 | 3.69 |
| AD-1210479 | 21.7 | 2.1 | 41.58 | 3.60 | 73.56 | 2.79 |
| AD-1216528 | 45.2 | 0.8 | 53.03 | 7.55 | 73.66 | 5.25 |
| AD-1213749 | 24.9 | 3.0 | 41.47 | 2.95 | 73.82 | 2.96 |
| AD-1216593 | 37.8 | 1.5 | 57.64 | 1.50 | 74.01 | 4.82 |
| AD-1213118 | 23.9 | 2.0 | 40.28 | 1.63 | 74.06 | 9.20 |
| AD-1216526 | 40.6 | 4.2 | 58.15 | 4.28 | 74.31 | 5.60 |
| AD-1216907 | 20.3 | 1.5 | 44.23 | 1.79 | 75.29 | 6.79 |
| AD-1216525 | 45.5 | 2.8 | 54.02 | 6.07 | 75.48 | 2.48 |
| AD-1216990 | 35.0 | 3.2 | 53.14 | 1.71 | 75.92 | 6.54 |
| AD-1213116 | 28.5 | 4.2 | 48.54 | 3.25 | 76.12 | 5.40 |
| AD-1216594 | 45.7 | 8.6 | 53.08 | 4.53 | 76.37 | 8.88 |

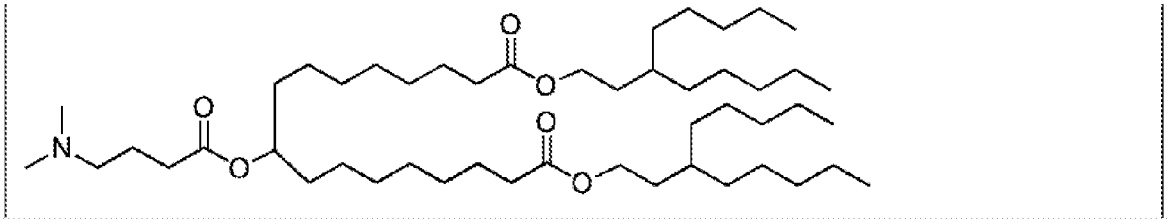
| Duplex | Avg 10 nM | SD | Avg 1 nM | SD | Avg 0.1 nM | SD |
|---|---|---|---|---|---|---|
| AD-1211677 | 18.5 | 2.2 | 37.22 | 4.04 | 77.49 | 6.22 |
| AD-1216940 | 29.4 | 5.5 | 49.40 | 3.49 | 77.55 | 4.88 |
| AD-1216595 | 45.6 | 2.2 | 59.98 | 4.33 | 78.30 | 6.20 |
| AD-1216594 | 43.7 | 2.7 | 52.17 | 5.56 | 78.38 | 9.40 |
| AD-1216131 | 29.4 | 4.5 | 40.22 | 5.08 | 78.57 | 3.24 |
| AD-1216942 | 27.5 | 5.1 | 50.47 | 2.40 | 78.95 | 7.99 |
| AD-1216559 | 47.5 | 3.5 | 58.31 | 5.29 | 79.57 | 4.34 |
| AD-1210480 | 27.4 | 1.4 | 55.25 | 6.03 | 80.03 | 7.65 |
| AD-1213115 | 21.8 | 1.8 | 50.16 | 8.38 | 80.29 | 7.30 |
| AD-1216786 | 29.8 | 3.2 | 49.96 | 7.35 | 81.03 | 9.54 |
| AD-1216895 | 26.5 | 2.6 | 52.63 | 5.98 | 81.50 | 11.93 |
| AD-1216132 | 34.0 | 5.1 | 49.52 | 2.35 | 81.50 | 3.94 |
| AD-1216596 | 43.8 | 1.7 | 60.51 | 7.52 | 81.59 | 2.06 |
| AD-1216906 | 31.6 | 2.9 | 51.31 | 7.11 | 81.65 | 4.04 |
| AD-1210732 | 27.5 | 0.7 | 55.20 | 5.63 | 82.56 | 11.46 |
| AD-1216989 | 29.2 | 2.9 | 50.63 | 3.85 | 82.99 | 5.31 |
| AD-1217004 | 29.5 | 3.0 | 53.78 | 3.96 | 83.37 | 7.09 |
| AD-1216592 | 56.6 | 2.7 | 65.11 | 5.53 | 83.40 | 5.10 |
| AD-1216941 | 39.4 | 4.4 | 62.94 | 4.12 | 83.43 | 0.29 |
| AD-1212721 | 54.0 | 6.9 | 65.65 | 8.12 | 83.48 | 3.38 |
| AD-1216561 | 48.4 | 4.4 | 63.17 | 7.36 | 83.52 | 9.05 |
| AD-1216506 | 46.0 | 1.9 | 56.58 | 2.41 | 83.91 | 3.34 |
| AD-1216596 | 47.9 | 2.8 | 61.91 | 2.53 | 84.41 | 7.38 |
| AD-1213119 | 34.2 | 2.7 | 60.89 | 5.55 | 85.22 | 6.55 |
| AD-1216560 | 52.4 | 6.1 | 60.41 | 1.71 | 85.93 | 1.24 |
| AD-1215018 | 28.4 | 3.1 | 51.14 | 6.12 | 86.11 | 2.76 |
| AD-1216595 | 54.3 | 5.3 | 63.21 | 5.04 | 86.30 | 9.38 |
| AD-1216349 | 41.1 | 2.3 | 64.12 | 7.16 | 86.49 | 5.12 |
| AD-1215017 | 42.1 | 3.4 | 61.25 | 4.57 | 86.93 | 4.89 |
| AD-1212721 | 52.4 | 2.6 | 70.04 | 5.96 | 86.98 | 5.97 |
| AD-1217007 | 41.0 | 3.4 | 61.63 | 5.54 | 87.47 | 8.88 |
| AD-1217007 | 39.5 | 3.3 | 66.47 | 2.70 | 87.52 | 4.62 |
| AD-1216350 | 42.5 | 0.8 | 65.23 | 5.55 | 87.58 | 8.36 |
| AD-1216938 | 43.4 | 5.9 | 58.25 | 4.31 | 87.74 | 1.60 |
| AD-1216944 | 47.0 | 5.4 | 64.14 | 3.95 | 87.75 | 2.03 |
| AD-1216560 | 57.5 | 2.0 | 78.55 | 10.47 | 88.32 | 5.75 |
| AD-1216345 | 28.2 | 3.2 | 54.21 | 5.91 | 88.89 | 11.42 |
| AD-1216558 | 52.0 | 2.9 | 66.25 | 12.01 | 90.56 | 3.45 |
| AD-1211582 | 40.8 | 4.1 | 68.58 | 7.37 | 90.76 | 4.63 |
| AD-1210734 | 66.0 | 8.0 | 75.91 | 5.09 | 92.38 | 5.61 |
| AD-1210734 | 56.8 | 1.9 | 83.84 | 6.72 | 93.89 | 2.46 |
| AD-1211578 | 81.4 | 6.2 | 91.70 | 1.40 | 96.01 | 0.86 |
| AD-1213117 | 41.0 | 7.4 | 65.32 | 9.19 | 97.54 | 7.35 |

| Duplex | Avg 10 nM | SD | Avg 1 nM | SD | Avg 0.1 nM | SD |
|---|---|---|---|---|---|---|
| AD-1216352 | 44.5 | 3.0 | 71.22 | 7.34 | 98.29 | 10.71 |

**Example 4. *in Vivo* Assessment of CTNNB1 siRNAs in Non-Human Primates**

Duplexes identified from the above *in vitro* analyses were assessed for their ability to inhibit CTNNB1 expression *in vivo*. Briefly, duplexes were formulated in lipid particles comprising a biodegradable lipid, *e.g.,* cationic lipid and intravenously administered to non-human primates.

In particular, duplexes AD-167990 (Negative Control), AD-1548393, AD-1548488, and AD-1548459 were combined with a cationic lipid having the structure below and DSPC/Chol/PEG-DMG in a ratio of 50:12:36:2, respectively.



At Day -10 pre-dose, percutaneous needle liver biopsies were obtained and the level of CTNNB1 mRNA was determined as described above.

At Day 0, cynomolgus monkeys were intravenously administered a single 0.1 mg/kg or 0.3 mg/kg dose of the lipid formulated duplex and at Days 5, 15, and 29, percutaneous needle liver biopsies were obtained and the level of CTNNB1 mRNA was determined as described above. The study design is provided in Table 8 below.

As shown in FIGs. 1A-1C, all three duplexes potently inhibit CTNNB1 expression at 0.1 mg/kg and 0.3 mg/kg.

**Table 8. Study Design**

| Group | siRNA | LNP | N | Dose Route | Dose Day | Dose Level (mg/kg) | Biopsies |
|---|---|---|---|---|---|---|---|
| 1 | AD-167990 (Negative Control) | 50/12/36/2 (AF-105) | 3 | IV | Day 1 | 0.3 | • Pre-dose<br>• Day 5<br>• Day 15<br>• Day 29 |
| 2 | AD-1548393.1 | AF-105 | 3 | IV | | 0.1 | |
| 3 | AD-1548393.1 | AF-105 | 3 | IV | | 0.3 | |
| 4 | AD-1548488.1 | AF-105 | 3 | IV | | 0.1 | |
| 5 | AD-1548488.1 | AF-105 | 3 | IV | | 0.3 | |
| 6 | AD-1548459.1 | AF-105 | 3 | IV | | 0.1 | |
| 7 | AD-1548459.1 | AF-105 | 3 | IV | | 0.3 | |

## 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_001904.4 Homo sapiens catenin beta 1 (CTNNB1), transcript variant 1, mRNA

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**SEQ ID NO: 2 REVERSE COMPLEMENT OF SEQ ID NO: 1**

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**SEQ ID NO: 3**

>NM_007614.3 Mus musculus catenin (cadherin associated protein), beta 1 (Ctnnb1), transcript variant 1, mRNA

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**SEQ ID NO: 4 REVERSE COMPLEMENT OF SEQ ID NO: 3**

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CTGGCCAGAATGATGAGCTTGCTCTCTTGATTGCCATAAGCTAAGATCTGAAGGCAGTCTGTTGTAATAGCCAAG
AATTTCACGTTTGTTTTGTTGAGCAAAGCAACCATTTTCTGCAGTCCACCAGCTAGGCGCACTGCCATTTTAGCT
CCTTCCTGATGGAGCAGGAGATTATGCAGTGTCGTGATGGCGTAGAACAGTACAGAATCCACTGGTGACCCAAGC
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AGAGTCCCAGCAGTACAACGAGCTGTCTCTACATCATTTGTATTCTGCATGGTGCGTACAATGGCAGACACCATC
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TGATAGTTAATCAAATTGACAACTGCATGTTTCAACATCTGTGATGGTTCAGCCAAGCGCTGGACATTAGTGGGA
TGAGCAGCGTCAAACTGCGTGGATGGGATCTGCATGCCCTCATCTAGCGTCTCAGGGAACATGGCAGCTCGGACC
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TGCTCCCATTCATAAAGGACTTGGGAGGTGTCAACATCTTCTTCCTCAGGGTTGCCCTTGCCACTCAGGGAAGGA
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**SEQ ID NO: 5**

>NM_053357.2 Rattus norvegicus catenin beta 1 (Ctnnb1), mRNA

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GTGGATACGGCCAGGACGCCTTGGGGATGGACCCTATGATGGAGCATGAGATGGGTGGCCACCACCCTGG
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**SEQ ID NO: 6 REVERSE COMPLEMENT OF SEQ ID NO: 5**

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**SEQ ID NO: 7**

>NM_001319394.1 Macaca fascicularis catenin beta 1 (CTNNB1), mRNA

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**SEQ ID NO: 8 REVERSE COMPLEMENT OF SEQ ID NO: 7**

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**SEQ ID NO: 9**

>NM_001257918.1 Macaca mulatta catenin beta 1 (CTNNB1), mRNA

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**SEQ ID NO: 10 REVERSE COMPLEMENT OF SEQ ID NO: 9**

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GCATATTAAAAAGGAAACTAATTGGACCATTTTCTATTTGTCTATTTTATACAAAAAGGCTACACAATGTTACAC
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GGAGCTCTTATAAGTCGCCCAAAAGCTGCC

**We claim:**

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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 CTNNB1, and wherein the region of complementarity comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences in any one of Tables 2, 3, 5, or 6.

2. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) 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 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequence of nucleotides 603-625, 1489-1511, or 1739-1761 of SEQ ID NO: 1, and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides, from the corresponding nucleotide sequence of SEQ ID NO:2.

3. The dsRNA agent of claim 1 or 2, wherein the the antisense strand comprises at least 15 contiguous nucleotides differing by no more than three nucleotides from any one of the antisense strand nucleotide sequences of a duplex selected from the group consisting of AD-1548393, AD-1548488, and AD-1548459.

4. The dsRNA agent of any one of claims 1-3, wherein the dsRNA agent comprises at least one modified nucleotide.

5. The dsRNA agent of any one of claims 1-4, wherein 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.

6. The dsRNA agent of any one of claims 1-5, wherein 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.

7. The dsRNA agent of any one of claims 4-6, wherein at least one of the modified nucleotides is selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxythimidine (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.

8. The dsRNA agent of any one of claims 4-6, 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, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and glycol; and combinations thereof.

9. The dsRNA agent of any one of claims 4-6, 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, a glycol modified nucleotide (GNA), a nucleotide comprising a 2' phosphate, a nucleotide comprising a phosphorothioate group, and a vinyl-phosphonate nucleotide; and combinations thereof.

10. The dsRNA agent of any one of claims 4-6, wherein at least one of the modified nucleotides is a nucleotide modified with a thermally destabilizing nucleotide modification.

11. The dsRNA agent of claim 10, 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).

12. The dsRNA agent of any one of claims 1-11, wherein the double stranded region is 19-30 nucleotide pairs in length.

13. The dsRNA agent of claim 12, wherein the double stranded region is 19-25 nucleotide pairs in length.

14. The dsRNA agent of claim 12, wherein the double stranded region is 19-23 nucleotide pairs in length.

15. The dsRNA agent of claim 12, wherein the double stranded region is 23-27 nucleotide pairs in length.

16. The dsRNA agent of claim 12, wherein the double stranded region is 21-23 nucleotide pairs in length.

17. The dsRNA agent of any one of claims 1-16, wherein each strand is independently no more than 30 nucleotides in length.

18. The dsRNA agent of any one of claims 1-17, wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length.

19. The dsRNA agent of any one of claims 1-18, wherein the region of complementarity is at least 17 nucleotides in length.

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

21. The dsRNA agent of any one of claims 1-20, wherein the region of complementarity is 19 nucleotides in length.

22. The dsRNA agent of any one of claims 1-21, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide.

23. The dsRNA agent of any one of claims 1-21, wherein at least one strand comprises a 3' overhang of at least 2 nucleotides.

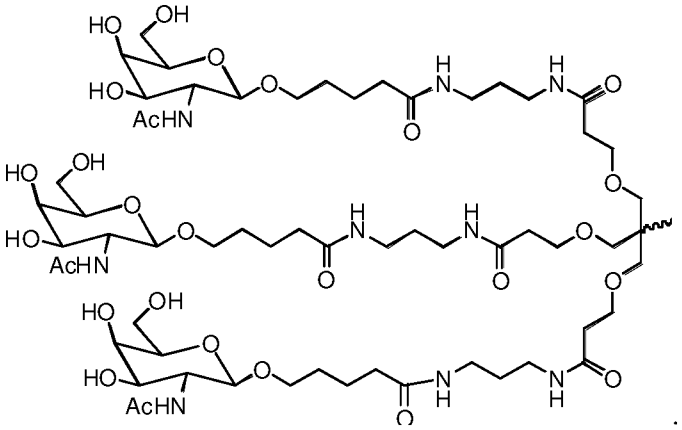
24. The dsRNA agent of any one of claims 1-23, further comprising a ligand.

25. The dsRNA agent of claim 24, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

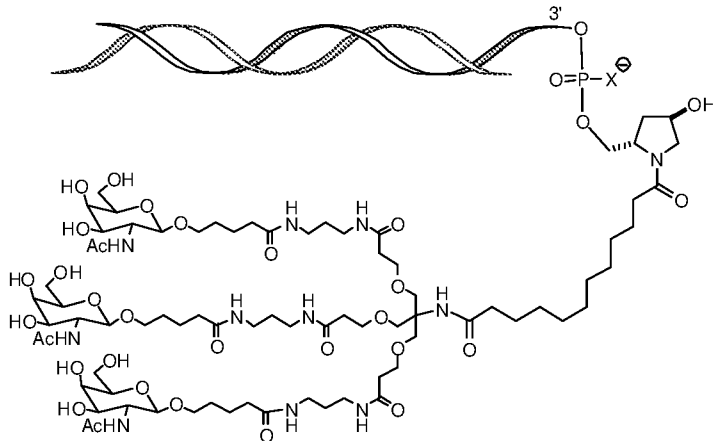
26. The dsRNA agent of claim 24 or 25, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

27. The dsRNA agent of any one of claims 24-26, wherein the ligand is one or more GalNAc derivatives attached through a monovalent, bivalent, or trivalent branched linker.

28. The dsRNA agent of claim 26 or 27, wherein the ligand is



29. The dsRNA agent of claim 28, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

30. The dsRNA agent of claim 29, wherein the X is O.

31. The dsRNA agent of any one of claims 1-30, wherein the dsRNA agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

32. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminus of one strand.

33. The dsRNA agent of claim 32, wherein the strand is the antisense strand.

34. The dsRNA agent of claim 32, wherein the strand is the sense strand.

35. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 5'-terminus of one strand.

36. The dsRNA agent of claim 35, wherein the strand is the antisense strand.

37. The dsRNA agent of claim 35, wherein the strand is the sense strand.

38. The dsRNA agent of claim 31, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at both the 5'- and 3'-terminus of one strand.

39. The dsRNA agent of claim 38, wherein the strand is the antisense strand.

40. The dsRNA agent of any one of claims 1-39, wherein the base pair at the 1 position of the 5′-end of the antisense strand of the duplex is an AU base pair.

41. The dsRNA agent of any one of claims 1-40, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

42. The dsRNA agent of any one of claims 1-40, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5'-UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprising at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

43. The dsRNA agent of claim 41 or 42, wherein the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

44. The dsRNA agent of claims 41 or 42, wherein the sense strand comprises the nucleotide sequence 5'- UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

45. The dsRNA agent of claims 44, wherein the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4 bases from the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

46. A double stranded RNA (dsRNA) agent for inhibiting expression of beta-catenin (CTNNB1) in a cell, comprising a sense strand comprising the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand comprises the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

47. The dsRNA agent of claim 46, further comprising a ligand.

48. A cell containing the dsRNA agent of any one of claims 1-47.

49. A pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49, wherein dsRNA agent is in an unbuffered solution.

51. The pharmaceutical composition of claim 50, wherein the unbuffered solution is saline or water.

52. The pharmaceutical composition of claim 49, wherein said dsRNA agent is in a buffer solution.

53. The pharmaceutical composition of claim 52, wherein the buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

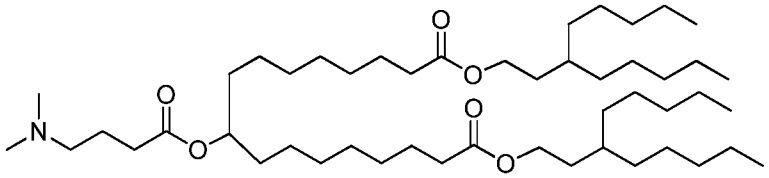
54. The pharmaceutical composition of claim 53, wherein the buffer solution is phosphate buffered saline (PBS).

55. A pharmaceutical composition for inhibiting expression of a gene encoding beta-catenin (CTNNB1) comprising the dsRNA agent of any one of claims 1-47 and a lipid.

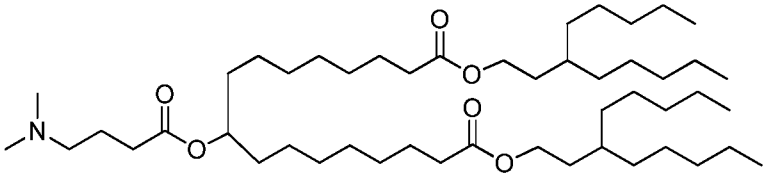
56. The pharmaceutical composition of claim 55, wherein the lipid is a cationic lipid.

57. The pharmaceutical composition of claim 56, wherein the cationic lipid comprises one or more biodegradable groups.

58. The pharmaceutical composition of claim 57, wherein the lipid comprises the structure



59. The pharmaceutical composition of claim 58, comprising

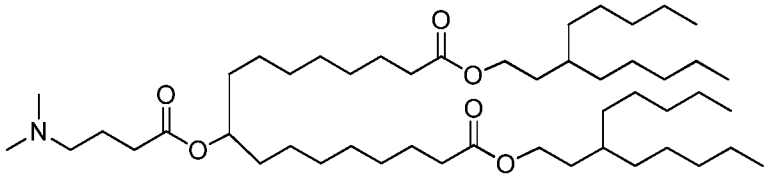
(a) ;

(b) cholesterol;

(c) DSPC; and

(d) PEG-DMG.

60. The pharmaceutical composition of claim 59, wherein the

, DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2, respectively.

61. A pharmaceutical composition comprising a dsRNA agent for inhibiting expression of a gene encoding beta-catenin (CTNNB1), comprising a sense strand and an antisense strand forming a double stranded region, wherein the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' - UTUCGAAUCAATCCAACAGUAGC -3', and a lipid.

62. The pharmaceutical composition of claim 61, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5'- UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence 5' - UTUCGAAUCAATCCAACAGUAGC -3'.

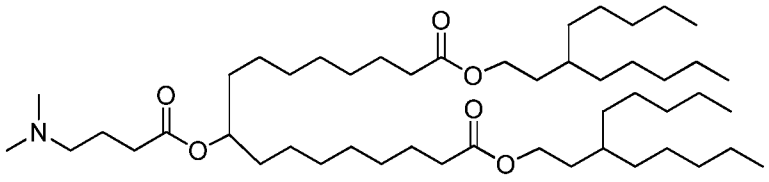
63. The pharmaceutical composition of claim 61 or 62, wherein the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

64. The pharmaceutical composition of claim 61 or 62, wherein the sense strand comprises the nucleotide sequence 5'- UACUGUUGGAUUGAUUCGAAA -3' and the antisense strand comprises the nucleotide sequence 5' -UTUCGAAUCAATCCAACAGUAGC -3'.

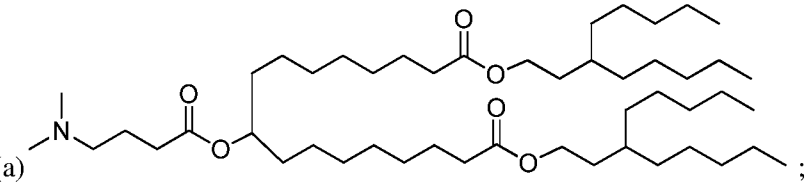
65. The pharmaceutical composition of claim 64, wherein the sense strand differs by no more than 4 bases from the nucleotide sequence 5'-usascuguugGfAfUfugauucgasasa-3' and the antisense strand differs by no more than 4 bases from the nucleotide sequence 5' -VPudTucdGadAucaadTcCfaacaguasgsc -3',

wherein a, g, c and u are 2′-O-methyl (2′-OMe) A, G, C, and U respectively; Af, Gf, Cf and Uf are 2′-fluoro A, G, C and U respectively; s is a phosphorothioate linkage; VP is a vinyl phosphonate; dT is 2`-deoxythimidine -3`-phosphate; dG is 2`-deoxyguanosine-3`-phosphate; and dA is 2`-deoxyadenosine-3`-phosphate.

66. The pharmaceutical composition of any one of claims 61-65, wherein the lipid comprises the structure



67. The pharmaceutical composition of claim 66, comprising

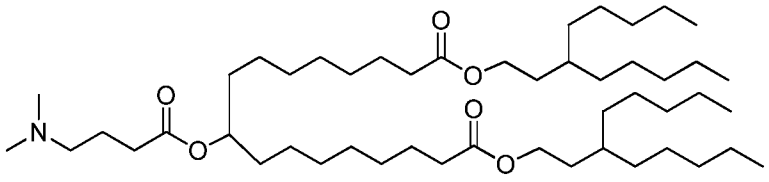
(a) ;

(b) cholesterol;

(c) DSPC; and

(d) PEG-DMG.

68. The pharmaceutical composition of claim 66, wherein the

, DSPC, cholesterol, and PEG-DMG are present in a molar ratio of 50:12:36:2 respectively.

69. A method of inhibiting expression of a beta-catenin (CTNNB1) gene in a cell, the method comprising contacting the cell with the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby inhibiting expression of the CTNNB1 gene in the cell.

70. The method of claim 69, wherein the cell is within a subject.

71. The method of claim 70, wherein the subject is a human.

72. The method of claim 70, wherein the subject has a CTNNB1-associated disorder.

73. The method of claim 72, wherein the CTNNB1-associated disorder is a cancer.

74. The method of claim 73, wherein the cancer is hepatocellular carcinoma.

75. The method of any one of claims 69-74, wherein contacting the cell with the dsRNA agent inhibits the expression of CTNNB1 by at least 50%, 60%, 70%, 80%, 90%, or 95%.

76. The method of any one of claims 69-75, wherein inhibiting expression of CTNNB1 decreases CTNNB1 protein level in serum of the subject by at least 50%, 60%, 70%, 80%, 90%, or 95%.

77. A method of treating a subject having a disorder that would benefit from reduction in beta-catenin (CTNNB1) expression, comprising administering to the subject a therapeutically effective amount of the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby treating the subject having the disorder that would benefit from reduction in CTNNB1 expression.

78. A method of preventing at least one symptom in a subject having a disorder that would benefit from reduction in beta-catenin (CTNNB1) expression, comprising administering to the subject a prophylactically effective amount of the dsRNA agent of any one of claims 1-47, or the pharmaceutical composition of any one of claims 49-68, thereby preventing at least one symptom in the subject having the disorder that would benefit from reduction in CTNNB1 expression.

79. The method of claim 77 or 78, wherein the disorder is a CTNNB1-associated disorder.

80. The method of claim 79, wherein the CTNNB1-associated disorder is a cancer.

81. The method of claim 80, wherein the cancer is hepatocellular carcinoma.

82. The method of claim 79 or 80, wherein the subject is a human.

83. The method of any one of claims 79-82, wherein administration of the dsRNA agent to the subject causes a decrease in CTNNB1 protein accumulation in the subject.

84. The method of any one of claims 79-83, wherein the dsRNA agent is administered to the subject at a dose of about 0.01 mg/kg to about 50 mg/kg.

85. The method of any one of claims 79-84, wherein the dsRNA agent is administered to the subject subcutaneously.

86. The method of any one of claims 79-84, wherein the dsRNA agent is administered to the subject intravenously.

87. The method of any one of claims 79-86, further comprising determining the level of CTNNB1 in a sample(s) from the subject.

88. The method of claim 87, wherein the level of CTNNB1 in the subject sample(s) is a CTNNB1 protein level in a blood or serum or liver tissue sample(s).

89. The method of any one of claims 79-88, further comprising administering to the subject an additional therapeutic agent for treatment of a CTNNB1-associated disorder.

90. The method of claim 89, wherein the additional therapeutic agent is selected from the group consisting of a chemotherapeutic agent, a growth inhibitory agent, an anti-angiogenesis agent, an anti-neoplastic composition and a combination of any of the foregoing.

91. A kit comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.

92. A vial comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.

93. A syringe comprising the dsRNA agent of any one of claims 1-47 or the pharmaceutical composition of any one of claims 49-68.

94. An RNA-induced silencing complex (RISC) comprising an antisense strand of any of the dsRNA agents of claims 1-47.