# COMPOSITIONS AND METHODS FOR INHIBITING CYTOCHROME P450 FAMILY 7 SUBFAMILY A MEMBER 1 (CYP7A1) EXPRESSION

## RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No. 63/568,617, filed March 22, 2024, entitled "COMPOSITIONS AND METHODS FOR INHIBITING CYTOCHROME P450 FAMILY 7 SUBFAMILY A MEMBER 1 (CYP7A1) EXPRESSION", the entire contents of which are incorporated herein by reference.

## REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

The content of the electronic sequence listing (T083370043WO00-SEQ-ZJG.xml; Size: 2,462,553 bytes; and Date of Creation: March 18, 2025) is herein incorporated by reference in its entirety.

## FIELD OF THE INVENTION

The invention relates to compositions (e.g., RNAi agent compositions) for inhibiting gene expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1).

## BACKGROUND OF INVENTION

Primary sclerosing cholangitis (PSC) is a rare progressive liver disease characterized by inflammation of the bile ducts, liver fibrosis, cirrhosis, and end-stage liver disease. Patients frequently present with concurrent inflammatory bowel disease (IBD). The risk factors and mechanisms driving pathogenesis are unknown, but include human leukocyte antigen (HLA) genetics, immune dysfunction, gut-to-liver lymphocyte homing, dysbiosis and intra-hepatic accumulation of toxic bile acids. Diagnosis is based on elevated alkaline phosphatase (ALP) and characteristic magnetic resonance cholangiography (MRC) findings (beading and stricturing of bile ducts). Fatigue and pruritus are the most common early symptoms, with jaundice, abdominal pain, fever, weight loss and signs of portal hypertension occurring in later stages. The median survival from diagnosis of PSC until liver transplantation or PSC-related death is estimated to be 13-21 years. Although it is a rare condition, with a prevalence of PSC about ~1-12 per 100,000 worldwide and approximately 80,000 patients in major markets, PSC is the fifth most common indication for liver transplantation in the US and a leading indication in other countries. Furthermore, PSC represents a very significant health burden due to increased risk of colorectal cancer in children and young adults, bacterial cholangitis and the risk of all-cause hepatopancreatobiliary malignancy, cholangiocarcinoma and premature mortality compared to patients with IBD and the general population.

There are no approved therapies for the treatment of PSC and, to date, no investigational drug that prevents progression of liver disease in PSC patients has progressed to pivotal clinical trials. Current clinical practice is limited to off-label use of ursodeoxycholic acid (UDCA), despite evidence of long-term negative outcomes of UDCA in PSC patients, and managing complications, such as dilatation of dominant strictures and early diagnosis of cholangiocarcinoma. Liver transplantation is currently the only life-extending therapy for PSC patients progressing to liver failure. However, liver transplantation does not preclude recurrence of the disease.

CYP7A1 is a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This endoplasmic reticulum membrane protein catalyzes the first reaction in the cholesterol catabolic pathway in the liver, which converts cholesterol to bile acids. This reaction is the rate limiting step and the major site of regulation of bile acid synthesis, which is the primary mechanism for the removal of cholesterol from the body.

Certain liver disorders are characterized by perturbed bile acid homeostasis. These disorders include PSC, and additional cholestatic diseases such as progressive familial intrahepatic cholestasis (PFIC) and primary biliary cholangitis (PBC), and other liver diseases such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD). Liver disorders with perturbed bile acid homeostasis may benefit from the inhibition of CYP7A1 activities.

Accordingly, there is a need in the art for compositions and methods for treating PSC and other disorders by inhibiting CYP7A1 activities.

## SUMMARY OF INVENTION

The present disclosure, in some aspects, provides RNAi agents which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a gene encoding Cytochrome P450 family 7 subfamily A member 1 (CYP7A1). The CYP7A1 RNA transcript may be within a cell, e.g., a cell within a subject, such as a human subject. Compositions comprising such RNAi agents and methods of using such (e.g., for treating a CYP7A1-associated disease) are also provided.

Some aspects of the present disclosure provide RNAi agents for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the

antisense strand comprises a region of complementarity of at least 15 nucleosides (e.g., at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleosides) to a CYP7A1 target sequence in Table 2, wherein the region of complementarity comprises a nucleobase sequence that contains no more than 3 (e.g., 0, 1, 2, or 3) mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

Some aspects of the present disclosure provide RNAi agents for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleosides (e.g., at least 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleosides) to a CYP7A1 target sequence of nucleotides 113-133, 221-241, 249-269, 290-310, 301-321, 475-495, 476-496, 504-524, 593-613, 600-620, 671-691, 779-799, 839-859, 842-862, 1003-1023, 1009-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1227, 1215-1235, 1225-1245, 1226-1246, 1235-1255, 1289-1309, 1296-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1, wherein the region of complementarity comprises a nucleobase sequence that contains no more than 3 (e.g., 0, 1, 2, or 3) mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, the region of complementarity is at least 17 nucleosides (e.g., at least 17, 18, or 19 nucleosides) in length. In some embodiments, the region of complementarity is 19-21 (e.g., 19, 20, or 21) nucleosides in length.

In some embodiments, the antisense strand comprises at least 15 consecutive nucleobases (e.g., at least 15, 16, 17, 18, 19 nucleobases) of any one of SEQ ID NOs: 777-1190. In some embodiments, the sense strand comprises at least 15 consecutive nucleobases (e.g., at least 15, 16, 17, 18, 19 nucleobases) of any one of SEQ ID NOs: 393-776.

In some embodiments, the sense strand is 21 nucleosides in length and the antisense strand is 23 nucleosides in length, optionally wherein the RNAi agent comprises a 3' overhang of at least 1 nucleoside (e.g., 1, 2, or 3) on a least one strand (e.g., on the sense and/or antisense strand).

In some embodiments, the antisense strand comprises nucleobases 2-21 (counting 5'→3') of any one of SEQ ID NOs: 777-1190 and the sense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776. In some embodiments, the antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190 and the sense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776.

In some embodiments, the RNAi agent comprises the nucleobases sequences of an siRNA selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272'.

In some embodiments, the antisense strand comprises nucleobases 2-21 (counting 5'→3') of a nucleobase sequence selected from (5'→3'):

(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)

(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)

(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)

(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)

(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)

(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)

(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)

(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)

(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)

(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)

(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and

(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

In some embodiments, the antisense strand comprises a nucleobase sequence selected from (5'→3'):

(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)

(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)

(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)

(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)

(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)

(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)

(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)

(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)

(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)

(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)

(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and

(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

In some embodiments, the sense strand comprises a nucleobase sequence selected from (5' →3'):

(i) UCUUGAGUUCCUCAGAGCAAA (SEQ ID NO: 419);

(ii) GCGCAUGUUUCUCAAUGACAA (SEQ ID NO: 517);

(iii) UAGCUCUUUACCCACAGUUAA (SEQ ID NO: 597);

(iv) ACUGAAUGACCUGCCAGUAUA (SEQ ID NO: 566);

(v) UGUCCAUUUCAUCACAAAUCA (SEQ ID NO: 439);

(vi) CCCACAGUUAAUGCACUUAGA (SEQ ID NO: 604);

(vii) AGCUCUUUACCCACAGUUAAA (SEQ ID NO: 598);

(viii) UUGUCUAUGGCUUAUUCUUGA (SEQ ID NO: 404);

(ix) CCUUGAAUUCCCUCACGGAAA (SEQ ID NO: 464);

(x) UCACAAAUCCCUUGUCAUACA (SEQ ID NO: 443);

(xi) AUGUUUGAAGCUGGGUAUUUA (SEQ ID NO: 493); and

(xii) GAAAACCUCCAACGUAUCAUA (SEQ ID NO: 473).

In some embodiments, the RNAi agent comprises one or more (e.g., any integers between 1-45) modified nucleosides. In some embodiments, each nucleoside of the antisense strand is a modified nucleoside and each nucleoside of the sense strand is a modified nucleoside. In some embodiments, the one or more modified nucleosides are 2' modified nucleosides. In some embodiments, the 2'-modified nucleoside is selected from 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside and combinations thereof. In some embodiments, the 2'-modified nucleoside is selected from a 2'-O-methyl (2'-O-Me) modified nucleoside, a 2'-fluoro (2'-F) modified nucleoside, 2'-MOE modified nucleoside, and any combinations thereof. In some embodiments, each nucleoside of the antisense strand is selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside, and each nucleoside of the sense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5'→3') of the sense strand are nucleosides with the same 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside, or 2'-deoxy

nucleoside and the nucleosides at positions 8 or 13 (counting 5'→3') are nucleosides with different 2' chemistry in the sugar moiety including, e.g., unmodified nucleosides, i.e., 2'-hydroxy nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and the nucleosides at positions 8 and 13 (counting 5'→3') have a different 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside or 2'-deoxy nucleoside.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 8 and 13 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the sense strand that are not 2'-F modified nucleosides (e.g., nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand) are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the sense strand that are not 2'-F modified nucleosides (e.g., nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand) are 2'-O-Me modified nucleosides.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides positions 2 and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, and one or more of positions 5, 6, and 8 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides and the nucleosides at other positions are not 2'-F modified nucleoside, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides,

2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides and all that are not 2'-F modified are 2'-O-Me modified nucleoside.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at one or more positions at 1, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 of the antisense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides positions at one or more positions 1, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides positions at 1, 4, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 of the antisense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides positions at 1, 4, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, the RNAi agent comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) modified internucleoside linkages. In some embodiments, the RNAi agent comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more), phosphorothioate internucleoside linkages in at least one strand (e.g., sense strand and/or antisense strand).

In some embodiments, the RNAi agent comprises two phosphorothioate internucleoside linkages in the sense strand, optionally wherein the two phosphorothioate internucleoside linkages are the first two internucleoside linkages in the sense strand from 5'→3'. In some embodiments, the RNAi agent comprises four phosphorothioate internucleoside linkages in the antisense strand, wherein the four phosphorothioate internucleoside linkages are the first two internucleoside linkages and the last two internucleoside linkages in the antisense strand from 5'→3'.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all

nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, the RNAi agent comprises an antisense strand and a sense strand, each comprising a structure as provided in Table 5B, Table 7B, or Table 9. In some embodiments, the RNAi agent comprises an antisense strand comprising a structure as set forth in any one of SEQ ID NOs: 1605-2054. In some embodiments, the RNAi agent comprises a sense strand comprising a structure as set forth in any one of SEQ ID NOs: 1191-1604. In some embodiments, the RNAi agent comprises an antisense strand comprising a structure as

set forth in any one of SEQ ID NOs: 1605-2054 and a sense strand comprising a structure as set forth in any one of SEQ ID NOs: 1191-1604. In some embodiments, the CYP7A1 RNAi agent is selected from any one of the siRNAs listed in Tables 5B, 7B, and 9.

In some embodiments, the RNAi agent comprises:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure (5'→3') of [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;

fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and

s is a phosphorothioate linkage; and/or

a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure (5'→3') of [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;

fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and

s is a phosphorothioate linkage.

In some embodiments, the RNAi agent comprises:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure (5'→3') of [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;

fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and

s is a phosphorothioate linkage; and/or

a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure (5'→3') of
[mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

In some embodiments, the RNAi agent comprises:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5'→3') of
[mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage; and/or

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5'→3') of
[mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

In some embodiments, the RNAi agent comprises:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure (5'→3') of

[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;

fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and

s is a phosphorothioate linkage; and/or

a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure (5'→3') of

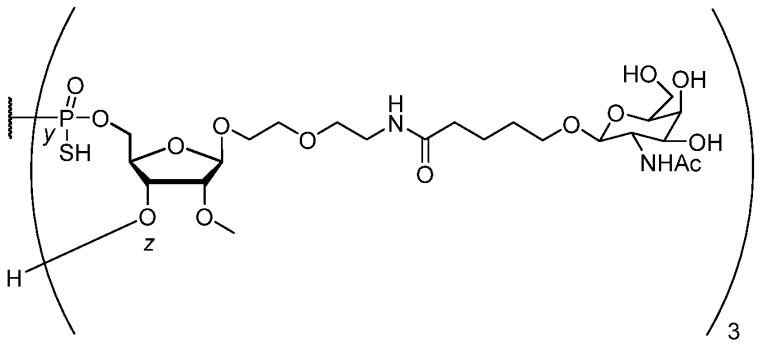
[mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;

fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and

s is a phosphorothioate linkage.

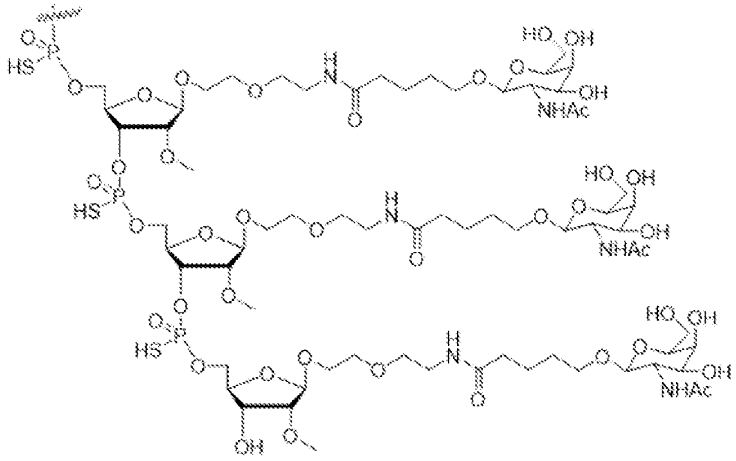
In some embodiments, the RNAi agent further comprises a targeting moiety. In some embodiments, the targeting moiety is conjugated to the 3' end of the sense strand of the RNAi agent. In some embodiments, the targeting moiety comprises N-acetyl-galactosamine (GalNAc). In some embodiments, the targeting moiety comprises a GalNAc trimer. In some embodiments, the targeting moiety comprises one or more instances of GalNAc attached through a monovalent, bivalent, trivalent, or tetravalent branched linker. In some embodiments, the targeting moiety comprises a structure of Formula ($Z^6$):



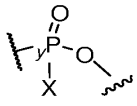
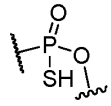

($Z^6$);

or a pharmaceutically acceptable salt thereof, wherein the ✂ indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the

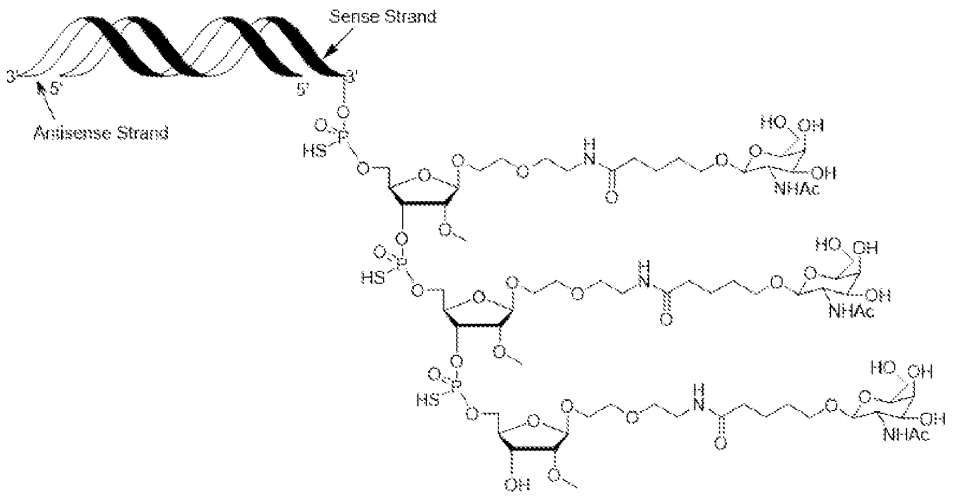
sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled *z* is bound to the phosphorous labeled *y* of the adjacent unit. In some embodiments, the targeting moiety comprises a structure of formula:



, or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand.

It is to be understood that in an RNAi agent (e.g., CYP7A1 RNAi agent) that further comprises a targeting moiety conjugated to the 3' terminal nucleoside of the sense strand, the 3'-terminal nucleoside is linked to the targeting moiety via a phosphorothioate linkage. As such, when the 3'-terminal nucleoside of the sense strand is represented as [mAs] (e.g., the sense strands of the siRNAs provided in Table 9 and elsewhere in the present disclosure), the "s" corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or ($Z^1$)-($Z^{16}$).

In some embodiments, the resulting conjugate comprises a structure of:




,

or a pharmaceutically acceptable salt thereof.

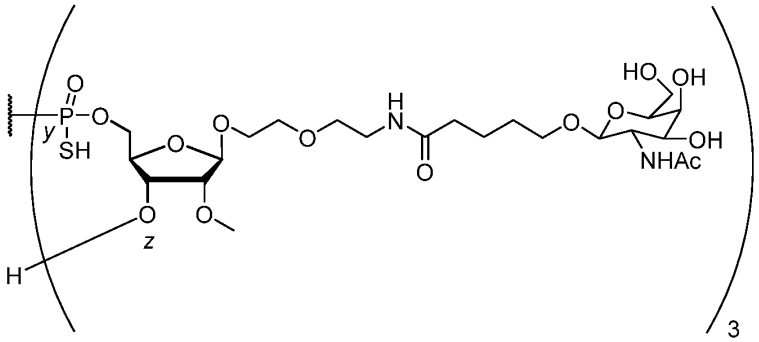
Further provided herein is an RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure (5' → 3') of [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016); and

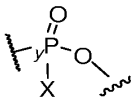
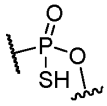
a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure (5' → 3') of [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



$_3$ ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

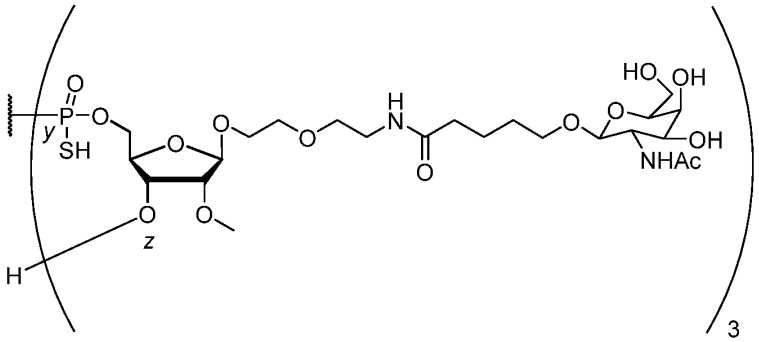
Further provided herein is an RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure (5' → 3') of [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020); and

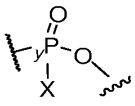
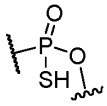
a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure (5' → 3') of [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



$(Z^6)$,

or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula $(Z^6)$.

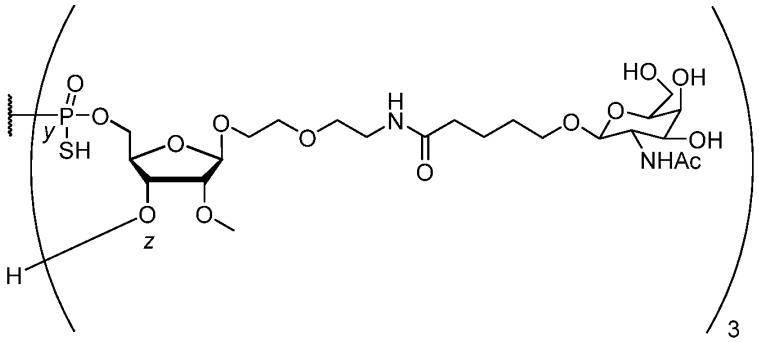
Further provided herein is an RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029); and

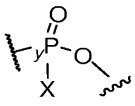
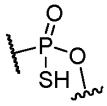
a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula $(Z^6)$



$_3$ ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

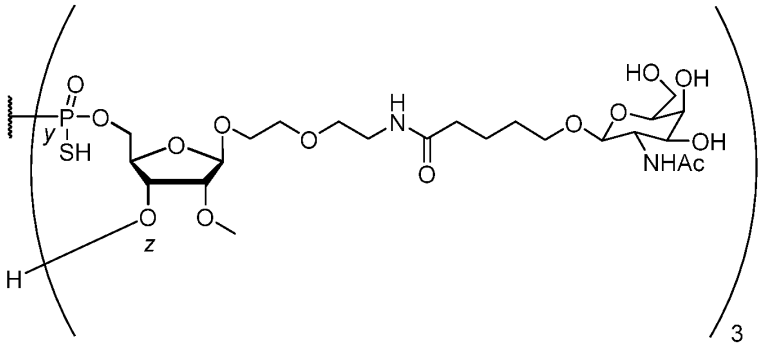
Further provided herein is an RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure (5' → 3') of [mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033); and

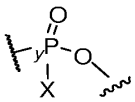
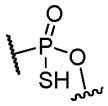
a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure (5' → 3') of [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



$_3$ ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

Other aspects of the present disclosure provide pharmaceutical composition for inhibiting expression of a gene encoding CYP7A1 comprising the RNAi agent described herein.

Other aspects of the present disclosure provide methods of inhibiting expression of a CYP7A1 gene in a cell, the method comprising contacting the cell with the RNAi agent or the pharmaceutical composition provided herein, thereby inhibiting expression of the CYP7A1 gene in the cell. In some embodiments, the cell is within a subject, optionally wherein the subject is human. In some embodiments, the subject has a CYP7A1 disease or CYP7A1-associated disease.

Other aspects of the present disclosure provide methods of treating a subject having a CYP7A1 disease or CYP7A1-associated disease, comprising administering to the subject a therapeutically effective amount of the RNAi agent or the pharmaceutical composition provided herein, thereby treating the subject having the CYP7A1 disease or CYP7A1-associated disease.

Other aspects of the present disclosure provide methods of treating at least one

symptom in a subject having a CYP7A1 disease or CYP7A1-associated disease, comprising administering to the subject an effective amount of the RNAi agent or the pharmaceutical composition provided herein, thereby treating at least one symptom in the subject having the CYP7A1 disease or CYP7A1-associated disease, optionally wherein the CYP7A1 disease or CYP7A1-associated disease is a liver disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a cholestatic liver diseases such as primary sclerosing cholangitis (PSC). In some embodiments, the subject is human. In some embodiments, the method further comprises administering an agent for the treatment of a CYP7A1 disease or CYP7A1-associated disease.

## BRIEF DESCRIPTION OF DRAWINGS

The foregoing and other features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of embodiments when read together with the accompanying drawings, in which:

FIG. 1 is a schematic that illustrates 4 modification patterns (MOD1, MOD2, MOD3, and MOD4) used in siRNA synthesized for *in vivo* study. As used in FIG. 1 , mN = a 2'-O-methyl (2'-O-Me) modified nucleoside, fN = a 2'-fluoro (2'-F) modified nucleoside, The bolded "|" between two nucleosides means phosphorothioate internucleoside linkage. Chemically modified siRNAs having the modification patterns illustrated in FIG. 1 may further be conjugated to targeting moieties (e.g., targeting moieties comprising GalNAc). For example, the targeting moiety having a structure of Formula ($Z^6$) may be conjugated to the 3' end nucleoside of the sense strand of such an siRNA.

## DETAILED DESCRIPTION OF INVENTION

Unless otherwise defined herein, scientific and technical terms used herein have the meanings that are commonly understood by those of ordinary skill in the art. In the event of any latent ambiguity, definitions provided herein take precedence over any dictionary or extrinsic definition. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. The use of "or" means "and/or" unless stated otherwise. The use of the term "including", as well as other forms of the term, is not limiting.

Generally, nomenclatures used in connection with cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The

methods and techniques provided herein are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as otherwise described herein. The nomenclatures, laboratory procedures and techniques of analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery, and treatment of patients.

Any of the methods for gene therapy available in the art can be used in the methods provided herein. For general reviews of the methods of gene therapy, see Goldspiel et al. (1993) Clin. Pharmacy 12:488-505; Wu and Wu (1991) Biotherapy 3:87-95; Tolstoshev (1993) Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan (1993) Science 260:926- 932; Morgan and Anderson (1993) Ann. Rev. Biochem. 62:191-217; and May (1993) TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy are disclosed in US Patent Publication No. US20050042664.

**Definitions**

In order that the present disclosure may be more readily understood, certain terms are first defined. Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition.

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 part of this disclosure.

As used herein, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly stated otherwise. The terms "comprising, "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited

to") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value recited or falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited.

The term "about" or "approximately," as applied to one or more values provided herein, refers to a value that is similar to a stated reference value. In some embodiments, the term "about" or "approximately" refers to a range of values that fall within and include 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context. In some embodiments, "about" or "approximately" can be understood as about 2 standard deviations from the mean. In some embodiments, "about" or "approximately" means up to and including ±10% (e.g., ±10%, ±9%, ±8%, ±7%, ±6%, ±5%, ±4%, ±3%, ±2%, ±1%, or less). In some embodiments, "about" or "approximately" means ±5%. When "about" or "approximately" is present before a series of numbers or a range, it is understood that it can modify each of the numbers in the series or range.

The term "administering" or "administration of," as used herein, means to provide an agent, e.g., RNAi agent or conjugate to a subject. In some embodiments, "administering" or "administration of " means to provide an RNAi agent or conjugate to a subject in a manner that is physiologically and/or (e.g., and) pharmacologically useful (e.g., to treat a condition in the subject). Non-limiting examples of routes of administration include intravenous, intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal routes. In some embodiments, the route of administration is subcutaneous.

The term "antisense strand" or "guide strand," as used herein, refers to a single stranded nucleic acid molecule, which is one strand of a double stranded RNAi molecule, and which comprises a region of complementarity to a target sequence (e.g., a target gene sequence, RNA sequence, or mRNA sequence). The antisense strand may contain modified nucleosides with base analogs and is not necessarily 100% complementary over its entire length to the target sequence, but must at least be sufficiently complementary to hybridize with a target RNA and result in RNA interference.

The term "at least" 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 or nucleosides 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.

The term "biological activity" means any biological property of a molecule, whether present naturally *in vivo*, or provided or enabled by recombinant means. Biological activities include, but are not limited to, binding to a receptor, inducing cell proliferation, inhibiting cell growth, inducing other cytokines, inducing apoptosis, and enzymatic activity.

"Blunt" or "blunt end" means that there are no unpaired nucleosides at that end of a double stranded RNAi agent, i.e., no nucleoside overhang. A "blunt ended" double stranded RNAi agent is double stranded over its entire length, i.e., no nucleoside overhang at either end of the molecule. The RNAi agents of the disclosure include RNAi agents with no nucleoside overhang at one end (i.e., agents with one overhang and one blunt end) or with no nucleoside overhangs at either end.

The term "complementary," as used herein, refers to the capacity for base pairing between two nucleobases or two nucleobase sequences. In particular, complementary is a term that characterizes an extent of hydrogen bond pairing that brings about binding between two nucleobases or two nucleobase sequences. For example, if a base at one position of a nucleobase sequence (e.g., antisense strand of an RNAi agent) is capable of hydrogen bonding with a base at the corresponding position of another nucleobase sequence (e.g., RNAi agent sense strand or target mRNA), then the bases are considered to be complementary to each other at that position. The nucleic acid molecules (e.g., antisense strand and sense strand of an RNAi agent) whose nucleobase sequences are complementary may comprise one or more modified nucleosides and modified internucleoside linkages, which do not affect the capacity of base paring between the nucleobases and do not affect the "complementarity" between two nucleobase sequences. The nucleic acid molecules (e.g., antisense strand and sense strand of an RNAi agent) whose nucleobase sequences are complementary may also comprise nucleobase analogous that result in bases at certain positions not being complementary, but the nucleobase sequences of the two molecules must be sufficiently complementary over the entire length to result in a desired biological activity (e.g., RNA interference).

Complementary sequences include Watson-Crick base pairs or non-Watson-Crick base pairs (e.g., Wobble base pairs and Hoogsteen base pairs) and may include natural or modified nucleosides or nucleoside mimics. For example, in some embodiments, for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as 3-nitropyrrole or 5-nitroindole can hybridize to and are

considered complementary to any A, C, U, or T. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U or T.

Complementarity is independent of modifications in the sugar of a nucleoside. For example, 2'-modified A, as defined herein, are complementary to U (or T) and identical to A for the purposes of determining identity or complementarity.

The term "perfectly complementary" or "fully complementary" means that all (100%) of the nucleobases, nucleosides, or nucleotides in a contiguous sequence of a first nucleotide sequence (e.g., antisense strand of an RNAi agent) will hybridize with the same number of nucleobases, nucleosides, or nucleotides in a contiguous sequence of second nucleotide sequence (e.g., RNAi agent sense strand or target mRNA). The contiguous sequence may comprise all or a part of a first or second nucleotide sequence. The term "partially complementary" means that in a hybridized pair of nucleobase, nucleosides, or nucleotide sequences, at least 70%, but not all, of the bases in a contiguous sequence of a first nucleotide sequence (e.g., antisense strand of an RNAi agent) will hybridize with the same number of bases in a contiguous sequence of a second nucleotide sequence (e.g., RNAi agent sense strand or target mRNA). The term "sufficiently complementary" or "substantially complementary" means that in a hybridized pair of nucleobase, nucleosides, or nucleotide sequences, at least 85%, but not all, of the bases in a contiguous sequence of a first nucleotide sequence (e.g., antisense strand of an RNAi agent) will hybridize with the same number of bases in a contiguous sequence of a second nucleotide sequence (e.g., RNAi agent sense strand or target mRNA). The terms "complementary," "fully complementary," "partially complementary," and "sufficiently/substantially complementary" herein are used with respect to the nucleobase, nucleosides, or nucleotide matching between the sense strand and the antisense strand of an RNAi agent, or between the antisense strand of an RNAi agent and a target mRNA sequence (e.g., CYP7A1 mRNA).

The term "conjugate," as used herein, refers to an RNAi agent described herein (e.g., a CYP7A1 RNAi agent) linked (e.g., covalently linked) to a conjugate group (e.g., a targeting moiety). In general, conjugate groups modify one or more properties of the RNAi agent to which they are attached, including, but not limited to pharmacodynamic, pharmacokinetic, binding, absorption, cellular distribution, cellular uptake, charge and/or clearance properties.

The term "contiguous" in the context of an oligonucleotide (e.g., RNAi agent) refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other in a sequence.

The term "control" or "reference," when referring to a substance, means a composition used as a standard or a point of comparison against which other test results are measured. In some embodiments, a "control" or "reference" is a composition known to not contain analyte ("negative control") or to contain analyte ("positive control"). A positive control can comprise a known concentration of analyte. "Control," and "positive control," may be used to refer to a composition comprising a known concentration of analyte. A "positive control" can be used to establish assay performance characteristics and is a useful indicator of the integrity of reagents (e.g., analytes). In some embodiments, an appropriate "control" or "reference" is where only one element is changed in order to determine the effect of the one element. In some embodiments, a control is a level of a target gene (e.g., in a cell or in a subject) before treatment (e.g., with an RNAi agent described herein).

The term "control" or "reference" also means a baseline level of a measurement depending upon the context, in which the term is used. A baseline level of a measurement is a standard or a point of comparison against which the measurement is compared. In some embodiments, a "control" or a "reference" refers to a level of a measurement for certain biological activity or substance in a cell, a tissue, an organ, or a subject, e.g., the expression level of a gene, copy number of mRNA for such gene, or level of protein encoded by such gene, without treatment of the cell, the tissue, the organ, or the subject, with an agent, e.g., an RNAi agent. In some embodiments, a "control" or a "reference" refers to a level of an average measurement for a certain biological activity or substance in a cell, a tissue, an organ, or a subject, e.g., certain enzyme activity of the liver, among a group of healthy subjects, e.g., the general population within in certain geographic or demographic limits or any other limits that may be appropriate for the study of certain disease or disorder, that does not have certain disease or disorder, e.g., liver disease.

The term "reference" may also be used in "reference sequence." The term "reference sequence" refers to a sequence, e.g., a nucleic acid sequence or an amino acid sequence, used as a basis for sequence comparison. In certain embodiments, a reference sequence is the mRNA sequence, e.g., human CYP7A1 mRNA sequence (NM_000780.4, SEQ ID NO: 1), upon which the design of the siRNA is based.

The term "cross-reactive" means the ability of a binding molecule (e.g., an RNAi agent) to bind a target molecule (e.g., an mRNA) other than that against which it was designed or generated. For example, the binding molecule is capable of specifically binding to more than one target molecule of a similar type or class (e.g., mRNA variants or mRNA homologus from closely related species) with similar affinity. Generally, a binding molecule will bind its

target molecule with an appropriately high affinity, but can bind to the same target molecule of another species or display a low affinity for non-target molecules. In some embodiments, an RNAi agent that is cross-reactive against human and non-human primate CYP7A1 comprises an antisense strand comprising a region of complementarity to human and non-human primate CYP7A1 mRNA, and/or inhibits the expression of human and non-human primate CYP7A1. Individual binding molecules are generally selected to meet two criteria: (1) tissue staining appropriate for the known expression of the target or (2) similar staining pattern between human and tox species (mouse and cynomolgus monkey) tissues from the same organ. These and other methods of assessing cross-reactivity are known to one skilled in the art.

The term "effective amount" or "therapeutically effective amount," as used herein, refers to that amount of an RNAi agent to produce a molecular (e.g., reduced expression of CYP7A1), biological (e.g., reduced accumulation of toxic bile acids), pharmacological, therapeutic (e.g., treatment of a CYP7A1 associated disease), or preventive result. The amount administered will likely depend on such variables as the overall health status of the patient, the relative biological efficacy of the compound delivered, the formulation of the drug, the presence and types of excipients in the formulation, and the route of administration. Also, it is to be understood that the initial dosage administered can, in some instances, be increased beyond the above upper level to rapidly achieve the desired blood-level or tissue level, or the initial dosage can, in some instances, be smaller than the optimum.

The term "GalNAc" refers to N-Acetylgalactosamine (GalNAc), which is a monosaccharide and amino sugar derivative of galactose. GalNAc may also be referred to in the art as 2-(Acetylamino)-2-deoxy-D-galactopyranose, 2-(Acetylamino)-2-deoxy-D-galactose, N-Acetylchondrosamine, and N-Acetyl-D-galactosamine. Galactose derivatives such as GalNAc have been used to target molecules to hepatocytes *in vivo* through their binding to the asialoglycoprotein receptor expressed on the surface of hepatocytes. Binding of asialoglycoprotein receptor ligands to the asialoglycoprotein receptor(s) facilitates cell-specific targeting to target cells (e.g., hepatocytes) and endocytosis of the molecule into the target cells (e.g., hepatocytes). In some embodiments, any one of the targeting moieties described herein includes an asialoglycoprotein receptor ligand comprising GalNAc. In some embodiments, the asialoglycoprotein receptor ligand comprises a GalNAc trimer. Asialoglycoprotein receptor ligands can be monomeric (e.g., having a single GalNAc) or multimeric (e.g., having multiple GalNAcs). The targeting moiety may comprise one or more GalNAcs attached to the 3' or 5' end of the sense or antisense strand of the RNAi agent using methods known in the art. In some embodiments, the targeting moiety comprises one or more (e.g., 1, 2, 3, 4, or more)

GalNAc, each of which are linked via phosphorothioate linkages. GalNAc targeting moieties, which comprise one or more GalNAc, have been described, for example, in the following references:

| Reference No. | Application No. | Filing Date |
|---|---|---|
| US 8,106,022 | US 12/328,528 | Dec. 4, 2008 |
| US 10,246,709 | US 15/452,423 | Mar. 7, 2017 |
| US 5,994,517 | US 08/755,062 | Nov. 22, 1996 |
| US 6,906,182 | US 09/998,497 | Nov. 30, 2001 |
| US 10,294,474 | US 15/452,324 | Mar. 7, 2017 |
| US 2017305956 | US 15/621,395 | Jun. 13, 2017 |
| US 10,233,448 | US 15/504,855 | Feb. 17, 2017 |
| US 2016122761 | US 14/898,873 | Jun. 23, 2014 |
| US 10,808,246 | US 14/901,945 | Dec. 29, 2015 |
| WO 2022/159158A1 | PCT/US2021/057016 | Oct. 28, 2021 |

The disclosures in these references related to GalNAc are hereby incorporated herein by reference.

The terms “hybridize” and “hybridization” refer to the pairing of complementary compounds (e.g., an RNAi agent and its target nucleic acid). While not limited to a particular mechanism, the most common mechanism of pairing involves hydrogen bonding, which may be Watson-Crick, Wobble, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

The term “internucleoside linkage,” as used herein, means a covalent linkage between adjacent nucleosides in an oligonucleotide (e.g., RNAi agent such as a CYP7A1 RNAi agent described herein). An internucleoside linkage may be a natural phosphodiester internucleoside linkage, or may be a modified (non-natural) internucleoside linkage. Modified internucleoside that may be used in an RNAi agent disclosed herein include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3’alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3’-amino phosphoramidate and aminoalkylphosphoramidates, mesyl phosphoramidates, 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’; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 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; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

The term "linker" in the context of a conjugate refers to a bond (e.g., covalent bond) or chemical moiety between two components of the conjugate, e.g., between an RNAi agent and a targeting moiety. In some embodiments, the linker facilitates covalent linkage of an RNAi agent to a targeting moiety. In some embodiments, the linker is conjugated to the 5' or 3'end of the sense strand or antisense strand of an RNAi agent. In some embodiments, the linker is conjugated to the 5' or 3' end the sense strand of an RNAi agent. In some embodiments, a linker is conjugated to the 5' end of the sense strand of an RNAi agent. In some embodiments, a linker is conjugated to the 3' end of the sense strand of an RNAi agent. The linker can be any suitable group for coupling the RNAi agent to the ligand. In some embodiments, the linker is a monovalent, bivalent, trivalent, or tetravalent branched linker. In some embodiments, the linker is a phosphorus-containing linker (e.g., phosphate, phosphodiester, phosphorothioate, phosphorodithioate, phosphoroamidate, etc), or other linker. Non-limiting examples of other linkers can include, but are not limited to: reactive groups such a primary amines and alkynes, alkyl groups, abasic nucleotides, ribitol (abasic ribose), and/or PEG groups. In some embodiments, the linker is a phosphorothioate linkage.

The term, "nucleoside," as used herein, refers to a compound comprising a nucleobase moiety and a sugar moiety. Nucleosides include, but are not limited to, naturally occurring nucleosides (as found in DNA and RNA) and modified nucleosides. Nucleosides may be linked to a phosphate moiety. The term "nucleoside" encompasses a natural nucleoside and chemically modified nucleosides (e.g., with modifications in the base and/or sugar moiety).

The term "nucleotide," as used herein, refers to a compound comprising a nucleoside linked to a phosphate group. As used herein, "linked nucleosides" may or may not be linked by phosphate linkages and thus includes, but is not limited to "linked nucleotides." As used herein, "linked nucleosides" are nucleosides that are connected in a continuous sequence (i.e., no additional nucleosides are present between those that are linked). The term "nucleotide" encompasses a natural nucleotide and chemically modified nucleotides (e.g., with modifications in the base, sugar moiety, and/or phosphate group).

The term "nucleobase," as used herein, refers to nitrogen-containing compounds that can be linked to a sugar moiety to create a nucleoside that is capable of incorporation into an oligonucleotide, and wherein the compound is capable of bonding with a complementary naturally occurring nucleobase of another oligonucleotide or nucleic acid. Nucleobases may be naturally occurring or may be modified. As used herein a "naturally occurring nucleobase"

is adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G). The term "nucleobase" encompasses 5'-methylated bases (e.g., 5'-methyl cytosine or 5'-methyl guanine).

The term "modified internucleoside linkage" refers to a linkage between two nucleosides (e.g., in an oligonucleotide or in a strand of an RNAi agent) that is not the natural phosphodiester linkage. Non-limiting examples of modified internucleoside linkages include phosphorothioates, phosphorodiamidates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages.

The term "nucleoside modification" or "modified nucleoside" means a nucleoside that has one or more modifications to the nucleoside, including modifications to the nucleobase moiety and/or the sugar moiety. Any of the modified chemistries or formats of nucleosides described herein can be combined with each other. Non-limiting examples of modified nucleosides includes 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA), locked nucleic acid (LNA, methylene-bridged nucleic acid), unlocked nucleic acid (UNA), ethylene-bridged nucleic acid (ENA), and (S)-constrained ethyl-bridged nucleic acid (cEt) modified nucleosides. Further non-limiting examples of modified nucleosides include a conformationally restricted nucleoside, an abasic nucleoside, a 2'-amino-modified nucleoside, a morpholino nucleoside, a phosphoramidate, a non-natural base comprising nucleoside, a tetrahydropyran modified nucleoside, a 1,5-anhydrohexitol modified nucleoside (HNA), a cyclohexenyl modified nucleoside (CeNA), a nucleoside comprising a phosphorothioate group, a nucleoside comprising a methylphosphonate group, a nucleoside comprising a 5'-phosphate, a nucleoside comprising a 5'-phosphate mimic, a thermally destabilizing nucleoside, a glycol modified nucleoside (GNA).

The term "2'-modified nucleoside" refers to a nucleoside having a sugar moiety modified at the 2' position, meaning the sugar moiety comprises at least one 2'-substituent group other than H or OH. Non-limiting examples of 2'-modified nucleosides include: 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-deoxy, 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleosides. In some embodiments, any one of the 2'-modified nucleosides described herein are high-affinity modified nucleosides and a modified RNAi agent has increased affinity to target sequences, relative to an unmodified

RNAi agent. In some embodiments, at least one modified nucleoside is a 2' modified nucleoside. In some embodiments the 2' modified nucleoside is a 2'-O-methyl (2'-O-Me) modified nucleoside or a 2'-fluoro (2'-F) modified nucleoside or combinations thereof.

The term "modified oligonucleotide" or "modified RNAi agent" or "modified siRNA" refers to oligonucleotides, modified RNAi agents, or modified siRNAs that comprise one or more modified nucleosides and/or one or more modified internucleoside linkages. In some embodiments, a "modified oligonucleotide" or "modified RNAi agent" or "modified siRNA" comprises a mix of modified nucleosides and unmodified nucleosides and/or a mix of modified internucleoside linkages and unmodified modified internucleoside linkages. In some embodiments, each nucleoside of a modified oligonucleotide" or "modified RNAi agent" or "modified siRNA" is a modified nucleoside, and/or each internucleoside linkage of a modified oligonucleotide" or "modified RNAi agent" or "modified siRNA" is a modified internucleoside linkage.

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

In some embodiments, at least one strand of an RNAi agent comprises a 3' overhang of at least 1 nucleoside. In some embodiments, at least one strand comprises a 3' overhang of at least 2 nucleosides, e.g., 2, 3, 4, 5, etc. nucleosides. In some embodiments, at least one strand of an RNAi agent comprises a 5' overhang of at least 1 nucleoside. In some embodiments, at least one strand comprises a 5' overhang of at least 2 nucleosides, e.g., 2, 3, 4, 5, etc. nucleosides. In some embodiments, both the 3' and the 5' end of one strand of an RNAi agent comprise an overhang of at least 1 nucleoside.

In some embodiments, the antisense strand of an RNAi agent has a 1-10 nucleoside, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleoside, overhang at the 3'-end or the 5'-end. In some embodiments, the sense strand of an RNAi agent has a 1-10 nucleoside, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleoside, overhang at the 3'-end or the 5'-end. In another embodiment, one or more of the nucleosides in the overhang is replaced with a nucleoside thiophosphate.

In some embodiments, the antisense strand of an RNAi agent has a 1-10 nucleosides, e.g., a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleoside, overhang at the 3'-end or the 5'-end. In some embodiments, the antisense strand of an RNAi agent has a 1-3 nucleosides, e.g., a 1, 2, or 3 nucleoside, overhang at the 3'-end.

In some embodiments, the nucleosides in an overhang that is immediately adjacent to the duplex region is linked to the adjacent nucleoside in the duplex region via a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. In some embodiments, the overhang comprises two or more nucleosides and the nucleosides in an overhang are linked via a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage.

The term "passenger strand" or "sense strand," as used herein, refers to a single stranded nucleic acid molecule which is one strand of a double stranded RNAi molecule, and which has a sequence that is at least substantially complementary (e.g., at least 85% complementary) to that of the guide strand/antisense strand. The sense strand need not be fully complementary over the entire length of the antisense strand, but must at least be sufficiently complementary to hybridize with the antisense strand and result in RNA interference.

The term "repeat unit" refers to a part of a compound whose repetition would produce the complete compound (except for the end-groups, e.g., the RNAi agent and $R^4$ as disclosed herein) by linking the repeat units together successively along the chain.

The term "region of complementarity," as used herein, refers to a nucleobase sequence, e.g., of an RNAi agent, that is sufficiently complementary to a cognate nucleobase sequence, e.g., of a target nucleic acid, such that the two nucleobase sequences are capable of annealing to one another under physiological conditions (e.g., in a cell). In some embodiments, a region of complementarity is fully complementary to a cognate nucleobase sequence of target nucleic acid. However, in some embodiments, a region of complementarity is partially complementary to a cognate nucleobase sequence of target nucleic acid (e.g., at least 80%, 90%, 95% or 99% complementarity). In some embodiments, a region of complementarity contains 1, 2, 3, 4, or 5 mismatches compared with a cognate nucleobase sequence of a target nucleic acid.

The term "RNAi agent," or "RNA interference agent" means a composition that contains an RNA or RNA-like (e.g., chemically modified RNA) oligonucleotide molecule that is capable of degrading or inhibiting translation of messenger RNA (mRNA) transcripts of a target mRNA in a sequence specific manner. As used herein, RNAi agents may operate through the RNA interference mechanism (i.e., inducing RNA interference through interaction with the RNA interference pathway machinery (RNA-induced silencing complex or RISC) of

mammalian cells), or by any alternative mechanism(s) or pathway(s). While it is believed that RNAi agents, as that term is used herein, operate primarily through the RNA interference mechanism, the disclosed RNAi agents are not bound by or limited to any particular pathway or mechanism of action. An RNAi agent modulates, e.g., inhibits, the expression of a CYP7A1 in a cell, e.g., a cell within a subject, such as a mammalian subject. RNAi agents include, but are not limited to: single-stranded oligonucleotides, single-stranded antisense oligonucleotides, short interfering RNAs (siRNAs), double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), and dicer substrates. Any one of the RNAi agents described herein comprises a strand that is at least partially complementary to the mRNA being targeted. In some embodiments, an RNAi agent is single stranded (e.g., it can be an antisense oligonucleotide). In some embodiments, an RNAi agent is double stranded. In some embodiments, the double stranded RNAi agent is a double stranded siRNA.

In some embodiments, an RNAi agent described herein is double-stranded, and comprises an antisense strand and a sense strand, wherein the antisense strand is at least partially complementary to the mRNA being targeted (e.g., CYP7A1 mRNA), and the sense strand is at least partially complementary to the antisense strand. It is not necessary that there be perfect complementarity between the RNAi agent and the target, but the correspondence is preferably sufficient to enable the RNAi agent to direct sequence specific silencing, e.g., by RNAi cleavage of the target RNA, e.g., CYP7A1 mRNA. An RNAi agent described herein may comprise one or more modified nucleosides and/or one or more modified (e.g., non-phosphodiester) internucleoside linkages.

Modification to stabilize one or more 3'- or 5'-terminus of an RNAi agent, e.g., against exonucleases may also be present in an RNAi agent described herein. Other modifications can include C3 (or C6, C7, C12) amino linkers, thiol linkers, carboxyl linkers, non-nucleotidic spacers (C3, C6, C9, C12, abasic, triethylene glycol, hexaethylene glycol), special biotin or fluorescein reagents that come as phosphoramidites and that have another DMT-protected hydroxyl group, allowing multiple couplings during RNA synthesis. Modifications can also include, e.g., the use of modifications at the 2' OH group of the ribose sugar, e.g., the use of deoxyribonucleosides, e.g., deoxythymidine, instead of ribonucleosides, and modifications in the internucleoside linkages, e.g., phosphothioate internucleoside linkages. In some embodiments, the different strands will include different modifications. In some embodiments, an RNAi agent of the disclosure includes a short interfering RNA (siRNA) that interacts with a target RNA sequence, e.g., a CYP7A1 target sequence, to direct the cleavage of the target

RNA. In some embodiments, an RNAi agent described herein is a small interfering RNA (siRNA).

The term "siRNA," as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary (e.g., at least 85% complementary) nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target sequence, i.e., a CYP7A1 sequence. Each strand of the siRNA may optionally and independently comprise ribonucleosides (RNA), RNA analog(s) (e.g., chemically modified ribonucleosides), and/or deoxyribonucleosides (DNA). Each strand of an siRNA comprises between 15 and 30 nucleosides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleosides). In some embodiments, each strand of an siRNA comprises between 18 and 28 nucleosides (e.g., 18-28, 19-25, 19-23, 19-21). In some embodiments, each strand of an siRNA is 19, 20, 21, 22, or 23 nucleosides in length. . In some embodiments of the disclosure, an siRNA induces 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 some embodiments, any one of the CYP7A1 RNAi agents (e.g., siRNAs) disclosed herein comprises a duplex region of 10-30 base pairs in length (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs in length). In some embodiments, an RNAi agent of the present disclosure is blunt ended. In some embodiments, an RNAi agent of the present disclosure has overhangs on one or both strands. The overhang may include 1 – 10 (e.g., 1-10, 1-8, 1-5, 1-3, 1-2) nucleosides, such that the duplex region in the RNAi agent comprises 17-21 nucleosides, or 19 nucleosides. 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.

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).

In one aspect, the disclosure relates to a single stranded RNA generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, i.e., CYP7A1 gene. In some embodiments, an RNAi agent may be a single-stranded RNA (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 RNAi agents are generally 15-30 nucleosides and may be chemically modified. The design and testing of single-stranded RNAi agents 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.

The term "sequence identity," as used herein, refers to the extent that sequences are identical (independent of chemical modification) on a nucleobase-by- nucleobase basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, Wis., USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., Nucl. Acids Res. 25:3389, 1997.

The terms "silence," "reduce," "inhibit," "down-regulate," or "knockdown" when referring to expression of a given gene (e.g., CYP7A1), mean that the expression of the gene, as measured by the level of RNA transcribed from the gene or the level of polypeptide, protein or protein subunit translated from the mRNA in a cell, group of cells, tissue, organ, or subject in which the gene is transcribed, is reduced when the cell, group of cells, tissue, organ, or subject is treated with an RNAi agent or conjugate described herein as compared to a control or reference cell, group of cells, tissue, organ or a subject, e.g., a second cell, group of cells, tissue, organ, or subject that has not or have not been so treated. In some embodiments, when a

cell, group of cells, tissue, organ, or subject is treated with an RNAi agent or conjugate described herein, expression of a target gene (e.g., CYP7A1) is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% relative to a control, e.g., baseline level of gene expression prior to treatment.

The term "subject," as used herein, refers to a mammal. In some embodiments, a subject is non-human primate, or rodent. In some embodiments, a subject is a human. In some embodiments, a subject is a patient, e.g., a human patient that has or is suspected of having a disease. In some embodiments, the subject is a human patient who has or is suspected of having a CYP7A1 disease or CYP7A1-associated disease.

The term "specificity" means the ability to inhibit the target RNA without manifest effects on other genes of the cell. The consequences of inhibition can be confirmed by examination of the outward properties of the cell or organism or by biochemical techniques such as RNA solution hybridization, nuclease protection, Northern hybridization, reverse transcription, gene expression monitoring with a microarray, antibody binding, enzyme linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay (RIA), other immunoassays, and fluorescence activated cell analysis (FACS).

The term "symptom" as used herein, refers to any manifestation or indication of an underly disease. A symptom can be any biochemical, cellular, genetic, histological, and/or physiological observation, measurement, and/or test result in a subject that deviate from those of a control or reference. For example, a symptom may be an elevated level of a liver enzyme, such as aminotransferases, as compared to a normal reference range.

The term "target sequence," as used herein, refers to a nucleoside sequence whose expression or activity is to be modulated. In some embodiments, the target sequence is a contiguous portion of the nucleoside sequence of a gene, a cDNA, or an mRNA molecule formed during the transcription of a target gene, e.g., CYP7A1 gene, including a unprocessed pre-mRNA transcript and mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for RNAi-directed cleavage at or near that portion of the nucleoside sequence of an mRNA molecule formed during the transcription of the target gene, e.g., CYP7A1 gene. In some embodiment, the target sequence is within the protein coding region of the target gene, e.g., CYP7A1.

The term "treat," "treatment," as used herein, mean the methods or steps taken to provide relief from or alleviation of the number, severity, and/or frequency of one or more

symptoms of a disease (e.g., a CYP7A1 disease or CYP7A1-associated disease) in a subject. As used herein, "treat" and treatment" may include the prevention, management, prophylactic treatment, and/or inhibition of the number, severity, and/or frequency of one or more symptoms of a disease (e.g., a CYP7A1 disease or CYP7A1-associated disease) in a subject.

The term "variant" means a molecule (e.g., nucleic acid or polypeptide) that differs from a given molecule (e.g., a reference nucleic acid or polypeptide) in sequence (nucleic acid or amino acid respectively) by the addition (e.g., insertion), deletion, or conservative substitution of nucleic acids or amino acids, respectively, but that retains the biological activity of the given molecule. Nucleic acid variants are closely related overall and, in many regions, identical. Changes in the reference nucleic acid sequence of the variant may be silent. That is, they may not alter the amino acid sequence encoded by the nucleic acid. Alternatively, changes in the nucleoside sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Such nucleoside changes may result in amino acid substitutions, additions, deletions, fusions, and truncations in the polypeptide encoded by the reference sequence. The term "variant" encompasses fragments of a variant unless otherwise defined. A variant may be 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, or 75% identical to the reference sequence. The degree of homology (percent identity) between a native and a variant sequence can be determined, for example, by comparing the two sequences using freely available computer programs commonly employed for this purpose on the world wide web (e.g., BLASTn with default settings).

Definitions of specific functional groups and chemical terms are described in more detail below. The chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, *Handbook of Chemistry and Physics*, 75$^{th}$ Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in *Organic Chemistry*, Thomas Sorrell, University Science Books, Sausalito, 1999; Smith and March, *March's Advanced Organic Chemistry*, 5$^{th}$ Edition, John Wiley & Sons, Inc., New York, 2001; Larock, *Comprehensive Organic Transformations*, VCH Publishers, Inc., New York, 1989; and Carruthers, *Some Modern Methods of Organic Synthesis*, 3$^{rd}$ Edition, Cambridge University Press, Cambridge, 1987.

Compounds described herein can comprise one or more asymmetric centers, and thus can exist in various stereoisomeric forms, e.g., enantiomers and/or diastereomers. For example, the compounds described herein can be in the form of an individual enantiomer, diastereomer

or geometric isomer, or can be in the form of a mixture of stereoisomers, including racemic mixtures and mixtures enriched in one or more stereoisomer. Isomers can be isolated from mixtures by methods known to those skilled in the art, including chiral high pressure liquid chromatography (HPLC) and the formation and crystallization of chiral salts; or preferred isomers can be prepared by asymmetric syntheses. See, for example, Jacques et al., *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen et al., *Tetrahedron* 33:2725 (1977); Eliel, E.L., *Stereochemistry of Carbon Compounds* (McGraw-Hill, NY, 1962); and Wilen, S.H., *Tables of Resolving Agents and Optical Resolutions* p. 268 (E.L. Eliel, Ed., Univ. of Notre Dame Press, Notre Dame, IN 1972). The invention additionally encompasses compounds as individual isomers substantially free of other isomers, and alternatively, as mixtures of various isomers.

In a formula, ~~~ is a single bond where the stereochemistry of the moieties immediately attached thereto is not specified, - - - is absent or a single bond, and ═ or ═ is a single or double bond.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of hydrogen by deuterium or tritium, replacement of $^{19}F$ with $^{18}F$, or the replacement of $^{12}C$ with $^{13}C$ or $^{14}C$ are within the scope of the disclosure. Such compounds are useful, for example, as analytical tools or probes in biological assays.

When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example "$C_{1-6}$ alkyl" is intended to encompass, $C_1$, $C_2$, $C_3$, $C_4$, $C_5$, $C_6$, $C_{1-6}$, $C_{1-5}$, $C_{1-4}$, $C_{1-3}$, $C_{1-2}$, $C_{2-6}$, $C_{2-5}$, $C_{2-4}$, $C_{2-3}$, $C_{3-6}$, $C_{3-5}$, $C_{3-4}$, $C_{4-6}$, $C_{4-5}$, and $C_{5-6}$ alkyl.

The term "aliphatic" refers to alkyl, alkenyl, alkynyl, and carbocyclic groups. Likewise, the term "heteroaliphatic" refers to heteroalkyl, heteroalkenyl, heteroalkynyl, and heterocyclic groups.

The term "alkyl" refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 10 carbon atoms ("$C_{1-10}$ alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("$C_{1-9}$ alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("$C_{1-8}$ alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("$C_{1-7}$ alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("$C_{1-6}$ alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("$C_{1-5}$ alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("$C_{1-4}$ alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("$C_{1-3}$ alkyl"). In some embodiments, an alkyl group has 1

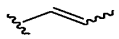
to 2 carbon atoms ("$C_{1-2}$ alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("$C_1$ alkyl"). In some embodiments, an alkyl group has 2 to 6 carbon atoms ("$C_{2-6}$ alkyl"). Examples of $C_{1-6}$ alkyl groups include methyl ($C_1$), ethyl ($C_2$), propyl ($C_3$) (e.g., n-propyl, isopropyl), butyl ($C_4$) (e.g., n-butyl, tert-butyl, sec-butyl, iso-butyl), pentyl ($C_5$) (e.g., n-pentyl, 3-pentanyl, amyl, neopentyl, 3-methyl-2-butanyl, tertiary amyl), and hexyl ($C_6$) (e.g., n-hexyl). Additional examples of alkyl groups include n-heptyl ($C_7$), n-octyl ($C_8$), and the like. Unless otherwise specified, each instance of an alkyl group is independently unsubstituted (an "unsubstituted alkyl") or substituted (a "substituted alkyl") with one or more substituents (e.g., halogen, such as F). In certain embodiments, the alkyl group is an unsubstituted $C_{1-10}$ alkyl (such as unsubstituted $C_{1-6}$ alkyl, e.g., $-CH_3$ (Me), unsubstituted ethyl (Et), unsubstituted propyl (Pr, e.g., unsubstituted n-propyl (n-Pr), unsubstituted isopropyl (i-Pr)), unsubstituted butyl (Bu, e.g., unsubstituted n-butyl (n-Bu), unsubstituted tert-butyl (tert-Bu or t-Bu), unsubstituted sec-butyl (sec-Bu), unsubstituted isobutyl (i-Bu)). In certain embodiments, the alkyl group is a substituted $C_{1-10}$ alkyl (such as substituted $C_{1-6}$ alkyl, e.g., $-CF_3$, Bn).

The term "haloalkyl" is a substituted alkyl group, wherein one or more of the hydrogen atoms are independently replaced by a halogen, e.g., fluoro, bromo, chloro, or iodo. In some embodiments, the haloalkyl moiety has 1 to 8 carbon atoms ("$C_{1-8}$ haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 6 carbon atoms ("$C_{1-6}$ haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 4 carbon atoms ("$C_{1-4}$ haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 3 carbon atoms ("$C_{1-3}$ haloalkyl"). In some embodiments, the haloalkyl moiety has 1 to 2 carbon atoms ("$C_{1-2}$ haloalkyl"). Examples of haloalkyl groups include $-CHF_2$, $-CH_2F$, $-CF_3$, $-CH_2CF_3$, $-CF_2CF_3$, $-CF_2CF_2CF_3$, $-CCl_3$, $-CFCl_2$, $-CF_2Cl$, and the like.

The term "heteroalkyl" refers to an alkyl group, which further includes at least one heteroatom (e.g., 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (i.e., inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkyl group refers to a saturated group having from 1 to 20 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-20}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 18 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-18}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 16 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-16}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 14 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-14}$ alkyl"). In some embodiments, a

heteroalkyl group is a saturated group having 1 to 12 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-12}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 10 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-10}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 8 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-8}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 6 carbon atoms and 1 or more heteroatoms within the parent chain ("hetero$C_{1-6}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 4 carbon atoms and 1 or 2 heteroatoms within the parent chain ("hetero$C_{1-4}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 3 carbon atoms and 1 heteroatom within the parent chain ("hetero$C_{1-3}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 to 2 carbon atoms and 1 heteroatom within the parent chain ("hetero$C_{1-2}$ alkyl"). In some embodiments, a heteroalkyl group is a saturated group having 1 carbon atom and 1 heteroatom ("hetero$C_1$ alkyl"). In some embodiments, the heteroalkyl group defined herein is a partially unsaturated group having 1 or more heteroatoms within the parent chain and at least one unsaturated carbon, such as a carbonyl group. For example, a heteroalkyl group may comprise an amide or ester functionality in its parent chain such that one or more carbon atoms are unsaturated carbonyl groups. Unless otherwise specified, each instance of a heteroalkyl group is independently unsubstituted (an "unsubstituted heteroalkyl") or substituted (a "substituted heteroalkyl") with one or more substituents. In certain embodiments, the heteroalkyl group is an unsubstituted hetero$C_{1-20}$ alkyl. In certain embodiments, the heteroalkyl group is an unsubstituted hetero$C_{1-10}$ alkyl. In certain embodiments, the heteroalkyl group is a substituted hetero$C_{1-20}$ alkyl. In certain embodiments, the heteroalkyl group is an unsubstituted hetero$C_{1-10}$ alkyl.

The term "alkenyl" refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 10 carbon atoms and one or more carbon-carbon double bonds (e.g., 1, 2, 3, or 4 double bonds). In some embodiments, an alkenyl group has 2 to 9 carbon atoms ("$C_{2-9}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 8 carbon atoms ("$C_{2-8}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 7 carbon atoms ("$C_{2-7}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 6 carbon atoms ("$C_{2-6}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 5 carbon atoms ("$C_{2-5}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 4 carbon atoms ("$C_{2-4}$ alkenyl"). In some embodiments, an alkenyl group has 2 to 3 carbon atoms ("$C_{2-3}$ alkenyl"). In some embodiments, an alkenyl group has 2 carbon atoms ("$C_2$ alkenyl"). The one or more carbon-

carbon double bonds can be internal (such as in 2-butenyl) or terminal (such as in 1-butenyl). Examples of $C_{2-4}$ alkenyl groups include ethenyl ($C_2$), 1-propenyl ($C_3$), 2-propenyl ($C_3$), 1-butenyl ($C_4$), 2-butenyl ($C_4$), butadienyl ($C_4$), and the like. Examples of $C_{2-6}$ alkenyl groups include the aforementioned $C_{2-4}$ alkenyl groups as well as pentenyl ($C_5$), pentadienyl ($C_5$), hexenyl ($C_6$), and the like. Additional examples of alkenyl include heptenyl ($C_7$), octenyl ($C_8$), octatrienyl ($C_8$), and the like. Unless otherwise specified, each instance of an alkenyl group is independently unsubstituted (an "unsubstituted alkenyl") or substituted (a "substituted alkenyl") with one or more substituents. In certain embodiments, the alkenyl group is an unsubstituted $C_{2-10}$ alkenyl. In certain embodiments, the alkenyl group is a substituted $C_{2-10}$ alkenyl. In an alkenyl group, a C=C double bond for which the stereochemistry is not specified (e.g., $-CH=CHCH_3$ or  ) may be an (E)- or (Z)-double bond.

The term "heteroalkenyl" refers to an alkenyl group, which further includes at least one heteroatom (e.g., 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (i.e., inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkenyl group refers to a group having from 2 to 10 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-10}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 9 carbon atoms at least one double bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-9}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 8 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-8}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 7 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-7}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 6 carbon atoms, at least one double bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-6}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 5 carbon atoms, at least one double bond, and 1 or 2 heteroatoms within the parent chain ("hetero$C_{2-5}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 4 carbon atoms, at least one double bond, and 1 or 2 heteroatoms within the parent chain ("hetero$C_{2-4}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 3 carbon atoms, at least one double bond, and 1 heteroatom within the parent chain ("hetero$C_{2-3}$ alkenyl"). In some embodiments, a heteroalkenyl group has 2 to 6 carbon atoms, at least one double bond, and 1 or 2 heteroatoms within the parent chain ("hetero$C_{2-6}$ alkenyl"). Unless otherwise specified, each instance of a heteroalkenyl group is independently unsubstituted (an "unsubstituted heteroalkenyl") or substituted (a "substituted heteroalkenyl") with one or more substituents. In certain embodiments, the heteroalkenyl group is an

unsubstituted hetero$C_{2-10}$ alkenyl. In certain embodiments, the heteroalkenyl group is a substituted hetero$C_{2-10}$ alkenyl.

The term "alkynyl" refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 10 carbon atoms and one or more carbon-carbon triple bonds (e.g., 1, 2, 3, or 4 triple bonds) ("$C_{2-10}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 9 carbon atoms ("$C_{2-9}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 8 carbon atoms ("$C_{2-8}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 7 carbon atoms ("$C_{2-7}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 6 carbon atoms ("$C_{2-6}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 5 carbon atoms ("$C_{2-5}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 4 carbon atoms ("$C_{2-4}$ alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("$C_{2-3}$ alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("$C_2$ alkynyl"). The one or more carbon-carbon triple bonds can be internal (such as in 2-butynyl) or terminal (such as in 1-butynyl). Examples of $C_{2-4}$ alkynyl groups include, without limitation, ethynyl ($C_2$), 1-propynyl ($C_3$), 2-propynyl ($C_3$), 1-butynyl ($C_4$), 2-butynyl ($C_4$), and the like. Examples of $C_{2-6}$ alkenyl groups include the aforementioned $C_{2-4}$ alkynyl groups as well as pentynyl ($C_5$), hexynyl ($C_6$), and the like. Additional examples of alkynyl include heptynyl ($C_7$), octynyl ($C_8$), and the like. Unless otherwise specified, each instance of an alkynyl group is independently unsubstituted (an "unsubstituted alkynyl") or substituted (a "substituted alkynyl") with one or more substituents. In certain embodiments, the alkynyl group is an unsubstituted $C_{2-10}$ alkynyl. In certain embodiments, the alkynyl group is a substituted $C_{2-10}$ alkynyl.

The term "heteroalkynyl" refers to an alkynyl group, which further includes at least one heteroatom (e.g., 1, 2, 3, or 4 heteroatoms) selected from oxygen, nitrogen, or sulfur within (i.e., inserted between adjacent carbon atoms of) and/or placed at one or more terminal position(s) of the parent chain. In certain embodiments, a heteroalkynyl group refers to a group having from 2 to 10 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-10}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 9 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-9}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 8 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-8}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 7 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-7}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 6 carbon atoms, at least one triple bond, and 1 or more heteroatoms within the parent chain ("hetero$C_{2-6}$ alkynyl"). In some embodiments, a

heteroalkynyl group has 2 to 5 carbon atoms, at least one triple bond, and 1 or 2 heteroatoms within the parent chain ("hetero$C_{2-5}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 4 carbon atoms, at least one triple bond, and 1or 2 heteroatoms within the parent chain ("hetero$C_{2-4}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 3 carbon atoms, at least one triple bond, and 1 heteroatom within the parent chain ("hetero$C_{2-3}$ alkynyl"). In some embodiments, a heteroalkynyl group has 2 to 6 carbon atoms, at least one triple bond, and 1 or 2 heteroatoms within the parent chain ("hetero$C_{2-6}$ alkynyl"). Unless otherwise specified, each instance of a heteroalkynyl group is independently unsubstituted (an "unsubstituted heteroalkynyl") or substituted (a "substituted heteroalkynyl") with one or more substituents. In certain embodiments, the heteroalkynyl group is an unsubstituted hetero$C_{2-10}$ alkynyl. In certain embodiments, the heteroalkynyl group is a substituted hetero$C_{2-10}$ alkynyl.

The term "carbocyclyl" or "carbocyclic" refers to a radical of a non-aromatic cyclic hydrocarbon group having from 3 to 14 ring carbon atoms ("$C_{3-14}$ carbocyclyl") and zero heteroatoms in the non-aromatic ring system. In some embodiments, a carbocyclyl group has 3 to 10 ring carbon atoms ("$C_{3-10}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 8 ring carbon atoms ("$C_{3-8}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 7 ring carbon atoms ("$C_{3-7}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 3 to 6 ring carbon atoms ("$C_{3-6}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 4 to 6 ring carbon atoms ("$C_{4-6}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 6 ring carbon atoms ("$C_{5-6}$ carbocyclyl"). In some embodiments, a carbocyclyl group has 5 to 10 ring carbon atoms ("$C_{5-10}$ carbocyclyl"). Exemplary $C_{3-6}$ carbocyclyl groups include, without limitation, cyclopropyl ($C_3$), cyclopropenyl ($C_3$), cyclobutyl ($C_4$), cyclobutenyl ($C_4$), cyclopentyl ($C_5$), cyclopentenyl ($C_5$), cyclohexyl ($C_6$), cyclohexenyl ($C_6$), cyclohexadienyl ($C_6$), and the like. Exemplary $C_{3-8}$ carbocyclyl groups include, without limitation, the aforementioned $C_{3-6}$ carbocyclyl groups as well as cycloheptyl ($C_7$), cycloheptenyl ($C_7$), cycloheptadienyl ($C_7$), cycloheptatrienyl ($C_7$), cyclooctyl ($C_8$), cyclooctenyl ($C_8$), bicyclo[2.2.1]heptanyl ($C_7$), bicyclo[2.2.2]octanyl ($C_8$), and the like. Exemplary $C_{3-10}$ carbocyclyl groups include, without limitation, the aforementioned $C_{3-8}$ carbocyclyl groups as well as cyclononyl ($C_9$), cyclononenyl ($C_9$), cyclodecyl ($C_{10}$), cyclodecenyl ($C_{10}$), octahydro-1H-indenyl ($C_9$), decahydronaphthalenyl ($C_{10}$), spiro[4.5]decanyl ($C_{10}$), and the like. As the foregoing examples illustrate, in certain embodiments, the carbocyclyl group is either monocyclic ("monocyclic carbocyclyl") or polycyclic (e.g., containing a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic carbocyclyl") or tricyclic system ("tricyclic carbocyclyl")) and can be saturated or can contain one or more carbon-carbon

double or triple bonds. "Carbocyclyl" also includes ring systems wherein the carbocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups wherein the point of attachment is on the carbocyclyl ring, and in such instances, the number of carbons continue to designate the number of carbons in the carbocyclic ring system. Unless otherwise specified, each instance of a carbocyclyl group is independently unsubstituted (an "unsubstituted carbocyclyl") or substituted (a "substituted carbocyclyl") with one or more substituents. In certain embodiments, the carbocyclyl group is an unsubstituted $C_{3-14}$ carbocyclyl. In certain embodiments, the carbocyclyl group is a substituted $C_{3-14}$ carbocyclyl.

In some embodiments, "carbocyclyl" is a monocyclic, saturated carbocyclyl group having from 3 to 14 ring carbon atoms ("$C_{3-14}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 10 ring carbon atoms ("$C_{3-10}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 8 ring carbon atoms ("$C_{3-8}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms ("$C_{3-6}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 4 to 6 ring carbon atoms ("$C_{4-6}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 6 ring carbon atoms ("$C_{5-6}$ cycloalkyl"). In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms ("$C_{5-10}$ cycloalkyl"). Examples of $C_{5-6}$ cycloalkyl groups include cyclopentyl ($C_5$) and cyclohexyl ($C_5$). Examples of $C_{3-6}$ cycloalkyl groups include the aforementioned $C_{5-6}$ cycloalkyl groups as well as cyclopropyl ($C_3$) and cyclobutyl ($C_4$). Examples of $C_{3-8}$ cycloalkyl groups include the aforementioned $C_{3-6}$ cycloalkyl groups as well as cycloheptyl ($C_7$) and cyclooctyl ($C_8$). Unless otherwise specified, each instance of a cycloalkyl group is independently unsubstituted (an "unsubstituted cycloalkyl") or substituted (a "substituted cycloalkyl") with one or more substituents. In certain embodiments, the cycloalkyl group is an unsubstituted $C_{3-14}$ cycloalkyl. In certain embodiments, the cycloalkyl group is a substituted $C_{3-14}$ cycloalkyl.

The term "heterocyclyl" or "heterocyclic" refers to a radical of a 3- to 14-membered non-aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("3-14 membered heterocyclyl"). In heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. A heterocyclyl group can either be monocyclic ("monocyclic heterocyclyl") or polycyclic (e.g., a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl") or tricyclic system ("tricyclic heterocyclyl")), and can be saturated or can contain one or more carbon-carbon double or triple bonds. Heterocyclyl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined

above, is fused with one or more carbocyclyl groups wherein the point of attachment is either on the carbocyclyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. Unless otherwise specified, each instance of heterocyclyl is independently unsubstituted (an "unsubstituted heterocyclyl") or substituted (a "substituted heterocyclyl") with one or more substituents. In certain embodiments, the heterocyclyl group is an unsubstituted 3-14 membered heterocyclyl. In certain embodiments, the heterocyclyl group is a substituted 3-14 membered heterocyclyl.

In some embodiments, a heterocyclyl group is a 5-10 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-10 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-8 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-8 membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 5-6 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-6 membered heterocyclyl"). In some embodiments, the 5-6 membered heterocyclyl has 1-3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heterocyclyl has 1-2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heterocyclyl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur.

Exemplary 3-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azirdinyl, oxiranyl, and thiiranyl. Exemplary 4-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azetidinyl, oxetanyl, and thietanyl. Exemplary 5-membered heterocyclyl groups containing 1 heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl, tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl, and pyrrolyl-2,5-dione. Exemplary 5-membered heterocyclyl groups containing 2 heteroatoms include, without limitation, dioxolanyl, oxathiolanyl and dithiolanyl. Exemplary 5-membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazolinyl, oxadiazolinyl, and thiadiazolinyl. Exemplary 6-membered heterocyclyl groups containing 1 heteroatom include, without limitation, piperidinyl, tetrahydropyranyl, dihydropyridinyl, and thianyl. Exemplary 6-membered heterocyclyl groups

containing 2 heteroatoms include, without limitation, piperazinyl, morpholinyl, dithianyl, and dioxanyl. Exemplary 6-membered heterocyclyl groups containing 3 heteroatoms include, without limitation, triazinyl. Exemplary 7-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary 8-membered heterocyclyl groups containing 1 heteroatom include, without limitation, azocanyl, oxecanyl and thiocanyl. Exemplary bicyclic heterocyclyl groups include, without limitation, indolinyl, isoindolinyl, dihydrobenzofuranyl, dihydrobenzothienyl, tetrahydrobenzothienyl, tetrahydrobenzofuranyl, tetrahydroindolyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, decahydroisoquinolinyl, octahydrochromenyl, octahydroisochromenyl, decahydronaphthyridinyl, decahydro-1,8-naphthyridinyl, octahydropyrrolo[3,2-b]pyrrole, indolinyl, phthalimidyl, naphthalimidyl, chromanyl, chromenyl, 1H-benzo[e][1,4]diazepinyl, 1,4,5,7-tetrahydropyrano[3,4-b]pyrrolyl, 5,6-dihydro-4H-furo[3,2-b]pyrrolyl, 6,7-dihydro-5H-furo[3,2-b]pyranyl, 5,7-dihydro-4H-thieno[2,3-c]pyranyl, 2,3-dihydro-1H-pyrrolo[2,3-b]pyridinyl, 2,3-dihydrofuro[2,3-b]pyridinyl, 4,5,6,7-tetrahydro-1H-pyrrolo[2,3-b]pyridinyl, 4,5,6,7-tetrahydrofuro[3,2-c]pyridinyl, 4,5,6,7-tetrahydrothieno[3,2-b]pyridinyl, 1,2,3,4-tetrahydro-1,6-naphthyridinyl, and the like.

The term "aryl" refers to a radical of a monocyclic or polycyclic (e.g., bicyclic or tricyclic) 4n+2 aromatic ring system (e.g., having 6, 10, or 14 π electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system ("$C_{6-14}$ aryl"). In some embodiments, an aryl group has 6 ring carbon atoms ("$C_6$ aryl"; e.g., phenyl). In some embodiments, an aryl group has 10 ring carbon atoms ("$C_{10}$ aryl"; e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms ("$C_{14}$ aryl"; e.g., anthracyl). "Aryl" also includes ring systems wherein the aryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the radical or point of attachment is on the aryl ring, and in such instances, the number of carbon atoms continue to designate the number of carbon atoms in the aryl ring system. Unless otherwise specified, each instance of an aryl group is independently unsubstituted (an "unsubstituted aryl") or substituted (a "substituted aryl") with one or more substituents. In certain embodiments, the aryl group is an unsubstituted $C_{6-14}$ aryl. In certain embodiments, the aryl group is a substituted $C_{6-14}$ aryl.

"Aralkyl" is a subset of "alkyl" and refers to an alkyl group substituted by an aryl group, wherein the point of attachment is on the alkyl moiety.

The term "heteroaryl" refers to a radical of a 5-14 membered monocyclic or polycyclic (e.g., bicyclic, tricyclic) 4n+2 aromatic ring system (e.g., having 6, 10, or 14 π electrons shared

in a cyclic array) having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-14 membered heteroaryl"). In heteroaryl groups that contain one or more nitrogen atoms, the point of attachment can be a carbon or nitrogen atom, as valency permits. Heteroaryl polycyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more carbocyclyl or heterocyclyl groups wherein the point of attachment is on the heteroaryl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heteroaryl ring system. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring, and in such instances, the number of ring members designates the number of ring members in the fused polycyclic (aryl/heteroaryl) ring system. Polycyclic heteroaryl groups wherein one ring does not contain a heteroatom (e.g., indolyl, quinolinyl, carbazolyl, and the like) the point of attachment can be on either ring, i.e., either the ring bearing a heteroatom (e.g., 2-indolyl) or the ring that does not contain a heteroatom (e.g., 5-indolyl).

In some embodiments, a heteroaryl group is a 5-10 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-10 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5-8 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-8 membered heteroaryl"). In some embodiments, a heteroaryl group is a 5-6 membered aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5-6 membered heteroaryl"). In some embodiments, the 5-6 membered heteroaryl has 1-3 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heteroaryl has 1-2 ring heteroatoms selected from nitrogen, oxygen, and sulfur. In some embodiments, the 5-6 membered heteroaryl has 1 ring heteroatom selected from nitrogen, oxygen, and sulfur. Unless otherwise specified, each instance of a heteroaryl group is independently unsubstituted (an "unsubstituted heteroaryl") or substituted (a "substituted heteroaryl") with one or more substituents. In certain embodiments, the heteroaryl group is an unsubstituted 5-14 membered heteroaryl. In certain embodiments, the heteroaryl group is a substituted 5-14 membered heteroaryl.

Exemplary 5-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyrrolyl, furanyl, and thiophenyl. Exemplary 5-membered heteroaryl groups containing 2 heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5-membered heteroaryl groups containing 3 heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5-membered heteroaryl groups containing 4 heteroatoms include, without limitation, tetrazolyl. Exemplary 6-membered heteroaryl groups containing 1 heteroatom include, without limitation, pyridinyl. Exemplary 6-membered heteroaryl groups containing 2 heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6-membered heteroaryl groups containing 3 or 4 heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively. Exemplary 7-membered heteroaryl groups containing 1 heteroatom include, without limitation, azepinyl, oxepinyl, and thiepinyl. Exemplary 5,6-bicyclic heteroaryl groups include, without limitation, indolyl, isoindolyl, indazolyl, benzotriazolyl, benzothiophenyl, isobenzothiophenyl, benzofuranyl, benzoisofuranyl, benzimidazolyl, benzoxazolyl, benzisoxazolyl, benzoxadiazolyl, benzthiazolyl, benzisothiazolyl, benzthiadiazolyl, indolizinyl, and purinyl. Exemplary 6,6-bicyclic heteroaryl groups include, without limitation, naphthyridinyl, pteridinyl, quinolinyl, isoquinolinyl, cinnolinyl, quinoxalinyl, phthalazinyl, and quinazolinyl. Exemplary tricyclic heteroaryl groups include, without limitation, phenanthridinyl, dibenzofuranyl, carbazolyl, acridinyl, phenothiazinyl, phenoxazinyl, and phenazinyl.

"Heteroaralkyl" is a subset of "alkyl" and refers to an alkyl group substituted by a heteroaryl group, wherein the point of attachment is on the alkyl moiety.

The term "unsaturated" or "partially unsaturated" refers to a moiety that includes at least one double or triple bond.

The term "saturated" refers to a moiety that does not contain a double or triple bond, i.e., the moiety only contains single bonds.

Affixing the suffix "-ene" to a group indicates the group is a divalent moiety, e.g., alkylene is the divalent moiety of alkyl, alkenylene is the divalent moiety of alkenyl, alkynylene is the divalent moiety of alkynyl, heteroalkylene is the divalent moiety of heteroalkyl, heteroalkenylene is the divalent moiety of heteroalkenyl, heteroalkynylene is the divalent moiety of heteroalkynyl, carbocyclylene is the divalent moiety of carbocyclyl, heterocyclylene is the divalent moiety of heterocyclyl, arylene is the divalent moiety of aryl, and heteroarylene is the divalent moiety of heteroaryl.

A group is optionally substituted unless expressly provided otherwise. The term "optionally substituted" refers to being substituted or unsubstituted. In certain embodiments, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl groups are optionally substituted. "Optionally substituted" refers to a group which may be substituted or unsubstituted (e.g., "substituted" or "unsubstituted" alkyl, "substituted" or "unsubstituted" alkenyl, "substituted" or "unsubstituted" alkynyl, "substituted" or "unsubstituted" heteroalkyl, "substituted" or "unsubstituted" heteroalkenyl, "substituted" or "unsubstituted" heteroalkynyl, "substituted" or "unsubstituted" carbocyclyl, "substituted" or "unsubstituted" heterocyclyl, "substituted" or "unsubstituted" aryl or "substituted" or "unsubstituted" heteroaryl group). In general, the term "substituted" means that at least one hydrogen present on a group is replaced with a permissible substituent, e.g., a substituent which upon substitution results in a stable compound, e.g., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a "substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term "substituted" is contemplated to include substitution with all permissible substituents of organic compounds, and includes any of the substituents described herein that results in the formation of a stable compound. The present disclosure contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this disclosure, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety. The disclosure is not intended to be limited in any manner by the exemplary substituents described herein.

Exemplary carbon atom substituents include, but are not limited to, halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OR^{aa}$, $-ON(R^{bb})_2$, $-N(R^{bb})_2$, $-N(R^{bb})_3{}^+X^-$, $-N(OR^{cc})R^{bb}$, $-SH$, $-SR^{aa}$, $-SSR^{cc}$, $-C(=O)R^{aa}$, $-CO_2H$, $-CHO$, $-C(OR^{cc})_3$, $-CO_2R^{aa}$, $-OC(=O)R^{aa}$, $-OCO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-OC(=O)N(R^{bb})_2$, $-NR^{bb}C(=O)R^{aa}$, $-NR^{bb}CO_2R^{aa}$, $-NR^{bb}C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-OC(=NR^{bb})R^{aa}$, $-OC(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-OC(=NR^{bb})N(R^{bb})_2$, $-NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $-C(=O)NR^{bb}SO_2R^{aa}$, $-NR^{bb}SO_2R^{aa}$, $-SO_2N(R^{bb})_2$, $-SO_2R^{aa}$, $-SO_2OR^{aa}$, $-OSO_2R^{aa}$, $-S(=O)R^{aa}$, $-OS(=O)R^{aa}$, $-Si(R^{aa})_3$, $-OSi(R^{aa})_3$ $-C(=S)N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=S)SR^{aa}$, $-SC(=S)SR^{aa}$, $-SC(=O)SR^{aa}$, $-OC(=O)SR^{aa}$, $-SC(=O)OR^{aa}$, $-SC(=O)R^{aa}$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, $-OP(=O)(R^{aa})_2$, $-OP(=O)(OR^{cc})_2$, $-P(=O)(N(R^{bb})_2)_2$, $-OP(=O)(N(R^{bb})_2)_2$, $-NR^{bb}P(=O)(R^{aa})_2$,

$-NR^{bb}P(=O)(OR^{cc})_2$, $-NR^{bb}P(=O)(N(R^{bb})_2)_2$, $-P(R^{cc})_2$, $-P(OR^{cc})_2$, $-P(R^{cc})_3{}^+X^-$, $-P(OR^{cc})_3{}^+X^-$, $-P(R^{cc})_4$, $-P(OR^{cc})_4$, $-OP(R^{cc})_2$, $-OP(R^{cc})_3{}^+X^-$, $-OP(OR^{cc})_2$, $-OP(OR^{cc})_3{}^+X^-$, $-OP(R^{cc})_4$, $-OP(OR^{cc})_4$, $-B(R^{aa})_2$, $-B(OR^{cc})_2$, $-BR^{aa}(OR^{cc})$, $C_{1-10}$ alkyl, $C_{1-10}$ perhaloalkyl, $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$ alkyl, hetero$C_{2-10}$ alkenyl, hetero$C_{2-10}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups; wherein $X^-$ is a counterion; or two geminal hydrogens on a carbon atom are replaced with the group =O, =S, $=NN(R^{bb})_2$, $=NNR^{bb}C(=O)R^{aa}$, $=NNR^{bb}C(=O)OR^{aa}$, $=NNR^{bb}S(=O)_2R^{aa}$, $=NR^{bb}$, or $=NOR^{cc}$;

each instance of $R^{aa}$ is, independently, selected from $C_{1-10}$ alkyl, $C_{1-10}$ perhaloalkyl, $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$ alkyl, hetero$C_{2-10}$ alkenyl, hetero$C_{2-10}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl, or two $R^{aa}$ groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups;

each instance of $R^{bb}$ is, independently, selected from hydrogen, $-OH$, $-OR^{aa}$, $-N(R^{cc})_2$, $-CN$, $-C(=O)R^{aa}$, $-C(=O)N(R^{cc})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-C(=NR^{cc})OR^{aa}$, $-C(=NR^{cc})N(R^{cc})_2$, $-SO_2N(R^{cc})_2$, $-SO_2R^{cc}$, $-SO_2OR^{cc}$, $-SOR^{aa}$, $-C(=S)N(R^{cc})_2$, $-C(=O)SR^{cc}$, $-C(=S)SR^{cc}$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, $-P(=O)(N(R^{cc})_2)_2$, $C_{1-10}$ alkyl, $C_{1-10}$ perhaloalkyl, $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$ alkyl, hetero$C_{2-10}$ alkenyl, hetero$C_{2-10}$alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl, or two $R^{bb}$ groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups; wherein $X^-$ is a counterion;

each instance of $R^{cc}$ is, independently, selected from hydrogen, $C_{1-10}$ alkyl, $C_{1-10}$ perhaloalkyl, $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$ alkyl, hetero$C_{2-10}$ alkenyl, hetero$C_{2-10}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl, or two $R^{cc}$ groups are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups;

each instance of $R^{dd}$ is, independently, selected from halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OR^{ee}$, $-ON(R^{ff})_2$, $-N(R^{ff})_2$, $-N(R^{ff})_3^+X^-$, $-N(OR^{ee})R^{ff}$, $-SH$, $-SR^{ee}$, $-SSR^{ee}$, $-C(=O)R^{ee}$, $-CO_2H$, $-CO_2R^{ee}$, $-OC(=O)R^{ee}$, $-OCO_2R^{ee}$, $-C(=O)N(R^{ff})_2$, $-OC(=O)N(R^{ff})_2$, $-NR^{ff}C(=O)R^{ee}$, $-NR^{ff}CO_2R^{ee}$, $-NR^{ff}C(=O)N(R^{ff})_2$, $-C(=NR^{ff})OR^{ee}$, $-OC(=NR^{ff})R^{ee}$, $-OC(=NR^{ff})OR^{ee}$, $-C(=NR^{ff})N(R^{ff})_2$, $-OC(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}C(=NR^{ff})N(R^{ff})_2$, $-NR^{ff}SO_2R^{ee}$, $-SO_2N(R^{ff})_2$, $-SO_2R^{ee}$, $-SO_2OR^{ee}$, $-OSO_2R^{ee}$, $-S(=O)R^{ee}$, $-Si(R^{ee})_3$, $-OSi(R^{ee})_3$, $-C(=S)N(R^{ff})_2$, $-C(=O)SR^{ee}$, $-C(=S)SR^{ee}$, $-SC(=S)SR^{ee}$, $-P(=O)(OR^{ee})_2$, $-P(=O)(R^{ee})_2$, $-OP(=O)(R^{ee})_2$, $-OP(=O)(OR^{ee})_2$, $C_{1-6}$ alkyl, $C_{1-6}$ perhaloalkyl, $C_{2-6}$ alkenyl, $C_{2-6}$ alkynyl, hetero$C_{1-6}$ alkyl, hetero$C_{2-6}$ alkenyl, hetero$C_{2-6}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-10 membered heterocyclyl, $C_{6-10}$ aryl, 5-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{gg}$ groups, or two geminal $R^{dd}$ substituents can be joined to form =O or =S; wherein $X^-$ is a counterion;

each instance of $R^{ee}$ is, independently, selected from $C_{1-6}$ alkyl, $C_{1-6}$ perhaloalkyl, $C_{2-6}$ alkenyl, $C_{2-6}$ alkynyl, hetero$C_{1-6}$ alkyl, hetero$C_{2-6}$ alkenyl, hetero$C_{2-6}$ alkynyl, $C_{3-10}$ carbocyclyl, $C_{6-10}$ aryl, 3-10 membered heterocyclyl, and 3-10 membered heteroaryl, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{gg}$ groups;

each instance of $R^{ff}$ is, independently, selected from hydrogen, $C_{1-6}$ alkyl, $C_{1-6}$ perhaloalkyl, $C_{2-6}$ alkenyl, $C_{2-6}$ alkynyl, hetero$C_{1-6}$ alkyl, hetero$C_{2-6}$ alkenyl, hetero$C_{2-6}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-10 membered heterocyclyl, $C_{6-10}$ aryl and 5-10 membered heteroaryl, or two $R^{ff}$ groups are joined to form a 3-10 membered heterocyclyl or 5-10 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{gg}$ groups; and

each instance of $R^{gg}$ is, independently, halogen, $-CN$, $-NO_2$, $-N_3$, $-SO_2H$, $-SO_3H$, $-OH$, $-OC_{1-6}$ alkyl, $-ON(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_2$, $-N(C_{1-6}$ alkyl$)_3^+X^-$, $-NH(C_{1-6}$ alkyl$)_2^+X^-$, $-NH_2(C_{1-6}$ alkyl$)^+X^-$, $-NH_3^+X^-$, $-N(OC_{1-6}$ alkyl$)(C_{1-6}$ alkyl$)$, $-N(OH)(C_{1-6}$ alkyl$)$, $-NH(OH)$, $-SH$, $-SC_{1-6}$ alkyl, $-SS(C_{1-6}$ alkyl$)$, $-C(=O)(C_{1-6}$ alkyl$)$, $-CO_2H$, $-CO_2(C_{1-6}$ alkyl$)$, $-OC(=O)(C_{1-6}$ alkyl$)$, $-OCO_2(C_{1-6}$ alkyl$)$, $-C(=O)NH_2$, $-C(=O)N(C_{1-6}$ alkyl$)_2$, $-OC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)(C_{1-6}$ alkyl$)$, $-N(C_{1-6}$ alkyl$)C(=O)($ $C_{1-6}$ alkyl$)$, $-NHCO_2(C_{1-6}$ alkyl$)$, $-NHC(=O)N(C_{1-6}$ alkyl$)_2$, $-NHC(=O)NH(C_{1-6}$ alkyl$)$, $-NHC(=O)NH_2$, $-C(=NH)O(C_{1-6}$ alkyl$)$, $-OC(=NH)(C_{1-6}$ alkyl$)$, $-OC(=NH)OC_{1-6}$ alkyl, $-C(=NH)N(C_{1-6}$ alkyl$)_2$, $-C(=NH)NH(C_{1-6}$ alkyl$)$, $-C(=NH)NH_2$, $-OC(=NH)N(C_{1-6}$ alkyl$)_2$, $-OC(=NH)NH(C_{1-}$

$_{6}$ alkyl), –OC(=NH)$NH_2$, –NHC(=NH)N($C_{1-6}$ alkyl)$_2$, –NHC(=NH)$NH_2$, –$NHSO_2$($C_{1-6}$ alkyl), –$SO_2$N($C_{1-6}$ alkyl)$_2$, –$SO_2$NH($C_{1-6}$ alkyl), –$SO_2NH_2$, –$SO_2$($C_{1-6}$ alkyl), –$SO_2$O($C_{1-6}$ alkyl), –$OSO_2$($C_{1-6}$ alkyl), –SO($C_{1-6}$ alkyl), –Si($C_{1-6}$ alkyl)$_3$, –OSi($C_{1-6}$ alkyl)$_3$ –C(=S)N($C_{1-6}$ alkyl)$_2$, C(=S)NH($C_{1-6}$ alkyl), C(=S)$NH_2$, –C(=O)S($C_{1-6}$ alkyl), –C(=S)S$C_{1-6}$ alkyl, –SC(=S)S$C_{1-6}$ alkyl, –P(=O)(O$C_{1-6}$ alkyl)$_2$, –P(=O)($C_{1-6}$ alkyl)$_2$, –OP(=O)($C_{1-6}$ alkyl)$_2$, –OP(=O)(O$C_{1-6}$ alkyl)$_2$, $C_{1-6}$ alkyl, $C_{1-6}$ perhaloalkyl, $C_{2-6}$ alkenyl, $C_{2-6}$ alkynyl, hetero$C_{1-6}$ alkyl, hetero$C_{2-6}$ alkenyl, hetero$C_{2-6}$ alkynyl, $C_{3-10}$ carbocyclyl, $C_{6-10}$ aryl, 3-10 membered heterocyclyl, 5-10 membered heteroaryl; or two geminal $R^{gg}$ substituents can be joined to form =O or =S; wherein $X^-$ is a counterion.

In certain embodiments, an exemplary carbon atom substituent is halogen (*.e.g*, -F, -Cl, -Br, -I), –CN, –$NO_2$, –$N_3$, –$SO_2H$, –$SO_3H$, –OH, -O($C_{1-6}$ alkyl) (e.g., –OMe), –$NH_2$, –SH, -S($C_{1-6}$ alkyl) (e.g., –SMe), -C(=O)($C_{1-6}$ alkyl) (e.g., –C(=O)Me), –$CO_2H$, or –CHO.

The term "halo" or "halogen" refers to fluorine (fluoro, –F), chlorine (chloro, –Cl), bromine (bromo, –Br), or iodine (iodo, –I).

The term "hydroxyl" or "hydroxy" refers to the group –OH. The term "substituted hydroxyl" or "substituted hydroxyl," by extension, refers to a hydroxyl group wherein the oxygen atom directly attached to the parent molecule is substituted with a group other than hydrogen, and includes groups selected from –$OR^{aa}$, –ON($R^{bb}$)$_2$, –OC(=O)$SR^{aa}$, –OC(=O)$R^{aa}$, –$OCO_2R^{aa}$, –OC(=O)N($R^{bb}$)$_2$, –OC(=N$R^{bb}$)$R^{aa}$, –OC(=N$R^{bb}$)$OR^{aa}$, –OC(=N$R^{bb}$)N($R^{bb}$)$_2$, –OS(=O)$R^{aa}$, –$OSO_2R^{aa}$, –OSi($R^{aa}$)$_3$, –OP($R^{cc}$)$_2$, –OP($R^{cc}$)$_3^+X^-$, –OP($OR^{cc}$)$_2$, –OP($OR^{cc}$)$_3^+X^-$, –OP(=O)($R^{aa}$)$_2$, –OP(=O)($OR^{cc}$)$_2$, and –OP(=O)(N($R^{bb}$)$_2$)$_2$, wherein $X^-$, $R^{aa}$, $R^{bb}$, and $R^{cc}$ are as defined herein.

The term "amino" refers to the group –$NH_2$. The term "substituted amino," by extension, refers to a monosubstituted amino, a disubstituted amino, or a trisubstituted amino. In certain embodiments, the "substituted amino" is a monosubstituted amino or a disubstituted amino group.

The term "monosubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with one hydrogen and one group other than hydrogen, and includes groups selected from –NH($R^{bb}$), –NHC(=O)$R^{aa}$, –$NHCO_2R^{aa}$, –NHC(=O)N($R^{bb}$)$_2$, –NHC(=N$R^{bb}$)N($R^{bb}$)$_2$, –$NHSO_2R^{aa}$, –NHP(=O)($OR^{cc}$)$_2$, and –NHP(=O)(N($R^{bb}$)$_2$)$_2$, wherein $R^{aa}$, $R^{bb}$ and $R^{cc}$ are as defined herein, and wherein $R^{bb}$ of the group –NH($R^{bb}$) is not hydrogen.

The term "disubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with two groups other than hydrogen,

and includes groups selected from $-N(R^{bb})_2$, $-NR^{bb}C(=O)R^{aa}$, $-NR^{bb}CO_2R^{aa}$, $-NR^{bb}C(=O)N(R^{bb})_2$, $-NR^{bb}C(=NR^{bb})N(R^{bb})_2$, $-NR^{bb}SO_2R^{aa}$, $-NR^{bb}P(=O)(OR^{cc})_2$, and $-NR^{bb}P(=O)(N(R^{bb})_2)_2$, wherein $R^{aa}$, $R^{bb}$, and $R^{cc}$ are as defined herein, with the proviso that the nitrogen atom directly attached to the parent molecule is not substituted with hydrogen.

The term "trisubstituted amino" refers to an amino group wherein the nitrogen atom directly attached to the parent molecule is substituted with three groups, and includes groups selected from $-N(R^{bb})_3$ and $-N(R^{bb})_3{}^+X^-$, wherein $R^{bb}$ and $X^-$ are as defined herein.

The term "acyl" refers to a group having the general formula: $-C(=O)R^{X1}$, $-C(=O)OR^{X1}$, $-C(=O)-O-C(=O)R^{X1}$, $-C(=O)SR^{X1}$, $-C(=O)N(R^{X1})_2$, $-C(=S)R^{X1}$, $-C(=S)N(R^{X1})_2$, $-C(=S)O(R^{X1})$, $-C(=S)S(R^{X1})$, $-C(=NR^{X1})R^{X1}$, $-C(=NR^{X1})OR^{X1}$, $-C(=NR^{X1})SR^{X1}$, or $-C(=NR^{X1})N(R^{X1})_2$, wherein $R^{X1}$ is hydrogen; halogen; substituted or unsubstituted hydroxyl; substituted or unsubstituted thiol; substituted or unsubstituted amino; substituted or unsubstituted acyl, cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched heteroaliphatic; cyclic or acyclic, substituted or unsubstituted, branched or unbranched alkyl; cyclic or acyclic, substituted or unsubstituted, branched or unbranched alkenyl; substituted or unsubstituted alkynyl; substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, mono- or di- aliphaticamino, mono- or di- heteroaliphaticamino, mono- or di-alkylamino, mono- or di- heteroalkylamino, mono- or di-arylamino, or mono- or di-heteroarylamino; or two $R^{X1}$ groups taken together form a 5- to 6-membered heterocyclic ring. Exemplary acyl groups include aldehydes (–CHO), carboxylic acids ($-CO_2H$), ketones, acyl halides, esters, amides, imines, carbonates, carbamates, and ureas. Acyl substituents include, but are not limited to, any of the substituents described herein, that result in the formation of a stable moiety (e.g., aliphatic, alkyl, alkenyl, alkynyl, heteroaliphatic, heterocyclic, aryl, heteroaryl, acyl, oxo, imino, thiooxo, cyano, isocyano, amino, azido, nitro, hydroxyl, thiol, halo, aliphaticamino, heteroaliphaticamino, alkylamino, heteroalkylamino, arylamino, heteroarylamino, alkylaryl, arylalkyl, aliphaticoxy, heteroaliphaticoxy, alkyloxy, heteroalkyloxy, aryloxy, heteroaryloxy, aliphaticthioxy, heteroaliphaticthioxy, alkylthioxy, heteroalkylthioxy, arylthioxy, heteroarylthioxy, acyloxy, and the like, each of which may or may not be further substituted).

The term "oxo" refers to the group =O, and the term "thiooxo" refers to the group =S.

Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quaternary nitrogen atoms. Exemplary nitrogen atom substituents include, but are not limited to, hydrogen, –OH, $–OR^{aa}$, $–N(R^{cc})_2$, –CN, $–C(=O)R^{aa}$, $–C(=O)N(R^{cc})_2$, $–CO_2R^{aa}$, $–SO_2R^{aa}$, $–C(=NR^{bb})R^{aa}$, $–C(=NR^{cc})OR^{aa}$, $–C(=NR^{cc})N(R^{cc})_2$, $–SO_2N(R^{cc})_2$, $–SO_2R^{cc}$, $–SO_2OR^{cc}$, $–SOR^{aa}$, $–C(=S)N(R^{cc})_2$, $–C(=O)SR^{cc}$, $–C(=S)SR^{cc}$, $–P(=O)(OR^{cc})_2$, $–P(=O)(R^{aa})_2$, $–P(=O)(N(R^{cc})_2)_2$, $C_{1-10}$ alkyl, $C_{1-10}$ perhaloalkyl, $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$alkyl, hetero$C_{2-10}$alkenyl, hetero$C_{2-10}$alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl, or two $R^{cc}$ groups attached to an N atom are joined to form a 3-14 membered heterocyclyl or 5-14 membered heteroaryl ring, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups, and wherein $R^{aa}$, $R^{bb}$, $R^{cc}$ and $R^{dd}$ are as defined herein.

In certain embodiments, the substituent present on the nitrogen atom is a nitrogen protecting group (also referred to herein as an "amino protecting group"). Nitrogen protecting groups include, but are not limited to, –OH, $–OR^{aa}$, $–N(R^{cc})_2$, $–C(=O)R^{aa}$, $–C(=O)N(R^{cc})_2$, $–CO_2R^{aa}$, $–SO_2R^{aa}$, $–C(=NR^{cc})R^{aa}$, $–C(=NR^{cc})OR^{aa}$, $–C(=NR^{cc})N(R^{cc})_2$, $–SO_2N(R^{cc})_2$, $–SO_2R^{cc}$, $–SO_2OR^{cc}$, $–SOR^{aa}$, $–C(=S)N(R^{cc})_2$, $–C(=O)SR^{cc}$, $–C(=S)SR^{cc}$, $C_{1-10}$ alkyl (e.g., aralkyl, heteroaralkyl), $C_{2-10}$ alkenyl, $C_{2-10}$ alkynyl, hetero$C_{1-10}$ alkyl, hetero$C_{2-10}$ alkenyl, hetero$C_{2-10}$ alkynyl, $C_{3-10}$ carbocyclyl, 3-14 membered heterocyclyl, $C_{6-14}$ aryl, and 5-14 membered heteroaryl groups, wherein each alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carbocyclyl, heterocyclyl, aralkyl, aryl, and heteroaryl is independently substituted with 0, 1, 2, 3, 4, or 5 $R^{dd}$ groups, and wherein $R^{aa}$, $R^{bb}$, $R^{cc}$ and $R^{dd}$ are as defined herein. Nitrogen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

For example, nitrogen protecting groups such as amide groups (e.g., $–C(=O)R^{aa}$) include, but are not limited to, formamide, acetamide, chloroacetamide, trichloroacetamide, trifluoroacetamide, phenylacetamide, 3-phenylpropanamide, picolinamide, 3-pyridylcarboxamide, N-benzoylphenylalanyl derivative, benzamide, p-phenylbenzamide, o-nitophenylacetamide, o-nitrophenoxyacetamide, acetoacetamide, (N'-dithiobenzyloxyacylamino)acetamide, 3-(p-hydroxyphenyl)propanamide, 3-(o-nitrophenyl)propanamide, 2-methyl-2-(o-nitrophenoxy)propanamide, 2-methyl-2-(o-phenylazophenoxy)propanamide, 4-chlorobutanamide, 3-methyl-3-nitrobutanamide, o-

nitrocinnamide, N-acetylmethionine derivative, o-nitrobenzamide and o-(benzoyloxymethyl)benzamide.

Nitrogen protecting groups such as carbamate groups (e.g., –C(=O)OR$^{aa}$) include, but are not limited to, methyl carbamate, ethyl carbamate, 9-fluorenylmethyl carbamate (Fmoc), 9-(2-sulfo)fluorenylmethyl carbamate, 9-(2,7-dibromo)fluorenylmethyl carbamate, 2,7-di-t-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl carbamate (DBD-Tmoc), 4-methoxyphenacyl carbamate (Phenoc), 2,2,2-trichloroethyl carbamate (Troc), 2-trimethylsilylethyl carbamate (Teoc), 2-phenylethyl carbamate (hZ), 1-(1-adamantyl)-1-methylethyl carbamate (Adpoc), 1,1-dimethyl-2-haloethyl carbamate, 1,1-dimethyl-2,2-dibromoethyl carbamate (DB-t-BOC), 1,1-dimethyl-2,2,2-trichloroethyl carbamate (TCBOC), 1-methyl-1-(4-biphenylyl)ethyl carbamate (Bpoc), 1-(3,5-di-t-butylphenyl)-1-methylethyl carbamate (t-Bumeoc), 2-(2′- and 4′-pyridyl)ethyl carbamate (Pyoc), 2-(N,N-dicyclohexylcarboxamido)ethyl carbamate, t-butyl carbamate (BOC or Boc), 1-adamantyl carbamate (Adoc), vinyl carbamate (Voc), allyl carbamate (Alloc), 1-isopropylallyl carbamate (Ipaoc), cinnamyl carbamate (Coc), 4-nitrocinnamyl carbamate (Noc), 8-quinolyl carbamate, N-hydroxypiperidinyl carbamate, alkyldithio carbamate, benzyl carbamate (Cbz), p-methoxybenzyl carbamate (Moz), p-nitrobenzyl carbamate, p-bromobenzyl carbamate, p-chlorobenzyl carbamate, 2,4-dichlorobenzyl carbamate, 4-methylsulfinylbenzyl carbamate (Msz), 9-anthrylmethyl carbamate, diphenylmethyl carbamate, 2-methylthioethyl carbamate, 2-methylsulfonylethyl carbamate, 2-(p-toluenesulfonyl)ethyl carbamate, [2-(1,3-dithianyl)]methyl carbamate (Dmoc), 4-methylthiophenyl carbamate (Mtpc), 2,4-dimethylthiophenyl carbamate (Bmpc), 2-phosphonioethyl carbamate (Peoc), 2-triphenylphosphonioisopropyl carbamate (Ppoc), 1,1-dimethyl-2-cyanoethyl carbamate, m-chloro-p-acyloxybenzyl carbamate, p-(dihydroxyboryl)benzyl carbamate, 5-benzisoxazolylmethyl carbamate, 2-(trifluoromethyl)-6-chromonylmethyl carbamate (Tcroc), m-nitrophenyl carbamate, 3,5-dimethoxybenzyl carbamate, o-nitrobenzyl carbamate, 3,4-dimethoxy-6-nitrobenzyl carbamate, phenyl(o-nitrophenyl)methyl carbamate, t-amyl carbamate, S-benzyl thiocarbamate, p-cyanobenzyl carbamate, cyclobutyl carbamate, cyclohexyl carbamate, cyclopentyl carbamate, cyclopropylmethyl carbamate, p-decyloxybenzyl carbamate, 2,2-dimethoxyacylvinyl carbamate, o-(N,N-dimethylcarboxamido)benzyl carbamate, 1,1-dimethyl-3-(N,N-dimethylcarboxamido)propyl carbamate, 1,1-dimethylpropynyl carbamate, di(2-pyridyl)methyl carbamate, 2-furanylmethyl carbamate, 2-iodoethyl carbamate, isobornyl carbamate, isobutyl carbamate, isonicotinyl carbamate, p-(p'-methoxyphenylazo)benzyl carbamate, 1-methylcyclobutyl carbamate, 1-

methylcyclohexyl carbamate, 1-methyl-1-cyclopropylmethyl carbamate, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl carbamate, 1-methyl-1-(p-phenylazophenyl)ethyl carbamate, 1-methyl-1-phenylethyl carbamate, 1-methyl-1-(4-pyridyl)ethyl carbamate, phenyl carbamate, p-(phenylazo)benzyl carbamate, 2,4,6-tri-t-butylphenyl carbamate, 4-(trimethylammonium)benzyl carbamate, and 2,4,6-trimethylbenzyl carbamate.

Nitrogen protecting groups such as sulfonamide groups (e.g., $-S(=O)_2R^{aa}$) include, but are not limited to, p-toluenesulfonamide (Ts), benzenesulfonamide, 2,3,6-trimethyl-4-methoxybenzenesulfonamide (Mtr), 2,4,6-trimethoxybenzenesulfonamide (Mtb), 2,6-dimethyl-4-methoxybenzenesulfonamide (Pme), 2,3,5,6-tetramethyl-4-methoxybenzenesulfonamide (Mte), 4-methoxybenzenesulfonamide (Mbs), 2,4,6-trimethylbenzenesulfonamide (Mts), 2,6-dimethoxy-4-methylbenzenesulfonamide (iMds), 2,2,5,7,8-pentamethylchroman-6-sulfonamide (Pmc), methanesulfonamide (Ms), β-trimethylsilylethanesulfonamide (SES), 9-anthracenesulfonamide, 4-(4′,8′-dimethoxynaphthylmethyl)benzenesulfonamide (DNMBS), benzylsulfonamide, trifluoromethylsulfonamide, and phenacylsulfonamide.

Other nitrogen protecting groups include, but are not limited to, phenothiazinyl-(10)-acyl derivative, N′-p-toluenesulfonylaminoacyl derivative, N′-phenylaminothioacyl derivative, N-benzoylphenylalanyl derivative, N-acetylmethionine derivative, 4,5-diphenyl-3-oxazolin-2-one, N-phthalimide, N-dithiasuccinimide (Dts), N-2,3-diphenylmaleimide, N-2,5-dimethylpyrrole, N-1,1,4,4-tetramethyldisilylazacyclopentane adduct (STABASE), 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridone, N-methylamine, N-allylamine, N-[2-(trimethylsilyl)ethoxy]methylamine (SEM), N-3-acetoxypropylamine, N-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl)amine, quaternary ammonium salts, N-benzylamine, N-di(4-methoxyphenyl)methylamine, N-5-dibenzosuberylamine, N-triphenylmethylamine (Tr), N-[(4-methoxyphenyl)diphenylmethyl]amine (MMTr), N-9-phenylfluorenylamine (PhF), N-2,7-dichloro-9-fluorenylmethyleneamine, N-ferrocenylmethylamino (Fcm), N-2-picolylamino N'-oxide, N-1,1-dimethylthiomethyleneamine, N-benzylideneamine, N-p-methoxybenzylideneamine, N-diphenylmethyleneamine, N-[(2-pyridyl)mesityl]methyleneamine, N-(N',N'-dimethylaminomethylene)amine, N,N'-isopropylidenediamine, N-p-nitrobenzylideneamine, N-salicylideneamine, N-5-chlorosalicylideneamine, N-(5-chloro-2-hydroxyphenyl)phenylmethyleneamine, N-cyclohexylideneamine, N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, N-borane derivative, N-diphenylborinic acid derivative, N-[phenyl(pentaacylchromium- or tungsten)acyl]amine, N-copper chelate, N-zinc chelate, N-nitroamine, N-nitrosoamine, amine N-oxide,

diphenylphosphinamide (Dpp), dimethylthiophosphinamide (Mpt), diphenylthiophosphinamide (Ppt), dialkyl phosphoramidates, dibenzyl phosphoramidate, diphenyl phosphoramidate, benzenesulfenamide, o-nitrobenzenesulfenamide (Nps), 2,4-dinitrobenzenesulfenamide, pentachlorobenzenesulfenamide, 2-nitro-4-methoxybenzenesulfenamide, triphenylmethylsulfenamide, and 3-nitropyridinesulfenamide (Npys). In certain embodiments, a nitrogen protecting group is benzyl (Bn), tert-butyloxycarbonyl (BOC), carbobenzyloxy (Cbz), 9-flurenylmethyloxycarbonyl (Fmoc), trifluoroacetyl, triphenylmethyl, acetyl (Ac), benzoyl (Bz), p-methoxybenzyl (PMB), 3,4-dimethoxybenzyl (DMPM), p-methoxyphenyl (PMP), 2,2,2-trichloroethyloxycarbonyl (Troc), triphenylmethyl (Tr), tosyl (Ts), brosyl (Bs), nosyl (Ns), mesyl (Ms), triflyl (Tf), or dansyl (Ds).

In certain embodiments, the substituent present on an oxygen atom is an oxygen protecting group (also referred to herein as an "hydroxyl protecting group"). Oxygen protecting groups include, but are not limited to, $-R^{aa}$, $-N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=O)R^{aa}$, $-CO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(R^{cc})_3{}^+X^-$, $-P(OR^{cc})_2$, $-P(OR^{cc})_3{}^+X^-$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, and $-P(=O)(N(R^{bb})_2)_2$, wherein $X^-$, $R^{aa}$, $R^{bb}$, and $R^{cc}$ are as defined herein. Oxygen protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference.

Exemplary oxygen protecting groups include, but are not limited to, methyl, methoxylmethyl (MOM), methylthiomethyl (MTM), t-butylthiomethyl, (phenyldimethylsilyl)methoxymethyl (SMOM), benzyloxymethyl (BOM), p-methoxybenzyloxymethyl (PMBM), (4-methoxyphenoxy)methyl (p-AOM), guaiacolmethyl (GUM), t-butoxymethyl, 4-pentenyloxymethyl (POM), siloxymethyl, 2-methoxyethoxymethyl (MEM), 2,2,2-trichloroethoxymethyl, bis(2-chloroethoxy)methyl, 2-(trimethylsilyl)ethoxymethyl (SEMOR), tetrahydropyranyl (THP), 3-bromotetrahydropyranyl, tetrahydrothiopyranyl, 1-methoxycyclohexyl, 4-methoxytetrahydropyranyl (MTHP), 4-methoxytetrahydrothiopyranyl, 4-methoxytetrahydrothiopyranyl S,S-dioxide, 1-[(2-chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl (CTMP), 1,4-dioxan-2-yl, tetrahydrofuranyl, tetrahydrothiofuranyl, 2,3,3a,4,5,6,7,7a-octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl, 1-ethoxyethyl, 1-(2-chloroethoxy)ethyl, 1-methyl-1-methoxyethyl, 1-methyl-1-benzyloxyethyl, 1-methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-(phenylselenyl)ethyl, t-butyl, allyl, p-chlorophenyl, p-methoxyphenyl, 2,4-dinitrophenyl, benzyl (Bn), p-methoxybenzyl, 3,4-dimethoxybenzyl, o-nitrobenzyl, p-

nitrobenzyl, p-halobenzyl, 2,6-dichlorobenzyl, p-cyanobenzyl, p-phenylbenzyl, 2-picolyl, 4-picolyl, 3-methyl-2-picolyl N-oxido, diphenylmethyl, p,p'-dinitrobenzhydryl, 5-dibenzosuberyl, triphenylmethyl, α-naphthyldiphenylmethyl, p-methoxyphenyldiphenylmethyl, di(p-methoxyphenyl)phenylmethyl, tri(p-methoxyphenyl)methyl, 4-(4'-bromophenacyloxyphenyl)diphenylmethyl, 4,4',4″-tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4″-tris(levulinoyloxyphenyl)methyl, 4,4',4″-tris(benzoyloxyphenyl)methyl, 3-(imidazol-1-yl)bis(4',4″-dimethoxyphenyl)methyl, 1,1-bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl, 9-(9-phenyl-10-oxo)anthryl, 1,3-benzodithiolan-2-yl, benzisothiazolyl S,S-dioxido, trimethylsilyl (TMS), triethylsilyl (TES), triisopropylsilyl (TIPS), dimethylisopropylsilyl (IPDMS), diethylisopropylsilyl (DEIPS), dimethylthexylsilyl, t-butyldimethylsilyl (TBDMS), t-butyldiphenylsilyl (TBDPS), tribenzylsilyl, tri-p-xylylsilyl, triphenylsilyl, diphenylmethylsilyl (DPMS), t-butylmethoxyphenylsilyl (TBMPS), formate, benzoylformate, acetate, chloroacetate, dichloroacetate, trichloroacetate, trifluoroacetate, methoxyacetate, triphenylmethoxyacetate, phenoxyacetate, p-chlorophenoxyacetate, 3-phenylpropionate, 4-oxopentanoate (levulinate), 4,4-(ethylenedithio)pentanoate (levulinoyldithioacetal), pivaloate, adamantoate, crotonate, 4-methoxycrotonate, benzoate, p-phenylbenzoate, 2,4,6-trimethylbenzoate (mesitoate), methyl carbonate, 9-fluorenylmethyl carbonate (Fmoc), ethyl carbonate, 2,2,2-trichloroethyl carbonate (Troc), 2-(trimethylsilyl)ethyl carbonate (TMSEC), 2-(phenylsulfonyl) ethyl carbonate (Psec), 2-(triphenylphosphonio) ethyl carbonate (Peoc), isobutyl carbonate, vinyl carbonate, allyl carbonate, t-butyl carbonate (BOC or Boc), p-nitrophenyl carbonate, benzyl carbonate, p-methoxybenzyl carbonate, 3,4-dimethoxybenzyl carbonate, o-nitrobenzyl carbonate, p-nitrobenzyl carbonate, S-benzyl thiocarbonate, 4-ethoxy-1-napththyl carbonate, methyl dithiocarbonate, 2-iodobenzoate, 4-azidobutyrate, 4-nitro-4-methylpentanoate, o-(dibromomethyl)benzoate, 2-formylbenzenesulfonate, 2-(methylthiomethoxy)ethyl, 4-(methylthiomethoxy)butyrate, 2-(methylthiomethoxymethyl)benzoate, 2,6-dichloro-4-methylphenoxyacetate, 2,6-dichloro-4-(1,1,3,3-tetramethylbutyl)phenoxyacetate, 2,4-bis(1,1-dimethylpropyl)phenoxyacetate, chlorodiphenylacetate, isobutyrate, monosuccinoate, (E)-2-methyl-2-butenoate, o-(methoxyacyl)benzoate, α-naphthoate, nitrate, alkyl N,N,N',N'-tetramethylphosphorodiamidate, alkyl N-phenylcarbamate, borate, dimethylphosphinothioyl, alkyl 2,4-dinitrophenylsulfenate, sulfate, methanesulfonate (mesylate), benzylsulfonate, and tosylate (Ts). In certain embodiments, an oxygen protecting group is silyl. In certain embodiments, an oxygen protecting group is t-butyldiphenylsilyl (TBDPS), t-butyldimethylsilyl (TBDMS), triisoproylsilyl (TIPS), triphenylsilyl (TPS), triethylsilyl (TES),

trimethylsilyl (TMS), triisopropylsiloxymethyl (TOM), acetyl (Ac), benzoyl (Bz), allyl carbonate, 2,2,2-trichloroethyl carbonate (Troc), 2-trimethylsilylethyl carbonate, methoxymethyl (MOM), 1-ethoxyethyl (EE), 2-methyoxy-2-propyl (MOP), 2,2,2-trichloroethoxyethyl, 2-methoxyethoxymethyl (MEM), 2-trimethylsilylethoxymethyl (SEM), methylthiomethyl (MTM), tetrahydropyranyl (THP), tetrahydrofuranyl (THF), p-methoxyphenyl (PMP), triphenylmethyl (Tr), methoxytrityl (MMT), dimethoxytrityl (DMTr), allyl, p-methoxybenzyl (PMB), t-butyl, benzyl (Bn), allyl, or pivaloyl (Piv).

In certain embodiments, the substituent present on a sulfur atom is a sulfur protecting group (also referred to as a "thiol protecting group"). Sulfur protecting groups include, but are not limited to, $-R^{aa}$, $-N(R^{bb})_2$, $-C(=O)SR^{aa}$, $-C(=O)R^{aa}$, $-CO_2R^{aa}$, $-C(=O)N(R^{bb})_2$, $-C(=NR^{bb})R^{aa}$, $-C(=NR^{bb})OR^{aa}$, $-C(=NR^{bb})N(R^{bb})_2$, $-S(=O)R^{aa}$, $-SO_2R^{aa}$, $-Si(R^{aa})_3$, $-P(R^{cc})_2$, $-P(R^{cc})_3{}^+X^-$, $-P(OR^{cc})_2$, $-P(OR^{cc})_3{}^+X^-$, $-P(=O)(R^{aa})_2$, $-P(=O)(OR^{cc})_2$, and $-P(=O)(N(R^{bb})_2)_2$, wherein $R^{aa}$, $R^{bb}$, and $R^{cc}$ are as defined herein. Sulfur protecting groups are well known in the art and include those described in detail in *Protecting Groups in Organic Synthesis*, T. W. Greene and P. G. M. Wuts, 3rd edition, John Wiley & Sons, 1999, incorporated herein by reference. In certain embodiments, a sulfur protecting group is acetamidomethyl, t-Bu, 3-nitro-2-pyridine sulfenyl, 2-pyridine-sulfenyl, or triphenylmethyl.

A "counterion" or "anionic counterion" is a negatively charged group associated with a positively charged group in order to maintain electronic neutrality. An anionic counterion may be monovalent (i.e., including one formal negative charge). An anionic counterion may also be multivalent (i.e., including more than one formal negative charge), such as divalent or trivalent. Exemplary counterions include halide ions (e.g., $F^-$, $Cl^-$, $Br^-$, $I^-$), $NO_3^-$, $ClO_4^-$, $OH^-$, $H_2PO_4^-$, $HCO_3^-$, $HSO_4^-$, sulfonate ions (e.g., methansulfonate, trifluoromethanesulfonate, *p*–toluenesulfonate, benzenesulfonate, 10–camphor sulfonate, naphthalene–2–sulfonate, naphthalene–1–sulfonic acid–5–sulfonate, ethan–1–sulfonic acid–2–sulfonate, and the like), carboxylate ions (e.g., acetate, propanoate, benzoate, glycerate, lactate, tartrate, glycolate, gluconate, and the like), $BF_4^-$, $PF_4^-$, $PF_6^-$, $AsF_6^-$, $SbF_6^-$, $B[3,5\text{-}(CF_3)_2C_6H_3]_4]^-$, $B(C_6F_5)_4^-$, $BPh_4^-$, $Al(OC(CF_3)_3)_4^-$, and carborane anions (e.g., $CB_{11}H_{12}^-$ or $(HCB_{11}Me_5Br_6)^-$). Exemplary counterions which may be multivalent include $CO_3^{2-}$, $HPO_4^{2-}$, $PO_4^{3-}$, $B_4O_7^{2-}$, $SO_4^{2-}$, $S_2O_3^{2-}$, carboxylate anions (e.g., tartrate, citrate, fumarate, maleate, malate, malonate, gluconate, succinate, glutarate, adipate, pimelate, suberate, azelate, sebacate, salicylate, phthalates, aspartate, glutamate, and the like), and carboranes.

As used herein, the term "salt" refers to any and all salts, and encompasses pharmaceutically acceptable salts.

The term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and/or animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and $N^+(C_{1-4}\ alkyl)_4^-$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

It is also to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed "isomers". Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers".

Stereoisomers that are not mirror images of one another are termed "diastereomers" and those that are non-superimposable mirror images of each other are termed "enantiomers". When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute

configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (i.e., as (+) or (−)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a "racemic mixture".

The term "Cytochrome P450 family 7 subfamily A member 1," used interchangeably with the term "CYP7A1," refers to the well-known gene and polypeptide, also known in the art as Cytochrome P450 family 7 subfamily A member 1.

The term "CYP7A1" includes human (*Homo sapiens)* CYP7A1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI: 1581**,** NCBI Accession Nos. NP_000771.2 and NM_000780.4, UniProt ID: P22680; mouse (*Mus musculus)* CYP7A1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI: 13122, NCBI Accession Nos. NP_031850.2 and NM_007824.3; UniProt ID: Q64505; rat (*Rattus norvegicus)* CYP7A1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI: 25428**,** NCBI Accession No. NP_037074.1 and NM_012942.2, UniProt ID: P18125; and chimpanzee *(Pan troglodytes*) CYP7A1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI: 464191, NCBI Accession No. XM_519773.3, UniProt ID: H2QW73. The term "CYP7A1" also includes cynomolgus monkey (*Macaca fascicularis*) CYP7A1, the amino acid and nucleotide sequence of which may be found in, for example, GenBank Accession No. GI: 102132452, NCBI Accession No. XP_005563427.1 and XM_005563370.2, UniProt ID: G7PBW5. Additional examples of CYP7A1 mRNA sequences are readily available using, e.g., GenBank, UniProt, OMIM, and the *Macaca* genome project web site. Exemplary CYP7A1 nucleotide and amino acid sequences may also be found in Table 1, SEQ ID NOs: 1-8.

The term "CYP7A1," as used herein, also refers to naturally occurring DNA sequence variations of the CYP7A1 gene. Numerous sequence variations within the CYP7A1 gene have been identified and may be found at, for example, NCBI dbSNP and UniProt (see, e.g., ncbi.nlm.nih.gov/snp).

Further information on CYP7A1 can be found, for example, at ncbi.nlm.nih.gov/gene/1581. 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.

Table 1 below summarizes exemplary amino acid sequences of CYP7A1 proteins and DNA sequence of CYP7A1 genes of various mammals.

Table 1: Exemplary Amino Acid and DNA Sequences of CYP7A1

| | Sequence | SEQ ID NO |
|---|---|---|
| Human CYP7A1 Nucleic Acid (NM_000780.4) | AGTGGCATCCTTCCCTTTCTAATCAGAGATTTTCTTCCTCAGAGATTTTGGCCTAGATTT<br>GCAAAATGATGACCACATCTTTGATTTGGGGGATTGCTATAGCAGCATGCTGTTGTCTAT<br>GGCTTATTCTTGGAATTAGGAGAAGGCAAACGGGTGAACCACCTCTAGAGAATGGATTAA<br>TTCCATACCTGGGCTGTGCTCTGCAATTTGGTGCCAATCCTCTTGAGTTCCTCAGAGCAA<br>ATCAAAGGAAACATGGTCATGTTTTTACCTGCAAACTAATGGGAAAATATGTCCATTTCA<br>TCACAAATCCCTTGTCATACCATAAGGTGTTGTGCCACGGAAAATATTTTGATTGGAAAA<br>AATTTCACTTTGCTACTTCTGCGAAGGCATTTGGGCACAGAAGCATTGACCCGATGGATG<br>GAAATACCACTGAAAACATAAACGACACTTTCATCAAAACCCTGCAGGGCCATGCCTTGA<br>ATTCCCTCACGGAAAGCATGATGGAAAACCTCCAACGTATCATGAGACCTCCAGTCTCCT<br>CTAACTCAAAGACCGCTGCCTGGGTGACAGAAGGGATGTATTCTTTCTGCTACCGAGTGA<br>TGTTTGAAGCTGGGTATTTAACTATCTTTGGCAGAGATCTTACAAGGCGGGACACACAGA<br>AAGCACATATTCTAAACAATCTTGACAACTTCAAGCAATTCGACAAAGTCTTTCCAGCCC<br>TGGTAGCAGGCCTCCCCATTCACATGTTCAGGACTGCGCACAATGCCCGGGAGAAACTGG<br>CAGAGAGCTTGAGGCACGAGAACCTCCAAAAGAGGGAAAGCATCTCAGAACTGATCAGCC<br>TGCGCATGTTTCTCAATGACACTTTGTCCACCTTTGATGATCTGGAGAAGGCCAAGACAC<br>ACCTCGTGGTCCTCTGGGCATCGCAAGCAAACACCATTCCAGCGACTTTCTGGAGTTTAT<br>TTCAAATGATTAGGAACCCAGAAGCAATGAAAGCAGCTACTGAAGAAGTGAAAAGAACAT<br>TAGAGAATGCTGGTCAAAAAGTCAGCTTGGAAGGCAATCCTATTTGTTTGAGTCAAGCAG<br>AACTGAATGACCTGCCAGTATTAGATAGTATAATCAAGGAATCGCTGAGGCTTTCCAGTG<br>CCTCCCTCAACATCCGGACAGCTAAGGAGGATTTCACTTTGCACCTTGAGGACGGTTCCT<br>ACAACATCCGAAAAGATGACATCATAGCTCTTTACCCACAGTTAATGCACTTAGATCCAG<br>AAATCTACCCAGACCCTTTGACTTTTAAATATGATAGGTATCTTGATGAAAACGGGAAGA<br>CAAAGACTACCTTCTATTGTAATGGACTCAAGTTAAAGTATTACTACATGCCCTTTGGAT<br>CGGGAGCTACAATATGTCCTGGAAGATTGTTCGCTATCCACGAAATCAAGCAATTTTTGA<br>TTCTGATGCTTTCTTATTTTGAATTGGAGCTTATAGAGGGCCAAGCTAAATGTCCACCTT<br>TGGACCAGTCCCGGGCAGGCTTGGGCATTTTGCCGCCATTGAATGATATTGAATTTAAAT<br>ATAAATTCAAGCATTTGTGAATACATGGCTGGAATAAGAGGACACTAGATGATATTACAG<br>GACTGCAGAACACCCTCACCACACAGTCCCTTTGGACAAATGCATTTAGTGGTGGTAGAA<br>ATGATTCACCAGGTCCAATGTTGTTCACCAGTGCTTGCTTGTGAATCTTAACATTTTGGT<br>GACAGTTTCCAGATGCTATCACAGACTCTGCTAGTGAAAAGAACTAGTTTCTAGGAGCAC<br>AATAATTTGTTTTCATTTGTATAAGTCCATGAATGTTCATATAGCCAGGGATTGAAGTTT<br>ATTATTTTCAAAGGAAAACACCTTTATTTTATTTTTTTTCAAAATGAAGATACACATTAC<br>AGCCAGGTGTGGTAGCAGGCACCTGTAGTCTTAGCTACTCGAGAGGCCAAAGAAGGAGGA<br>TGGCTTGAGCCCAGGAGTTCAAGACCAGCCTGGACAGCTTAGTGAGATCCCGTCTCCGAA<br>GAAAAGATATGTATTCTAATTGGCAGATTGTTTTTTCCTAAGGAAACTGCTTTATTTTTA<br>TAAAACTGCCTGACAATTATGAAAAAATGTTCAAATTCACGTTCTAGTGAAACTGCATTA<br>TTTGTTGACTAGATGGTGGGGTTCTTCGGGTGTGATCATATATCATAAAGGATATTTCAA<br>ATGATTATGATTAGTTATGTCTTTTAATAAAAAGGAAATATTTTTCAACTTCTTCTATAT<br>CCAAAATTCAGGGCTTTAAACATGATTATCTTGATTTCCCAAAAACACTAAAGGTGGTTT<br>TATTTTCCCTTCATGTTTTAACTTATTGTTGCTGAAAACTCTATGTCCGGCTTTAACTAT<br>CTTCTCTATATTTTTATTTCATTCACATTAATGAGAAGAGTTTTCTCAGAGATTAAAAAA<br>GGTAGTTTTTCTGTCATTGTTAAATACACATTATCACTGAAAAAATGTAGCTTTTATGTG<br>ATATGTTTTAAAGTTAAAACTGGATGGAAATAGCCATTTGGAAGCTTTGGTTATGAAACA<br>TGTGGAGTGTATTAAGTGCAGCTTGACATTATGTTTTATTTAAATGCTTTTTATCGCTAA<br>ATGACTTGCAGATGAAAAAAACTAAGGTGACTCGAGTGTTTAAATGCTGTGTACAACAAT<br>GCTTTGATAAAATATTTTAAGTATGAGTTATCAGCTCTATGTCAATTGATATTTCTGTGT<br>AGTATTTATATTTAAATTATATTTACCTTTTTGCTTATTTTACAAATATTAAGAAAATAT<br>TCTAACATTTGATAATTTTGAAATGATTCATCTTTCAGAAATAAAAGTATGAATCTA | 1 |

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|---|---|---|
| Human CYP7A1 Protein (NP_000 771.2) | MMTTSLIWGIAIAACCCLWLILGIRRRQTGEPPLENGLIPYLGCALQFGANPLEFLRANQ<br>RKHGHVFTCKLMGKYVHFITNPLSYHKVLCHGKYFDWKKFHFATSAKAFGHRSIDPMDGN<br>TTENINDTFIKTLQGHALNSLTESMMENLQRIMRPPVSSNSKTAAWVTEGMYSFCYRVMF<br>EAGYLTIFGRDLTRRDTQKAHILNNLDNFKQFDKVFPALVAGLPIHMFRTAHNAREKLAE<br>SLRHENLQKRESISELISLRMFLNDTLSTFDDLEKAKTHLVVLWASQANTIPATFWSLFQ<br>MIRNPEAMKAATEEVKRTLENAGQKVSLEGNPICLSQAELNDLPVLDSIIKESLRLSSAS<br>LNIRTAKEDFTLHLEDGSYNIRKDDIIALYPQLMHLDPEIYPDPLTFKYDRYLDENGKTK<br>TTFYCNGLKLKYYYMPFGSGATICPGRLFAIHEIKQFLILMLSYFELELIEGQAKCPPLD<br>QSRAGLGILPPLNDIEFKYKFKHL | 2 |
| Mouse CYP7A1 Nucleic Acid (NM_007 824.3) | GGACCGTCTTGCTTTGCTAAGCACAGATTCTCCCCTTGGGACGTTTTCCTGCTTTTGCAA<br>AATGATGAGCATTTCTTTGATCTGGGGGATTGCTGTGGTAGTGAGCTGTTGCATATGGTT<br>TATCATTGGAATAAGGAGAAGGAAAGTAGGTGAACCTCCTTTGGACAACGGGTTGATTCC<br>ATACCTGGGCTGTGCTCTGAAGTTCGGATCCAATCCTCTTGAATTCCTAAGAGCAAAGCA<br>AAGGAAACATGGCCATGTTTTTACCTGCAAACTGATGGGGAAATATGTCCACTTCATCAC<br>AAACTCCCTGTCATACCACAAAGTCTTATGTCACGGAAAATATTTTGACTGGAAAAAATT<br>TCATTACACTACTTCTGCGAAGGCATTTGGACACAGAAGCATAGACCCAAGTGATGGAAA<br>TACCACGGAAAACATAAACAAGACTTTTAACAAAACCCTCCAGGGAGATGCTCTGTGTTC<br>ACTCTCTGAAGCCATGATGCAAAACCTCCAATCTGTCATGAGACCTCCGGGCCTTCCTAA<br>ATCAAAGAGCGCTGTCTGGGTCACGGAAGGGATGTATGCCTTCTGCTACCGAGTGATGTT<br>TGAAGCCGGATATCTAACGCTGTTTGGCAAAGATATTTCAAAGACAGACTCACAAAGAGC<br>ATTTATTCAAAACAACCTTGACAGCTTCAAACAATTTGACCAAGTATTTCCGGCACTAGT<br>GGCGGGCGTCCCTATTCACTTGTTCAAGACCGCACATAAAGCCCGGGAAAGGCTGGCTGA<br>GAGCTTGAAGCACAAGAACCTGTACATGAGGGACCAGGTCTCTGAACTGATCCGTCTACG<br>CATGTTTCTCAACGATACACTCTCCACCTTTGATGACATGGAGAAGGCTAAGACGCACCT<br>CGTGATCCTCTGGGCATCTCAAGCAAACACCATTCCTGCAACCTTCTGGAGCTTATTTCA<br>AATGATCAGGAGCCCTGAAGCAATGAAAGCAGCCTCTGAAGAAGTGAATGGAGCATTACA<br>GAGTGCTGGCCAAGAGCTCAGCTCTGGAGGGAATGCCATTTACTTGGATCAAGAGCAACT<br>AAACAACCTGCCAGTACTAGATAGCATCATCAAGGAGGCTCTGCGGCTCTCCAGTGCATC<br>CTTGAATATCCGGACAGCTAAGGAGGACTTCACTCTACACCTTGAGGATGGTTCCTATAA<br>CATTCGAAAAGATGACATCATAGCTCTTTACCCACAGTTAATGCACTTGGATCCTGAAAT<br>CTACCCAGACCCTTTGACTTTTAAATACGACCGGTACCTTGATGAAAGTGGGAAAGCAAA<br>GACCACCTTCTATAGAAATGGAAACAAGCTGAAGTATTTCTACATGCCCTTTGGATCAGG<br>AGCTACAATATGTCCTGGAAGACTATTTGCTGTCCAAGAAATCAAGCAATTTTTGATTCT<br>GATGCTGTCTTACTTTGAACTGGAGCTTGTAGAGAGCCACACCAAGTGTCCCCCTCTAGA<br>TCAGTCCCGGGCAGGCTTGGGAATTTTGCCACCACTAAATGATATTGAGTTTAAATATAA<br>ACTGAAACACTGATATGTGGTTGGAAGAAGAGGACACTGGATGATGTAACGACTGCTGAG<br>CGTTATCAGTAAACAGGCCTTTGGGACTAGTGCTCACTGAAGCCCCCTAGTAGCTGTATT<br>AGTGAGAAGAACTCTGTTCTTACTGCTCACGTTCCTGGGGATTCGTGTAGCTGGGGCCTG<br>AGTTTCATCACTTTCAGAGCAACGTCTTTTGTTTTTATTTTCAAAATGAAGATATTCCAA<br>TTGGCAGGGTTCTTTCCTAAGGAAATTGCTTTATATTTTTATGAAAACTACCGATTAATT<br>ATGGAAGTACTTCAAATTCACGTTTTAGTGAAATTATTAATTTTTCACTAGTAAGGTTCT<br>TCATGTGTGAGTATATTATAAAAATGTTTTAGCTGATCATATCATGCTTTGCATAAAGGG<br>AAAGGAAATTATTGTTCAGCTTTTTTTTTATGGTGGTGAGAGCTTGAAAATGATCTTACT<br>ATTCTAGAACTACTAGGGAAGTTTCGACATGCTCTCGCTATATTTTAATTTATTGTTGCT<br>GGAGATTTTTATTCCAGTTTTGAACTACTTTATCTTTCCTTCTTTTTGACACACATACCA<br>ATAAGAAGAGCATTTTTCAGAAATTATAAAGGCACCTCCAAGAACCACACCATGAGTCTT<br>TTAAGCCTTTAATTCCAGCTCCCATGAGGCAAAGCCAGGCAAATCTCTATGAGTCTGAGG<br>TTATTCTGGTCTACATCAGCTCTAGACAAGCCAGGACTACAGAAAGGACCTTGTCTAAAA<br>AGAAAAAAAAGTTAATTTCTATGTCATATTTGATTATGAATCAACATGAAATATAAATTTA<br>AAATCAGGACTCAGAGAAATGATCAATTAAAAAACTTAGCTATGAAGTATGTGGAGTTCT<br>TTAAGTACAAGTTGACATTATATGTTCTTTTTTTTATGGTTTGTTGTAGAAAGGCACGGAG<br>AAAGAAAGAAGGTAGAAAGAGAGAGACACCGGCCATGGCCACGTGGAGAGAGGGGGGAAG<br>GGAAAGAGAGAAGGAGGCCTAGAGAGTAAGAAAGGTGAGGGCTTAAAGAGCTATATGCTC<br>TTTAAAAATGTTTTATGTTTTTATCTCTAAATGCCTTAAAGATGAAGAACAATAATGAAA<br>GGCTGAGTAATAGTGTTTAAATACTGAGTGCAATAGTGCTTTAGTAATATACTTTAAAGA<br>GAGTTATTAGCTATGTCATTTTTACTGAAAATATATTTATATATAAATTATATTTATCTT<br>TTTCTTATACCATATATATAAAAATATTGTAACATTTAGTAATTTTAAAATTACACACCT<br>TTCAGAAATTAACATATGAATGTTCGTGTTTTAAACTTTGAACAGAACATTTAAATTATT<br>CATCTACTGGTAAGCTACAATAATTTTTCTCATATTTATTTAAAATATTCATATTTTCAA<br>GAAATCCAAAAATATCCAAAGTAATCACTCAGTCAAATAGATCCCTAAGATGACAGTAAA<br>TTCAATACCATGCTTTTGTCTGCGAGGGCTGGAGCAGGGTCCAGTGGATGCTGTGCAGGT<br>CCTCCAGCAGAGAGCTAAATCCACAGACTCCTCAAAATCTTAAAACCTTACCACTGAATA | 3 |

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| | CTGGCACAGAGTTATTACCTAGGTACGCTATGCTTCCTTCCTTTTTCATTTTGACAGAGG<br>CCCATAGCATCCCAGGCTGGAACATTCCGCGTAGCTTAAGCAAGCTTGGAACTCACTGTG<br>CTTCCTGCTTTGCCTTGTTATAGGAAGTAATTTTCAAGACCAAGAAAACTTAATTGTTTA<br>GATGAGCTATAAGAAAGACACAATTGTGTTGTATACTAATCTGTACTAAGCTAAATTTGT<br>TTTTAAAACAAGTTTTATGACTCCCTGAACTGAATGTATCCACATACCTTCCCATGGCTT<br>TTTAAAAGCATATTTTTCATACAGAATGATGGGTCTCCTGGTGGTGCCTTCTTCTCATAT<br>TTTTATTCTTGTGAAGTGATGGCTAGCAAACAATAGCCTGTCAACCAAAGTTACTCTTCC<br>CGTTTCTGTGCAGCCTAAGTGTTAAAAGTAATTTTTACATTTTTAAATGCTTAGGGGAAA<br>AAAACCACATGTCTATTTTGTGTCATATACGAAGTATATGAAATACAGACTAGCAAACCC<br>GTGACTAAAACTTTTATTGGGATATCAGAATCTGCCTAATTCTTTGCGTTATGTCTACAA<br>TTCCATTTTTTGACATGTGTATGTAGTGTGCATTTTTGTGGAAGCATGTGTGAGTGTGTG<br>TGCACATGTGGGTAAACATGTGTGCATGTGTGTAGAGGCTGGAGGTGATGTTGAGTGTTT<br>TCCTCCACTGTTCTCCCTTGAGGGTCTCTCCCTTGAGCCAGAGTCCAATGCTTAGGCTAG<br>CATAGCCAACTTGCCGCAGTGCCCTTGAATTGCAGGAGGTTGCCACATCCTCCTGGCATT<br>TACCCTGGTGTGAGATGAGCTTCAGAAGGGCAAGTGCTTTGTCTGCTAGTCTCAGTGTCT<br>ACAGCTCTTGCTGTGTAATTGTAAAGGCAGGGTAGTGTGACATAGACAGGAGGACACAGA<br>GCTTACCATTTCAAAAATCAGTTTCTTCAGAGT | |
| Mouse CYP7A1 Protein (NP_031 850.2) | MMSISLIWGIAVVVSCCIWFIIGIRRRKVGEPPLDNGLIPYLGCALKFGSNPLEFLRAKQ<br>RKHGHVFTCKLMGKYVHFITNSLSYHKVLCHGKYFDWKKFHYTTSAKAFGHRSIDPSDGN<br>TTENINKTFNKTLQGDALCSLSEAMMQNLQSVMRPPGLPKSKSAVWVTEGMYAFCYRVMF<br>EAGYLTLFGKDISKTDSQRAFIQNNLDSFKQFDQVFPALVAGVPIHLFKTAHKARERLAE<br>SLKHKNLYMRDQVSELIRLRMFLNDTLSTFDDMEKAKTHLVILWASQANTIPATFWSLFQ<br>MIRSPEAMKAASEEVNGALQSAGQELSSGGNAIYLDQEQLNNLPVLDSIIKEALRLSSAS<br>LNIRTAKEDFTLHLEDGSYNIRKDDIIALYPQLMHLDPEIYPDPLTFKYDRYLDESGKAK<br>TTFYRNGNKLKYFYMPFGSGATICPGRLFAVQEIKQFLILMLSYFELELVESHTKCPPLD<br>QSRAGLGILPPLNDIEFKYKLKH | 4 |
| Rat CYP7A1 Nucleic Acid (NM_012 942.2) | GTCTCCCCTTTGGAAATTTTCCTGCTTTTGCAAAATGATGACTATTTCTTTGATTTGGGG<br>AATTGCCGTGTTGGTGAGCTGTTGCATATGGTTTATTGTTGGAATAAGGAGAAGGAAAGC<br>TGGTGAACCTCCTTTGGAGAACGGGTTGATTCCGTACCTGGGCTGTGCTCTGAAATTTGG<br>ATCTAATCCTCTTGAGTTCCTAAGAGCTAATCAAAGGAAGCATGGTCACGTTTTTACCTG<br>CAAACTGATGGGGAAATATGTCCATTTCATCACAAACTCCCTGTCATACCACAAAGTCTT<br>ATGTCATGGAAAATATTTTGACTGGAAAAAATTTCATTACACTACTTCTGCGAAGGCATT<br>TGGACACAGAAGCATTGACCCAAATGATGGAAATACCACGGAAAATATAAACAACACTTT<br>TACCAAAACCCTCCAGGGAGATGCTCTGTGTTCACTTTCTGAAGCCATGATGCAAAACCT<br>CCAATCTGTCATGAGACCTCCTGGCCTTCCTAAATCAAAGAGCAATGCCTGGGTCACGGA<br>AGGGATGTATGCCTTCTGTTACCGAGTGATGTTTGAAGCTGGCTATCTAACACTGTTTGG<br>CAGAGATATTTCAAAGACAGACACACAAAAAGCACTTATTCTAAACAACCTTGACAACTT<br>CAAACAATTTGACCAAGTCTTTCCGGCACTGGTGGCAGGCCTTCCTATTCACTTGTTCAA<br>GACCGCACATAAAGCTCGGGAAAAGCTGGCTGAGGGATTGAAGCACAAGAACCTGTGTGT<br>GAGGGACCAGGTCTCTGAACTGATCCGTCTACGTATGTTTCTCAATGACACGCTCTCCAC<br>CTTTGACGACATGGAGAAGGCCAAGACGCACCTCGCTATTCTCTGGGCATCTCAAGCAAA<br>CACCATTCCTGCAACCTTTTGGAGCTTATTTCAAATGATCAGGAGTCCTGAAGCAATGAA<br>AGCAGCCTCTGAAGAAGTGAGTGGAGCTTTACAGAGTGCTGGCCAAGAGCTCAGCTCTGG<br>AGGGAGTGCCATTTACTTGGATCAAGTGCAACTGAATGACCTGCCGGTACTAGACAGCAT<br>CATCAAGGAGGCTCTGAGGCTTTCCAGTGCATCCTTGAATATCCGCACAGCTAAGGAGGA<br>CTTCACTCTCCATCTTGAGGACGGTTCCTATAACATCCGAAAAGATGACATGATAGCTCT<br>TTATCCACAGTTAATGCACTTGGATCCTGAAATCTACCCAGACCCTTTGACTTTCAAATA<br>TGACCGGTACCTTGATGAAAGCGGGAAAGCAAAGACCACCTTCTACAGTAATGGAAACAA<br>GCTGAAGTGTTTCTACATGCCCTTCGGATCAGGCGCGACAATATGTCCTGGAAGACTCTT<br>TGCCGTCCAAGAAATCAAGCAGTTTTTGATCCTGATGCTCTCCTGCTTTGAACTGGAGTT<br>TGTGGAGAGCCAAGTCAAGTGTCCCCCTCTAGACCAGTCCCGGGCAGGCTTGGGAATTTT<br>GCCACCACTACATGATATTGAGTTTAAATATAAACTGAAACACTGATACGTGGTTGGAAG<br>AAGCGAACACTGGATGATGTCACTTGGCGGCTGAGAGTCATCACTAAACAGGCCTTCGGG<br>ACCAATGCTCACTGATGCGCCCTAGCGACTGGATTAGTGGGAAGAACTTTGTTCTCGCTG<br>CCCACATTCCTGGGTGTTCACATAGCTGGGGCCAGAGCTTCATCACTTTCAGAAAGCAAT<br>GTCTTTTGTATTTATTTTCAAAATGAAGATATTCCAATTGGCAGGATATTTTTCCTAAGG<br>AAATTGCTTTATATTTTTATGAAAACTACCAATTAATTATGAAAGGGCTTGAAATTCACG<br>TTTTAGTGAAATTACTGATTTTTCACTAGTAAGGTTCTTCAGGTGTGAAACTGTATTATA<br>AAAATGTTGTAATGGGTCACACTGTGCTTTGCATAAAGGTAAAGGAAACTATGTTTCAGC<br>CTTTTCTGTGTCTATGAGCTTCGAAAATAATCTTACTGTTCTAGAAACACTGGGGAGGTT<br>TCGACATGCTCTCGCTATATTTTATTTTACTGTTGCTAGAAATTTTCATTCCAGTTTTCA<br>ACTACCTTATCTTTCCCCCATTTTGACATGCATGCCAATGAGAAGAGTATTTTTTAGGAA | 5 |

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| | TTAACAAGGCACCTCCCAGAACCCTACCCTGAGACTTTTAAGCCTTTAATCCCAGCACTC<br>GAGAAGTAGAGCCAGGCAGATCTCTGAGTCTGAGGTTATTCTGGTCTACATCAGCTCCAG<br>ACAAGCCAGGACTACAGAATGGGATCTTGTCTAAAAAATACAGCTAATCTTTATGTCATA<br>ACTGATTATGAATCAACCTAAAAGATAAATTTTCAATCAGGACTCAGAGAAAATGAGCAA<br>TTAAAAAACTTAGCTCTGAGGTATGTGGAATTCATTAAGTACAAGTTGACATTACATGTT<br>CTTTAAAAATAGTTTATGTTTTATCTCTAAATGCCCTGCAGATGAAGAATAATAATGAAA<br>AGTTGAATAATACTGTTTAAACACTAAGTGCAATAATGCTTTGGTAATGTACTTTAAGAG<br>AATCATTAGCCGTGCCAGTTTTACTAAAATATATTTATATGTAAATTATATTTATCTTTT<br>TCTTATACCATAAATATAAAAATATTGCAACATTTAGTAATTTTAAAATTATATACCTTT<br>CAGAAAATGATGTATGAATGTTTGTATGTTTTTTAACTTTGAACAGAACATTTAAATTAT<br>TCATCTACGGTGATTTTTATCTTATTTATTTCTTTTTGTCTCATTCATATCTTGAAGAAA<br>TCCAAAAATATCTGAAGGAATCGCTCACTCAAATGTCTCCCTATGGTTACAGAAAAATTC<br>AATACCATGTTTTTGTCCTCGGGGACTGAAGCAGGGTGTCGTGGGTGCCGAGCAGAGGCT<br>CCTGCTGCAGCGAGCTTTATCCACGGGACTCCTTAAACTTTTAAAATCTTATCACTATTA<br>TCATGCATTTATTACCTAAGTAGGATATTTCCCTTTCCTTTTTCATTTCAGCCGAGTCCC<br>TTAGCAACCCAGGCTGACTGGGACCCTCCATGTAGCTTAAGCTGTGAACTCACTGTACTT<br>CCTGTTTTCACTTATTTTAGGAAGTAATTTTCCCTATCAGAAATTTTAATTGTTTAGATG<br>ATGTATAAGAGTAACACAATTCTGTTATATACTAATCTGTAGTAAACTAAATTTGTTCTT<br>AGAACAAGTTTGATGACTCTCAAATTGAATGTATCCATACATCTTTCCATGGCTTCTTGA<br>ATGCCCATTTCTCATACACAGAATGATGGGTTTCACGGTGATGTCTTCCTTTCATGTCTT<br>TATTCTTGTGCGGTGATGGTTGGCAAATGATACCCATGGAGCAAGGTTACTCTTCCTATT<br>TCTGTGCAGCCTAAGTGTTAAGAATAATTTTTAAATACTTGGAGGGAAGGCACATTTTGT<br>GTCATATGTGAAGTGACATGTGACACACAGACTAGCAAATCCTTGAGTAAAATTTTATTG<br>GGAT | |
| Rat CYP7A1 Protein (NP_037 074.1) | MMTISLIWGIAVLVSCCIWFIVGIRRRKAGEPPLENGLIPYLGCALKFGSNPLEFLRANQ<br>RKHGHVFTCKLMGKYVHFITNSLSYHKVLCHGKYFDWKKFHYTTSAKAFGHRSIDPNDGN<br>TTENINNTFTKTLQGDALCSLSEAMMQNLQSVMRPPGLPKSKSNAWVTEGMYAFCYRVMF<br>EAGYLTLFGRDISKTDTQKALILNNLDNFKQFDQVFPALVAGLPIHLFKTAHKAREKLAE<br>GLKHKNLCVRDQVSELIRLRMFLNDTLSTFDDMEKAKTHLAILWASQANTIPATFWSLFQ<br>MIRSPEAMKAASEEVSGALQSAGQELSSGGSAIYLDQVQLNDLPVLDSIIKEALRLSSAS<br>LNIRTAKEDFTLHLEDGSYNIRKDDMIALYPQLMHLDPEIYPDPLTFKYDRYLDESGKAK<br>TTFYSNGNKLKCFYMPFGSGATICPGRLFAVQEIKQFLILMLSCFELEFVESQVKCPPLD<br>QSRAGLGILPPLHDIEFKYKLKH | 6 |
| Cyno CYP7A1 Nucleic Acid (XM_00 5563370 .2) | GTCAACAAGCTCAAGTTAATGGATCTGGGTACTATGTATATAAAAAAACCTAGCTTGAGT<br>CTCTTTTCAGTGACATCTTTGCCTTTCTAATCAGAGATTTTCTTCCTCAGAGATTTTGGC<br>CTAGATTTGCAAAATGATGACCATATCTTTGATTTGGGGGATTGCTATAGCAGCATGCTG<br>TTGTCTATGGCTTATTCTTGGAATAAGGAGAAGGCAAACGGGTGAACCACCTCTAGAGAA<br>TGGGTTGATTCCATACCTGGGCTGTGCGCTGCAATTTGGTGCCAATCCTCTTGAGTTCCT<br>CAGAGCAAATCAAAGGAAACATGGTCATGTTTTTACCTGCAAACTAATGGGAAAATATGT<br>CCATTTCATCACAAATCCCTTGTCATACCATAAGGTGTTGTGCCATGGAAAATACTTTGA<br>TTGGAAAAAATTTCACTTTGCTACTTCTGCAAAGGCTTTTGGGCACAGAAGCATTGACCC<br>AAAGGATGGAAATACCACTGAAAACATAAACAACACTTTCATCAAAACCCTGCAGGGCAA<br>TGCCTTGAATTCCCTCACGGAAAGCATGATGGAAAACCTCCAACGTATCATGAGACCTCC<br>AGTCTTCTCTAACTCAAAGACCGCTGCCTGGGTGACAGAAGGGATGTATTCCTTCTGCTA<br>CCGAGTGATGTTTGAAGCTGGGTATTTAACTATCTTTGGCAGGGATCTTACAAGGCAAGA<br>CACACAGAAAGCACATATTCTAAACAATCTTGACAACTTCAAGCAGTTCGACAAAGTCTT<br>TCCAGCCCTGGTAGCAGGCCTCCCCATTCACATGTTCAGGACCGCGCACAGTGCCCGGGA<br>GAAACTGGCAGAGAGCTTGAGGCACGAGAACCTCCAAAAGAGGGAAAGCGTCTCAGAACT<br>GATCAGACTGCGCATGTTTCTCAATGACACTTTGTCCACCTTTGATGACCTGGAGAAGGC<br>CAAGACGCACCTCGTGGTCCTCTGGGCATCGCAAGCAAACACCATTCCAGCAACTTTCTG<br>GAGTTTATTTCAAATGATTAGGAACCCGGAAGCAATGAAAGCAGCTACTGAAGAAGTGAA<br>AAGAACATTAGAGAATGCTGGTCAAAAAGTCAGCTTGGAAGGCAATCCCATTTGTTTGAG<br>TCAAACACAACTGAATGACCTGCCAGTATTAGAAAGCATAATCAAGGAATCACTGAGGCT<br>TTCCAGTGCCTCCCTCAACATTCGGACAGCTAAGGAGGATTTCACTTTGCACCTTGAGGA<br>CGGTTCCTACAACATCCGAAAAGATGACATCATAGCTCTTTACCCACAGTTAATGCACTT<br>AGATCCAGAAATCTACCCAGACCCTTTGATTTTTAAATATGATAGGTATCTTGATGAAAA<br>CGGGAAGACAAAGACTACCTTCTACTGTAATGGACTCAAATTAAAGTATTACTACATGCC<br>CTTTGGATCAGGAGCTACAATATGCCCTGGAAGAGTGTTTGCTATCCACGAAATCAAGCA<br>ATTTTTGGTTTTGATGCTTTCTTATTTTGAACTGGAGCTTGTCGAGGGCCAAGATAAATG<br>TCCGCCTTTGGACCAGTCCCGGGCAGGCTTGGGCATTTTGCCGCCATTATATGACATTGA<br>GTTTAAATATAAATTCAAGCATTTGTGAATACGTGGCTGAAATAAGAGGACACTAGATGA<br>TATTACAGGACTGCAGAACACCATCACCACACAGTCCCTTTGGATAAATGCATTTAGTGG | 7 |

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|---|---|---|
| | TGGTAGAAATGATTCACCAGGTCCAATGTTGTTCACCAATGCTTGCTTGTGAATCTTAAT<br>ATTTTGGTGACAGTTTCCAGATGGTGTCGCAGACTGTGCTAGTGAAAAGAACTAGTTTCT<br>AGGAGCACGATAATTTGTTTTCATCTGTATGAGTCCATGAATGTTCATATAGCCAGGGGT<br>TGAAGTTTATTTTTTTCAGAGGAAAACACCTTTTTTTTTTTCCCCCCCCCAAAATGAAG<br>ATACACATTCCAGCCAGGTATTGTAGCAGGCACCTGTAGTCTTAGCTACTCGAGAGGCCA<br>AAGAAGGAGGATGGCTTGAGCCCAGGAGTTCAAGACTAGCCTGGACAGCTTAGTGAGACC<br>CCGTCTCTAAAGAAAAGATACGTATTCTAATTGGCAGATTGTTTTTTCCTAAGGAAACTG<br>CTTTATTTTTATAAAACTGCCCAACAATTATGAAACATGTTCAAATTCACGTTCTAGTGA<br>AACTGCATTATTTTTTTACTAGATGTTGGGGTTCTTCAGGTGTGATCATATATCATAAAG<br>GATATTTCAAATGATTATGATTAGTTATGTCTTTTAATAAAAAGGAAATACTTTTCAACT<br>TTTTATATATCCAAAATTCAGGGCTTTAAAAATGATTATTTTGATTTCCCAAAAACACTA<br>AAGGTGGTTTTAATATTTTCCCTTTATGTTTTAACTTATTGTTGCTGAAAACTCTATGTC<br>CAGCTTTAACTATCTTCTCTGTATTTTTATTTCATTTCATATTAATGAGAAGAGTTTTTT<br>TCAGAGATTAAAAAAGGCAGTTTTTCTGTTATTGTTAAATACACACTATCACTGAACAAA<br>TGTAGCTTTTATGTGATCTGTTTTAAAGTTAAAACCGGATGGAAATAGCCGTTTGAAAGC<br>TTTGGTTATGAAACATGCGGAGTGTATTAAGTACGGCTTGACATTATGTTTTATTTAAAT<br>GCTTTTTATCGCTAAATGACTTGCAGATGAAAAAAAACTAAGGTGGCCTGAGAGTTTAAA<br>TGCTGTGTACAACAATGCTTTGATAATATATTTTAAGTATGAGTTAT<br>CAGCTATATGTCAATTGATATTTCTGTGCAGTATTTATATGTAAATTATATTTACCTTTT<br>TGCTTATTTTATAAATATTAAGAAAGTATTCTAACATTTCATAATTTTGAAATGATTCAT<br>CTTTCAGAAATAAAAGTATGAATCTA | |
| Cyno CYP7A1 Protein (XP_005563427.1) | MMTISLIWGIAIAACCCLWLILGIRRRQTGEPPLENGLIPYLGCALQFGANPLEFLRANQ<br>RKHGHVFTCKLMGKYVHFITNPLSYHKVLCHGKYFDWKKFHFATSAKAFGHRSIDPKDGN<br>TTENINNTFIKTLQGNALNSLTESMMENLQRIMRPPVFSNSKTAAWVTEGMYSFCYRVMF<br>EAGYLTIFGRDLTRQDTQKAHILNNLDNFKQFDKVFPALVAGLPIHMFRTAHSAREKLAE<br>SLRHENLQKRESVSELIRLRMFLNDTLSTFDDLEKAKTHLVVLWASQANTIPATFWSLFQ<br>MIRNPEAMKAATEEVKRTLENAGQKVSLEGNPICLSQTQLNDLPVLESIIKESLRLSSAS<br>LNIRTAKEDFTLHLEDGSYNIRKDDIIALYPQLMHLDPEIYPDPLIFKYDRYLDENGKTK<br>TTFYCNGLKLKYYYMPFGSGATICPGRVFAIHEIKQFLVLMLSYFELELVEGQDKCPPLD<br>QSRAGLGILPPLYDIEFKYKFKHL | 8 |

As used herein, the term "CYP7A1 disease" or "CYP7A1-associated disease," is a disease or disorder that is caused by, or associated with, CYP7A1 expression and/or activity. The term "CYP7A1-associated disease" includes a disease, disorder or condition that would benefit from a decrease in CYP7A1 gene expression, replication, or protein activity. In some embodiments, a subject having a CYP7A1 disease or a CYP7A1-associated disease may benefit from the reduction in expression of CYP7A1 gene. In some embodiments, a CYP7A1 disease or CYP7A1-associated disease is a disease that is characterized by accumulation of toxic bile acids. A CYP7A1 disease or CYP7A1-associated disease may be a liver disease. In some embodiments, a CYP7A1-associated disease is a cholestatic liver disease. Cholestasis is resulted from disrupted bile flow from the liver to the intestine tract, leading to accumulation of toxic bile acids and other metabolites in the liver, decreased bile acids in the intestine and increased bile acids in the systemic circulation. The accumulation of toxic bile acids in the hepatobiliary system damages bile duct epithelial cells and hepatocytes, causing liver injury and inflammation. Chronic cholestasis leads to fibrosis, cirrhosis and eventually liver failure or hepatocellular or cholangiocellular carcinomas. Non-limiting examples of CYP7A1-associated diseases include, for example, cholestatic liver diseases such as primary sclerosing cholangitis (PSC), familial intrahepatic cholestasis (PFIC, including Type 1, PFIC1, Type 2, PFIC2, and

Type 3, PFIC3) and primary biliary cholangitis (PBC), Alagille syndrome, biliary atresia, and other liver diseases such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD).

Further details regarding signs and symptoms of the various diseases or conditions are provided herein and are well known in the art.

**Compositions**

*CYP7A1 RNAi agents*

Some aspects of the present disclosure provide RNAi agents that target CYP7A1 (referred to herein as "CYP7A1 RNAi agent") In some embodiments, a CYP7A1 RNAi agent disclosed herein targets a CYP7A1 sequence (e.g., gene sequence, pre-mRNA sequence, or mRNA sequence). In some embodiments, a CYP7A1 RNAi agent disclosed herein is a double stranded siRNA comprising a sense strand and an antisense strand. In some embodiments, the antisense strand of a CYP7A1 RNAi agent disclosed herein comprises a region of complementary to a CYP7A1 RNA (e.g., pre-mRNA or mRNA) sequence. In some embodiments, a CYP7A1 RNAi agent disclosed herein are useful for reducing levels of CYP7A1 RNA and/or protein.

In some embodiments, a CYP7A1 RNAi agent disclosed herein target a CYP7A1 sequence at or near a position corresponding to a position provided in Table 2. In some embodiments, a CYP7A1 RNAi agent disclosed herein is a siRNA oligonucleotide comprising an antisense strand comprising a region of complementary to a CYP7A1 sequence at or near a position in a CYP7A1 sequence corresponding to a position provided in Table 2. "Near" a position, as used herein, means within 10 (e.g., 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1) nucleosides upstream (5' end) or downstream (3' end) of the position.

As used herein, the "position" of a target sequence is represented by the "position" of the first nucleoside of the target sequence in a reference sequence. For example, for the CYP7A1 target sequences provided in Table 2, the reference sequence used is human CYP7A1 mRNA transcript and its corresponding cDNA sequence as set forth in NM_000780.4 (the reference sequence). The "position" for each target sequence provided in Table 2 refers to the position of the first nucleoside of a target sequence in SEQ ID NO: 1 (NM_000780.4). For example, the first C of the target sequence as set forth in SEQ ID NO: 9 is the 34$^{th}$ nucleoside of SEQ ID NO: 1 (NM_000780.4). For the purposes of the present disclosure, when referring to RNAi agents that target a CYP7A1 sequence at or near a position (e.g., position 34), such RNAi agents encompasses RNAi agents that target a CYP7A1 sequence at or near the position

in the reference sequence (SEQ ID NO: 1; NM_000780.4), and RNAi agents that target at or near a position in another CYP7A1 sequence (e.g., gene sequence, pre-mRNA sequence, or a variant or a homologue of human CYP7A1 mRNA, e.g., a splicing variant or a mRNA from a closely related species, such as a cynomolgus monkey) that corresponds to the position in the reference sequence. To identify a position in another CYP7A1 sequence that corresponds to a certain position in the reference sequence (SEQ ID NO: 1; NM_000780.4), such other CYP7A1 sequences can be aligned to the reference sequence and the position of a nucleoside corresponding the nucleoside in the reference sequence (SEQ ID NO: 1; NM_000780.4) can be determined. For example, the nucleosides at positions 34-52 of SEQ ID NO: 1 (NM_000780.4; upper sequence) are aligned to the nucleosides at positions 102-120 of the cynomolgus CYP7A1 sequence SEQ ID NO: 7 (XM_005563370.2; lower sequence) as follows:

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CTTCCTCAGAGATTTTGGC
||||||||||||||||||||
CTTCCTCAGAGATTTTGGC
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In the example above, when referring to RNAi agents that target a CYP7A1 sequence at or near position 34, the present disclosure encompasses RNAi agents that target at or near position 34 of NM_000780.4 (SEQ ID NO: 1) and RNAi agents that target at or near position 102 of XM_005563370.2 (SEQ ID NO: 7). In the present disclosure, unless otherwise indicated, the position of a nucleoside on another sequence is designated as the position of the corresponding nucleoside on the reference sequence. For example, in the exemplary alignment above, the first "C" of XM_005563370.2 (SEQ ID NO: 7) is designated as position 34 although it is the 102th nucleoside in XM_005563370.2 (SEQ ID NO: 7). In determining the position of a nucleoside of another sequence in relation to a reference sequence, the other sequence and the reference sequence are optimally aligned over the window of comparison, which comprises sufficient number of nucleosides for the alignment. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, Wis., USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., Nucl. Acids Res. 25:3389, 1997.

One or more insertions in another CYP7A1 sequence (e.g., gene sequence, pre-mRNA sequence, or a variant or a homologue of human CYP7A1 mRNA, e.g., a splicing variant or a

mRNA from a closely related species, such as a cynomolgus monkey) relative to the reference sequence (SEQ ID NO: 1; NM_000780.4) are represented as position "N-M," wherein N represents the corresponding position immediately before the insertions in the reference sequence, and M represents the position of the nucleoside within the one or more insertions. For example, in the following alignment between positions 2336-2349 of the sequence set forth in SEQ ID NO: 1 (NM_000780.4; upper sequence) and positions 2406-2422 of the sequence set forth in SEQ ID NO: 7 (XM_005563370.2; lower sequence),

```
GGTTT---TATTTTCCC
|||||    |||||||||
GGTTTTAATATTTTCCC
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The insertions TAA in SEQ ID NO: 7 (XM_005563370.2) follows the position 2340 (bolded T in the alignment above) in SEQ ID NO: 1 (NM_000780.4). Accordingly, the positions of insertions TAA are designated as 2340-1, 2340-2, and 2340-3, respectively.

One or more deletions or mismatches in another CYP7A1 sequence (e.g., gene sequence, pre-mRNA sequence, or a variant or a homologue of human CYP7A1 mRNA, e.g., a splicing variant or a mRNA from a closely related species, such as a cynomolgus monkey) relative to the reference sequence (SEQ ID NO: 1; NM_000780.4) may result in the lack of a "corresponding position." In these instances, as long as the deletions/mismatches (e.g., no more than 5, 4, 3, 2, 1 mismatches) are within a stretch of sequences of sufficient length (e.g., at least 20, 25, 30, 35, or 40 nucleosides) that aligned between the two sequences, the mismatched positions are represented as the position in the reference sequence that directly aligned even if the nucleoside in the reference is different. The position for the deletion in the other sequence is "skipped."

For example, the first 13 nucleosides (positions 1-13) of the SEQ ID NO: 1 (NM_000780.4; upper sequence) are aligned to the nucleosides at positions 69 – 82 of the cynomolgus CYP7A1 sequence SEQ ID NO: 7 (XM_005563370.2; lower sequence) as follows:

```
AGTGGCATCCTTC
|||| |||| ||·
AGTGACATCTTTG
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The bolded G at position 5 of SEQ ID NO: 1 (NM_000780.4) aligns with the bolded A at position 73 of SEQ ID NO: 7 (XM_005563370.2). When referring to RNAi agents that target a CYP7A1 sequence at or near position 5, the present disclosure encompasses RNAi agents that target at or near position 5 of NM_000780.4 (SEQ ID NO: 1) and RNAi agents that target at or near position 73 of XM_005563370.2 (SEQ ID NO: 7).

In some embodiments, the antisense strand of a CYP7A1 RNAi agent disclosed herein

comprises a core stretch of sequence that is fully, substantially, or at least partially complementary to a CYP7A1 target sequence disclosed in Table 2. CYP7A1 target sequences disclosed in Table 2 are 19 nucleosides in length. The core stretch of sequence that is fully, substantially, or at least partially complementary to a CYP7A1 target sequence disclosed in Table 2 may be, for example, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or 21 nucleosides in length.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-30 (e.g., 8-30, 8-25, 8-20, 8-15, 8-10, 10-30, 10-25, 10-20, 10-15, 15-30, 15-25, 15-20, 20-30, 20-25, or 25-30) nucleosides in length to a CYP7A1 sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, and 7 (e.g., SEQ ID NO: 1). In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleosides in length to a CYP7A1 sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, and 7 (e.g., SEQ ID NO: 1). In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of at least 15 (e.g., 15, 16, 17, 18, 19, 20, or 21) nucleosides in length to a CYP7A1 target sequence as set forth in any one of SEQ ID NOs: 1, 3, 5, and 7 (e.g., SEQ ID NO: 1).

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 113-133, 221-241, 249-269, 290-321, 475496, 504-524, 593-620, 671-691, 779-799, 839-862, 1003-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1235, 1225-1246, 1235-1255, 1289-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 113-133, 221-241, 249-269, 290-310, 301-321, 475-495, 476-496, 504-524, 593-613, 600-620, 671-691, 779-799, 839-859, 842-862, 1003-1023, 1009-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1227, 1215-1235, 1225-1245, 1226-1246, 1235-1255, 1289-1309, 1296-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 113-133, 475-495, 1226-1246, or 1235-1255 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 113-133 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 475-495 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 1226-1246 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence of nucleotides 1235-1255 of SEQ ID NO: 1.

In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8-21 (e.g., 8-21, 8-15, 8-10, 10-21, 10-15, 15-21, or 19-21) nucleosides in length to a CYP7A1 target sequence as set forth in any one of SEQ ID NOs: 9-776. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a region of complementary of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 nucleosides in length to a CYP7A1 target sequence as set forth in any one of SEQ ID NOs: 9-776. For the purposes of the present disclosure, a region of complementary need not be 100% complementary to that of its target to be specifically hybridizable or specific for a CYP7A1 sequence. In some embodiments, the region of complementarity is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% complementary to a target region in a CYP7A1 sequence. In some

embodiments, the target region is a region of consecutive nucleosides in the CYP7A1 sequence. In some embodiments, the region of complementarity comprises a nucleoside sequence that contains no more than 1, 2, 3, 4, or 5 base mismatches compared to the complementary portion of a CYP7A1 sequence. In some embodiments, the region of complementarity comprises a nucleoside sequence that has up to 3 mismatches over 15 nucleosides, or up to 2 mismatches over 10 nucleosides. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the sense strand is complementary or substantially complementary to the antisense strand.

In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the antisense strand is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the antisense strand is 19, 20, 21, 22, or 23 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the sense strand is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the sense strand is 19, 20, 21, 22, or 23 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the antisense strand is 19, 20, 21, 22, or 23 nucleosides in length and the sense strand is 19, 20, or 21 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAis agent disclosed herein, the antisense strand and the sense strand are of the same length, e.g., 19, 20, 21, 22, or 23 nucleosides in length. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the antisense strand and the sense strand are of different lengths. In some embodiments, in any one of the CYP7A1 RNAi agents disclosed herein, the antisense strand is 23 nucleosides in length and the sense strand is 21 nucleosides in length.

In some embodiments, a CYP7A1 RNAi agent disclosed herein may be blunt-ended on both ends. In some embodiments, a CYP7A1 RNAi agent disclosed herein may be blunt-ended on one end and has an overhang (5' or 3' overhang) on the other end. In some embodiments, a CYP7A1 RNAi agent disclosed herein has an overhang (5' or 3' overhang) on both ends. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 base pairs in length. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 19, 20, or 21 base pairs in length. In some embodiments, a CYP7A1 RNAi agent comprises an overhang (5' or 3' overhang) of at least 1 nucleoside on at least one strand. In some embodiments, a CYP7A1 RNAi agent comprises a 3' overhang of at least 1 nucleoside

on at least one strand. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 19, 20, 21 base pairs in length and comprises an overhang (5' or 3' overhang) of 1, 2, or 3 nucleosides in length on the sense strand. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 19, 20, 21 base pairs in length and comprises an overhang (5' or 3' overhang) of 1, 2, or 3 nucleosides in length on the antisense strand. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 19, 20, 21 base pairs in length and comprises a 3' overhang of 1, 2, or 3 nucleosides in length on the antisense strand. In some embodiments, a CYP7A1 RNAi agent disclosed herein comprises a duplex region of 19, 20, 21 base pairs in length and comprises a 3' overhang of 2 nucleosides on the antisense strand.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises a region of complementarity to a target sequence as set forth in any one of SEQ ID NOs: 9-776, wherein the region of complementarity is at least 15 nucleosides (e.g., 15, 16, 17, 18, 19, 20, 21, 22, or 23 nucleosides) in length. In some embodiments, the antisense strand is 23 nucleosides in length and comprises a region of complementarity to a target sequence as set forth in any one of SEQ ID NOs: 9-776, wherein the region of complementarity is 19, 20, 21, 22, or 23 nucleosides in length. In some embodiments, the region of complementarity is fully complementarity with all or a portion of its target sequence. In some embodiments, the region of complementarity includes 1, 2, 3 or more mismatches.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 777-1190, and a sense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 9-776.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand comprising nucleobases 2-21 (counting 5' → 3') of any one of SEQ ID NOs: 777-1190 and a sense strand comprising the nucleobase sequence of any one of SEQ ID NOs: 393-776.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190, and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776. In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand and a

sense strand, wherein the antisense strand and the sense strand form a duplex region, and wherein the antisense strand is fully complementary to the sense strand within the duplex region. In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand and a sense strand, wherein the antisense strand and the sense strand form a duplex region, wherein the antisense strand is complementary to the sense strand within the duplex region, and wherein there are 1, 2, 3, 4, or 5 mismatches between the sense strand and antisense strand.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises nucleobases 2-21 (counting 5' → 3') of a nucleobase sequence selected from (5'→3'):

(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)
(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)
(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)
(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)
(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)
(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)
(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)
(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)
(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)
(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)
(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and
(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises a nucleobase sequence selected from (5'→3'):

(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)
(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)
(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)
(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)
(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)
(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)
(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)
(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)
(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)

(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)

(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and

(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

In some embodiments, a CYP7A1 RNAi agent described herein comprises a sense strand that comprises a nucleobase sequence selected from (5'→3'):

(i) UCUUGAGUUCCUCAGAGCAAA (SEQ ID NO: 419);

(ii) GCGCAUGUUUCUCAAUGACAA (SEQ ID NO: 517);

(iii) UAGCUCUUUACCCACAGUUAA (SEQ ID NO: 597);

(iv) ACUGAAUGACCUGCCAGUAUA (SEQ ID NO: 566);

(v) UGUCCAUUUCAUCACAAAUCA (SEQ ID NO: 439);

(vi) CCCACAGUUAAUGCACUUAGA (SEQ ID NO: 604);

(vii) AGCUCUUUACCCACAGUUAAA (SEQ ID NO: 598);

(viii) UUGUCUAUGGCUUAUUCUUGA (SEQ ID NO: 404);

(ix) CCUUGAAUUCCCUCACGGAAA (SEQ ID NO: 464);

(x) UCACAAAUCCCUUGUCAUACA (SEQ ID NO: 443);

(xi) AUGUUUGAAGCUGGGUAUUUA (SEQ ID NO: 493); and

(xii) GAAAACCUCCAACGUAUCAUA (SEQ ID NO: 473).

In some embodiments, a CYP7A1 RNAi agent described herein comprises nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNAs listed in Table 5A, Table 7A, and Table 9.

In some embodiments, a CYP7A1 RNAi agent described herein comprises nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNAs selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272'. The siRNA numbers correspond to the siRNA numbers in Table 5A and Table 7A.

In some embodiments, a CYP7A1 RNAi agent described herein comprises nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNA molecule selected from siRNA27', siRNA125', siRNA205', siRNA174', siRNA47', siRNA212', siRNA206', siRNA12', siRNA72', siRNA51', sRNA101', and siRNA81'. The

siRNA numbers correspond to the siRNA numbers in Table 7A.

In some embodiments, a CYP7A1 RNAi agent described herein comprises nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNA molecule selected from siRNA72', siRNA206', siRNA12', and siRNA212'. The siRNA numbers correspond to the siRNA numbers in Table 7A.

It is to be understood that, for the purposes of the present disclosure, a CYP7A1 RNAi agent comprising nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNAs listed in Table 5A and Table 7A encompasses CYP7A1 RNAi agents comprising such nucleobase sequences and comprising no chemical modifications (e.g., modified nucleosides and/or modified internucleoside linkages), and CYP7A1 RNAi agents comprising such nucleobase sequences and comprising chemical modifications (e.g., one or more modified nucleosides and/or one or more modified internucleoside linkages; e.g., those provided in Table 5B, Table 7B, and Table 9), and encompasses such modified or unmodified RNAi agents unconjugated or conjugated to a targeting moiety.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand and sense strand, each comprising a structure as provided in Table 5B, Table 7B, or Table 9. In some embodiments, the CYP7A1RNAi agent comprises an antisense strand comprising a structure as set forth in any one of SEQ ID NOs: 1605-2054. In some embodiments, the CYP7A1 RNAi agent comprises a sense strand comprising a structure as set forth in any one of SEQ ID NOs: 1191-1604. In some embodiments, the CYP7A1 RNAi agent comprises an antisense strand comprising a structure as set forth in any one of SEQ ID NOs: 1605-2054 and a sense strand comprising a structure as set forth in any one of SEQ ID NOs: 1191-1604. In some embodiments, the CYP7A1 RNAi agent is selected from any one of the siRNAs listed in Tables 5B, 7B, and 9.

In some embodiments, an RNAi agent described herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) comprises a sense strand of 21 nucleosides in length and an antisense strand of 23 nucleosides in length, resulting in an siRNA having a 2 nucleoside overhang at the 3' end of the antisense strand. In some embodiments, the 2 nucleoside overhang at the 3' end of the antisense strand is UU (e.g., the siRNAs listed in Tables 5A and 5B). In some embodiments, the 2 nucleoside overhang at the 3' end of the antisense strand is AG (e.g., the siRNAs listed in Tables 7A and 7B, and 9). It is to be understood that the present disclosure contemplates and encompasses any one of the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9 with the 2 nucleoside overhang at the 3' end of the antisense strand being replaced with an overhang of a different nucleobase sequence (e.g., any known overhangs such as UG,

TG or TT) with the same or different chemical modifications (e.g., in the sugar moieties and/or the internucleoside linkages).

In some embodiments, an RNAi agent described herein has a U as the 5' terminal nucleoside of the antisense strand (i.e., position 1 counting from 5' to 3') (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9). It is to be understood that the present disclosure contemplates and encompasses any one of the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9 with the 5' terminal U of the antisense strand replaced with a different nucleobase (e.g., A, T, G, or C) with the same or different chemical modification (e.g., in the sugar moiety and/or the internucleoside linkage).

In some embodiments, any one or more of the uracil bases (U's) in any one of the target sequences provided herein (e.g., the target sequences provided in Table 2) may independently and optionally be thymine bases (T's).

In some embodiments, any one or more of the uracil bases (U's) in any one of the siRNAs provided herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) may independently and optionally be thymine bases (T's).

In some embodiments, any one or more of the purine bases (G's or A's) in any one of the siRNAs provided herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) may independently and optionally be a base that can base pair with a pyrimidine base, e.g., inosine bases (I's).

In some embodiments, any one or more of the pyrimidine bases (C's or U's) in any one of the siRNAs provided herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) may independently and optionally be a base that can base pair with a purine base, e.g., inosine bases (I's).

In some embodiments, any one of the siRNAs provided herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) may comprise:

(i) a sense strand that comprises a 5'-OH or 5'-O-methyl (5'-O-Me);

(ii) a sense strand that comprises a 3'-OH;

(iii) an antisense strand that comprises a 5'-OH, 5'-phosphate, or 5'-vinylphosphonate; and/or

(iv) an antisense strand that comprises a 3'-OH.

In some embodiments, any one of the siRNAs provided herein (e.g., the siRNAs listed in Tables 5A, 5B, 7A, 7B, and 9) may comprise:

(i) a sense strand that comprises a 5'-OH, or 5'-O-methyl (5'-O-Me);

(ii) a sense strand that is conjugated at the 3' end to a targeting moiety (e.g., via a

phosphorothioate linkage);

(iii) an antisense strand that comprises a 5'-OH, 5'-phosphate, or 5'-vinylphosphonate; and/or

(iv) an antisense strand that comprises a 3'-OH.

Table 2. CYP7A1 19-mer mRNA Target Sequences‡ (taken from human CYP7A1 mRNA transcript and cDNA sequence set forth in GenBank Accession No.NM_000780.4 (the RNA version of SEQ ID NO: 1, with all T's in SEQ ID NO: 1 replaced by U's))

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 9 | 34 | CUUCCUCAGAGAUUUUGGC |
| 10 | 40 | CAGAGAUUUUGGCCUAGAU |
| 11 | 41 | AGAGAUUUUGGCCUAGAUU |
| 12 | 42 | GAGAUUUUGGCCUAGAUUU |
| 13 | 43 | AGAUUUUGGCCUAGAUUUG |
| 14 | 45 | AUUUUGGCCUAGAUUUGCA |
| 15 | 105 | GCAUGCUGUUGUCUAUGGC |
| 16 | 110 | CUGUUGUCUAUGGCUUAUU |
| 17 | 111 | UGUUGUCUAUGGCUUAUUC |
| 18 | 112 | GUUGUCUAUGGCUUAUUCU |
| 19 | 113 | UUGUCUAUGGCUUAUUCUU |
| 20 | 115 | GUCUAUGGCUUAUUCUUGG |
| 21 | 116 | UCUAUGGCUUAUUCUUGGA |
| 22 | 117 | CUAUGGCUUAUUCUUGGAA |
| 23 | 118 | UAUGGCUUAUUCUUGGAAU |
| 24 | 139 | GGAGAAGGCAAACGGGUGA |
| 25 | 141 | AGAAGGCAAACGGGUGAAC |
| 26 | 142 | GAAGGCAAACGGGUGAACC |
| 27 | 143 | AAGGCAAACGGGUGAACCA |
| 28 | 181 | UUCCAUACCUGGGCUGUGC |
| 29 | 182 | UCCAUACCUGGGCUGUGCU |
| 30 | 183 | CCAUACCUGGGCUGUGCUC |
| 31 | 201 | CUGCAAUUUGGUGCCAAUC |
| 32 | 203 | GCAAUUUGGUGCCAAUCCU |
| 33 | 210 | GGUGCCAAUCCUCUUGAGU |
| 34 | 214 | CCAAUCCUCUUGAGUUCCU |
| 35 | 223 | UUGAGUUCCUCAGAGCAAA |
| 36 | 225 | GAGUUCCUCAGAGCAAAUC |
| 37 | 229 | UCCUCAGAGCAAAUCAAAG |
| 38 | 230 | CCUCAGAGCAAAUCAAAGG |
| 39 | 236 | AGCAAAUCAAAGGAAACAU |
| 40 | 238 | CAAAUCAAAGGAAACAUGG |
| 41 | 242 | UCAAAGGAAACAUGGUCAU |
| 42 | 243 | CAAAGGAAACAUGGUCAUG |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 43 | 244 | AAAGGAAACAUGGUCAUGU |
| 44 | 245 | AAGGAAACAUGGUCAUGUU |
| 45 | 246 | AGGAAACAUGGUCAUGUUU |
| 46 | 251 | ACAUGGUCAUGUUUUUACC |
| 47 | 253 | AUGGUCAUGUUUUUACCUG |
| 48 | 254 | UGGUCAUGUUUUUACCUGC |
| 49 | 262 | UUUUUACCUGCAAACUAAU |
| 50 | 264 | UUUACCUGCAAACUAAUGG |
| 51 | 266 | UACCUGCAAACUAAUGGGA |
| 52 | 267 | ACCUGCAAACUAAUGGGAA |
| 53 | 268 | CCUGCAAACUAAUGGGAAA |
| 54 | 291 | GUCCAUUUCAUCACAAAUC |
| 55 | 292 | UCCAUUUCAUCACAAAUCC |
| 56 | 294 | CAUUUCAUCACAAAUCCCU |
| 57 | 300 | AUCACAAAUCCCUUGUCAU |
| 58 | 302 | CACAAAUCCCUUGUCAUAC |
| 59 | 303 | ACAAAUCCCUUGUCAUACC |
| 60 | 304 | CAAAUCCCUUGUCAUACCA |
| 61 | 305 | AAAUCCCUUGUCAUACCAU |
| 62 | 309 | CCCUUGUCAUACCAUAAGG |
| 63 | 310 | CCUUGUCAUACCAUAAGGU |
| 64 | 311 | CUUGUCAUACCAUAAGGUG |
| 65 | 312 | UUGUCAUACCAUAAGGUGU |
| 66 | 313 | UGUCAUACCAUAAGGUGUU |
| 67 | 315 | UCAUACCAUAAGGUGUUGU |
| 68 | 319 | ACCAUAAGGUGUUGUGCCA |
| 69 | 360 | AAAUUUCACUUUGCUACUU |
| 70 | 363 | UUUCACUUUGCUACUUCUG |
| 71 | 372 | GCUACUUCUGCGAAGGCAU |
| 72 | 375 | ACUUCUGCGAAGGCAUUUG |
| 73 | 377 | UUCUGCGAAGGCAUUUGGG |
| 74 | 390 | UUUGGGCACAGAAGCAUUG |
| 75 | 392 | UGGGCACAGAAGCAUUGAC |
| 76 | 394 | GGCACAGAAGCAUUGACCC |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 77 | 474 | GCCUUGAAUUCCCUCACGG |
| 78 | 475 | CCUUGAAUUCCCUCACGGA |
| 79 | 476 | CUUGAAUUCCCUCACGGAA |
| 80 | 477 | UUGAAUUCCCUCACGGAAA |
| 81 | 478 | UGAAUUCCCUCACGGAAAG |
| 82 | 482 | UUCCCUCACGGAAAGCAUG |
| 83 | 484 | CCCUCACGGAAAGCAUGAU |
| 84 | 486 | CUCACGGAAAGCAUGAUGG |
| 85 | 488 | CACGGAAAGCAUGAUGGAA |
| 86 | 501 | AUGGAAAACCUCCAACGUA |
| 87 | 503 | GGAAAACCUCCAACGUAUC |
| 88 | 505 | AAAACCUCCAACGUAUCAU |
| 89 | 506 | AAACCUCCAACGUAUCAUG |
| 90 | 507 | AACCUCCAACGUAUCAUGA |
| 91 | 509 | CCUCCAACGUAUCAUGAGA |
| 92 | 510 | CUCCAACGUAUCAUGAGAC |
| 93 | 511 | UCCAACGUAUCAUGAGACC |
| 94 | 512 | CCAACGUAUCAUGAGACCU |
| 95 | 513 | CAACGUAUCAUGAGACCUC |
| 96 | 514 | AACGUAUCAUGAGACCUCC |
| 97 | 539 | CUCUAACUCAAAGACCGCU |
| 98 | 552 | ACCGCUGCCUGGGUGACAG |
| 99 | 562 | GGGUGACAGAAGGGAUGUA |
| 100 | 563 | GGUGACAGAAGGGAUGUAU |
| 101 | 564 | GUGACAGAAGGGAUGUAUU |
| 102 | 585 | UUCUGCUACCGAGUGAUGU |
| 103 | 587 | CUGCUACCGAGUGAUGUUU |
| 104 | 588 | UGCUACCGAGUGAUGUUUG |
| 105 | 589 | GCUACCGAGUGAUGUUUGA |
| 106 | 593 | CCGAGUGAUGUUUGAAGCU |
| 107 | 594 | CGAGUGAUGUUUGAAGCUG |
| 108 | 595 | GAGUGAUGUUUGAAGCUGG |
| 109 | 602 | GUUUGAAGCUGGGUAUUUA |
| 110 | 605 | UGAAGCUGGGUAUUUAACU |
| 111 | 606 | GAAGCUGGGUAUUUAACUA |
| 112 | 607 | AAGCUGGGUAUUUAACUAU |
| 113 | 608 | AGCUGGGUAUUUAACUAUC |
| 114 | 609 | GCUGGGUAUUUAACUAUCU |
| 115 | 610 | CUGGGUAUUUAACUAUCUU |
| 116 | 612 | GGGUAUUUAACUAUCUUUG |
| 117 | 613 | GGUAUUUAACUAUCUUUGG |
| 118 | 653 | CACACAGAAAGCACAUAUU |
| 119 | 663 | GCACAUAUUCUAAACAAUC |
| 120 | 665 | ACAUAUUCUAAACAAUCUU |
| 121 | 666 | CAUAUUCUAAACAAUCUUG |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 122 | 667 | AUAUUCUAAACAAUCUUGA |
| 123 | 669 | AUUCUAAACAAUCUUGACA |
| 124 | 670 | UUCUAAACAAUCUUGACAA |
| 125 | 671 | UCUAAACAAUCUUGACAAC |
| 126 | 673 | UAAACAAUCUUGACAACUU |
| 127 | 679 | AUCUUGACAACUUCAAGCA |
| 128 | 712 | UUCCAGCCCUGGUAGCAGG |
| 129 | 771 | GAGAAACUGGCAGAGAGCU |
| 130 | 781 | CAGAGAGCUUGAGGCACGA |
| 131 | 788 | CUUGAGGCACGAGAACCUC |
| 132 | 841 | UGCGCAUGUUUCUCAAUGA |
| 133 | 844 | GCAUGUUUCUCAAUGACAC |
| 134 | 846 | AUGUUUCUCAAUGACACUU |
| 135 | 850 | UUCUCAAUGACACUUUGUC |
| 136 | 851 | UCUCAAUGACACUUUGUCC |
| 137 | 852 | CUCAAUGACACUUUGUCCA |
| 138 | 861 | ACUUUGUCCACCUUUGAUG |
| 139 | 862 | CUUUGUCCACCUUUGAUGA |
| 140 | 901 | ACCUCGUGGUCCUCUGGGC |
| 141 | 903 | CUCGUGGUCCUCUGGGCAU |
| 142 | 904 | UCGUGGUCCUCUGGGCAUC |
| 143 | 905 | CGUGGUCCUCUGGGCAUCG |
| 144 | 906 | GUGGUCCUCUGGGCAUCGC |
| 145 | 912 | CUCUGGGCAUCGCAAGCAA |
| 146 | 915 | UGGGCAUCGCAAGCAAACA |
| 147 | 916 | GGGCAUCGCAAGCAAACAC |
| 148 | 917 | GGCAUCGCAAGCAAACACC |
| 149 | 920 | AUCGCAAGCAAACACCAUU |
| 150 | 921 | UCGCAAGCAAACACCAUUC |
| 151 | 950 | CUGGAGUUUAUUUCAAAUG |
| 152 | 954 | AGUUUAUUUCAAAUGAUUA |
| 153 | 955 | GUUUAUUUCAAAUGAUUAG |
| 154 | 956 | UUUAUUUCAAAUGAUUAGG |
| 155 | 957 | UUAUUUCAAAUGAUUAGGA |
| 156 | 980 | AGAAGCAAUGAAAGCAGCU |
| 157 | 982 | AAGCAAUGAAAGCAGCUAC |
| 158 | 983 | AGCAAUGAAAGCAGCUACU |
| 159 | 988 | UGAAAGCAGCUACUGAAGA |
| 160 | 989 | GAAAGCAGCUACUGAAGAA |
| 161 | 992 | AGCAGCUACUGAAGAAGUG |
| 162 | 993 | GCAGCUACUGAAGAAGUGA |
| 163 | 994 | CAGCUACUGAAGAAGUGAA |
| 164 | 995 | AGCUACUGAAGAAGUGAAA |
| 165 | 996 | GCUACUGAAGAAGUGAAAA |
| 166 | 1005 | GAAGUGAAAAGAACAUUAG |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 167 | 1006 | AAGUGAAAAGAACAUUAGA |
| 168 | 1007 | AGUGAAAAGAACAUUAGAG |
| 169 | 1011 | AAAAGAACAUUAGAGAAUG |
| 170 | 1012 | AAAGAACAUUAGAGAAUGC |
| 171 | 1013 | AAGAACAUUAGAGAAUGCU |
| 172 | 1018 | CAUUAGAGAAUGCUGGUCA |
| 173 | 1019 | AUUAGAGAAUGCUGGUCAA |
| 174 | 1020 | UUAGAGAAUGCUGGUCAAA |
| 175 | 1021 | UAGAGAAUGCUGGUCAAAA |
| 176 | 1026 | AAUGCUGGUCAAAAAGUCA |
| 177 | 1027 | AUGCUGGUCAAAAAGUCAG |
| 178 | 1029 | GCUGGUCAAAAAGUCAGCU |
| 179 | 1036 | AAAAAGUCAGCUUGGAAGG |
| 180 | 1039 | AAGUCAGCUUGGAAGGCAA |
| 181 | 1042 | UCAGCUUGGAAGGCAAUCC |
| 182 | 1084 | UGAAUGACCUGCCAGUAUU |
| 183 | 1087 | AUGACCUGCCAGUAUUAGA |
| 184 | 1134 | UCCAGUGCCUCCCUCAACA |
| 185 | 1151 | CAUCCGGACAGCUAAGGAG |
| 186 | 1152 | AUCCGGACAGCUAAGGAGG |
| 187 | 1155 | CGGACAGCUAAGGAGGAUU |
| 188 | 1158 | ACAGCUAAGGAGGAUUUCA |
| 189 | 1160 | AGCUAAGGAGGAUUUCACU |
| 190 | 1162 | CUAAGGAGGAUUUCACUUU |
| 191 | 1165 | AGGAGGAUUUCACUUUGCA |
| 192 | 1168 | AGGAUUUCACUUUGCACCU |
| 193 | 1169 | GGAUUUCACUUUGCACCUU |
| 194 | 1174 | UCACUUUGCACCUUGAGGA |
| 195 | 1177 | CUUUGCACCUUGAGGACGG |
| 196 | 1178 | UUUGCACCUUGAGGACGGU |
| 197 | 1189 | AGGACGGUUCCUACAACAU |
| 198 | 1191 | GACGGUUCCUACAACAUCC |
| 199 | 1194 | GGUUCCUACAACAUCCGAA |
| 200 | 1196 | UUCCUACAACAUCCGAAAA |
| 201 | 1200 | UACAACAUCCGAAAAGAUG |
| 202 | 1201 | ACAACAUCCGAAAAGAUGA |
| 203 | 1203 | AACAUCCGAAAAGAUGACA |
| 204 | 1204 | ACAUCCGAAAAGAUGACAU |
| 205 | 1205 | CAUCCGAAAAGAUGACAUC |
| 206 | 1207 | UCCGAAAAGAUGACAUCAU |
| 207 | 1209 | CGAAAAGAUGACAUCAUAG |
| 208 | 1212 | AAAGAUGACAUCAUAGCUC |
| 209 | 1214 | AGAUGACAUCAUAGCUCUU |
| 210 | 1215 | GAUGACAUCAUAGCUCUUU |
| 211 | 1217 | UGACAUCAUAGCUCUUUAC |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 212 | 1226 | AGCUCUUUACCCACAGUUA |
| 213 | 1227 | GCUCUUUACCCACAGUUAA |
| 214 | 1228 | CUCUUUACCCACAGUUAAU |
| 215 | 1229 | UCUUUACCCACAGUUAAUG |
| 216 | 1230 | CUUUACCCACAGUUAAUGC |
| 217 | 1231 | UUUACCCACAGUUAAUGCA |
| 218 | 1233 | UACCCACAGUUAAUGCACU |
| 219 | 1236 | CCACAGUUAAUGCACUUAG |
| 220 | 1237 | CACAGUUAAUGCACUUAGA |
| 221 | 1238 | ACAGUUAAUGCACUUAGAU |
| 222 | 1239 | CAGUUAAUGCACUUAGAUC |
| 223 | 1240 | AGUUAAUGCACUUAGAUCC |
| 224 | 1241 | GUUAAUGCACUUAGAUCCA |
| 225 | 1242 | UUAAUGCACUUAGAUCCAG |
| 226 | 1248 | CACUUAGAUCCAGAAAUCU |
| 227 | 1249 | ACUUAGAUCCAGAAAUCUA |
| 228 | 1261 | AAAUCUACCCAGACCCUUU |
| 229 | 1283 | UUUUAAAUAUGAUAGGUAU |
| 230 | 1285 | UUAAAUAUGAUAGGUAUCU |
| 231 | 1286 | UAAAUAUGAUAGGUAUCUU |
| 232 | 1288 | AAUAUGAUAGGUAUCUUGA |
| 233 | 1290 | UAUGAUAGGUAUCUUGAUG |
| 234 | 1291 | AUGAUAGGUAUCUUGAUGA |
| 235 | 1292 | UGAUAGGUAUCUUGAUGAA |
| 236 | 1293 | GAUAGGUAUCUUGAUGAAA |
| 237 | 1296 | AGGUAUCUUGAUGAAAACG |
| 238 | 1297 | GGUAUCUUGAUGAAAACGG |
| 239 | 1298 | GUAUCUUGAUGAAAACGGG |
| 240 | 1299 | UAUCUUGAUGAAAACGGGA |
| 241 | 1300 | AUCUUGAUGAAAACGGGAA |
| 242 | 1301 | UCUUGAUGAAAACGGGAAG |
| 243 | 1302 | CUUGAUGAAAACGGGAAGA |
| 244 | 1303 | UUGAUGAAAACGGGAAGAC |
| 245 | 1306 | AUGAAAACGGGAAGACAAA |
| 246 | 1307 | UGAAAACGGGAAGACAAAG |
| 247 | 1311 | AACGGGAAGACAAAGACUA |
| 248 | 1313 | CGGGAAGACAAAGACUACC |
| 249 | 1314 | GGGAAGACAAAGACUACCU |
| 250 | 1353 | UUAAAGUAUUACUACAUGC |
| 251 | 1354 | UAAAGUAUUACUACAUGCC |
| 252 | 1358 | GUAUUACUACAUGCCCUUU |
| 253 | 1360 | AUUACUACAUGCCCUUUGG |
| 254 | 1361 | UUACUACAUGCCCUUUGGA |
| 255 | 1363 | ACUACAUGCCCUUUGGAUC |
| 256 | 1364 | CUACAUGCCCUUUGGAUCG |

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| 258 | 1386 | GCUACAAUAUGUCCUGGAA |
| 259 | 1388 | UACAAUAUGUCCUGGAAGA |
| 260 | 1389 | ACAAUAUGUCCUGGAAGAU |
| 261 | 1413 | GCUAUCCACGAAAUCAAGC |
| 262 | 1415 | UAUCCACGAAAUCAAGCAA |
| 263 | 1416 | AUCCACGAAAUCAAGCAAU |
| 264 | 1417 | UCCACGAAAUCAAGCAAUU |
| 265 | 1424 | AAUCAAGCAAUUUUUGAUU |
| 266 | 1425 | AUCAAGCAAUUUUUGAUUC |
| 267 | 1431 | CAAUUUUUGAUUCUGAUGC |
| 268 | 1433 | AUUUUUGAUUCUGAUGCUU |
| 269 | 1506 | CAGUCCCGGGCAGGCUUGG |
| 270 | 1507 | AGUCCCGGGCAGGCUUGGG |
| 271 | 1510 | CCCGGGCAGGCUUGGGCAU |
| 272 | 1511 | CCGGGCAGGCUUGGGCAUU |
| 273 | 1513 | GGGCAGGCUUGGGCAUUUU |
| 274 | 1518 | GGCUUGGGCAUUUUGCCGC |
| 275 | 1555 | UUAAAUAUAAAUUCAAGCA |
| 276 | 1556 | UAAAUAUAAAUUCAAGCAU |
| 277 | 1557 | AAAUAUAAAUUCAAGCAUU |
| 278 | 1558 | AAUAUAAAUUCAAGCAUUU |
| 279 | 1559 | AUAUAAAUUCAAGCAUUUG |
| 280 | 1561 | AUAAAUUCAAGCAUUUGUG |
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| 282 | 1594 | AUAAGAGGACACUAGAUGA |
| 283 | 1597 | AGAGGACACUAGAUGAUAU |
| 284 | 1599 | AGGACACUAGAUGAUAUUA |
| 285 | 1601 | GACACUAGAUGAUAUUACA |
| 286 | 1605 | CUAGAUGAUAUUACAGGAC |
| 287 | 1606 | UAGAUGAUAUUACAGGACU |
| 288 | 1609 | AUGAUAUUACAGGACUGCA |
| 289 | 1610 | UGAUAUUACAGGACUGCAG |
| 290 | 1614 | AUUACAGGACUGCAGAACA |
| 291 | 1638 | ACCACACAGUCCCUUUGGA |
| 292 | 1660 | AUGCAUUUAGUGGUGGUAG |
| 293 | 1661 | UGCAUUUAGUGGUGGUAGA |
| 294 | 1662 | GCAUUUAGUGGUGGUAGAA |
| 295 | 1663 | CAUUUAGUGGUGGUAGAAA |
| 296 | 1669 | GUGGUGGUAGAAAUGAUUC |
| 297 | 1671 | GGUGGUAGAAAUGAUUCAC |
| 298 | 1673 | UGGUAGAAAUGAUUCACCA |
| 299 | 1676 | UAGAAAUGAUUCACCAGGU |
| 300 | 1683 | GAUUCACCAGGUCCAAUGU |
| 301 | 1686 | UCACCAGGUCCAAUGUUGU |

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| 305 | 1712 | UGCUUGCUUGUGAAUCUUA |
| 306 | 1713 | GCUUGCUUGUGAAUCUUAA |
| 307 | 1769 | UGCUAGUGAAAAGAACUAG |
| 308 | 1770 | GCUAGUGAAAAGAACUAGU |
| 309 | 1824 | AGUCCAUGAAUGUUCAUAU |
| 310 | 1825 | GUCCAUGAAUGUUCAUAUA |
| 311 | 1826 | UCCAUGAAUGUUCAUAUAG |
| 312 | 1827 | CCAUGAAUGUUCAUAUAGC |
| 313 | 1828 | CAUGAAUGUUCAUAUAGCC |
| 314 | 1829 | AUGAAUGUUCAUAUAGCCA |
| 315 | 1830 | UGAAUGUUCAUAUAGCCAG |
| 316 | 1895 | UUUUUCAAAAUGAAGAUAC |
| 317 | 2052 | UAUUCUAAUUGGCAGAUUG |
| 318 | 2053 | AUUCUAAUUGGCAGAUUGU |
| 319 | 2075 | UUCCUAAGGAAACUGCUUU |
| 320 | 2127 | AUGUUCAAAUUCACGUUCU |
| 321 | 2128 | UGUUCAAAUUCACGUUCUA |
| 322 | 2132 | CAAAUUCACGUUCUAGUGA |
| 323 | 2133 | AAAUUCACGUUCUAGUGAA |
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| 329 | 2140 | CGUUCUAGUGAAACUGCAU |
| 330 | 2144 | CUAGUGAAACUGCAUUAUU |
| 331 | 2189 | GGUGUGAUCAUAUAUCAUA |
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| 333 | 2191 | UGUGAUCAUAUAUCAUAAA |
| 334 | 2192 | GUGAUCAUAUAUCAUAAAG |
| 335 | 2193 | UGAUCAUAUAUCAUAAAGG |
| 336 | 2194 | GAUCAUAUAUCAUAAAGGA |
| 337 | 2195 | AUCAUAUAUCAUAAAGGAU |
| 338 | 2199 | UAUAUCAUAAAGGAUAUUU |
| 339 | 2209 | AGGAUAUUUCAAAUGAUUA |
| 340 | 2210 | GGAUAUUUCAAAUGAUUAU |
| 341 | 2211 | GAUAUUUCAAAUGAUUAUG |
| 342 | 2215 | UUUCAAAUGAUUAUGAUUA |
| 343 | 2216 | UUCAAAUGAUUAUGAUUAG |
| 344 | 2217 | UCAAAUGAUUAUGAUUAGU |
| 345 | 2218 | CAAAUGAUUAUGAUUAGUU |
| 346 | 2220 | AAUGAUUAUGAUUAGUUAU |

| SEQ ID NO. | Position | Sequence |
|---|---|---|
| 347 | 2221 | AUGAUUAUGAUUAGUUAUG |
| 348 | 2223 | GAUUAUGAUUAGUUAUGUC |
| 349 | 2224 | AUUAUGAUUAGUUAUGUCU |
| 350 | 2225 | UUAUGAUUAGUUAUGUCUU |
| 351 | 2313 | GAUUUCCCAAAAACACUAA |
| 352 | 2316 | UUCCCAAAAACACUAAAGG |
| 353 | 2317 | UCCCAAAAACACUAAAGGU |
| 354 | 2319 | CCAAAAACACUAAAGGUGG |
| 355 | 2320 | CAAAAACACUAAAGGUGGU |
| 356 | 2321 | AAAAACACUAAAGGUGGUU |
| 357 | 2353 | AUGUUUUAACUUAUUGUUG |
| 358 | 2355 | GUUUUAACUUAUUGUUGCU |
| 359 | 2356 | UUUUAACUUAUUGUUGCUG |
| 360 | 2362 | CUUAUUGUUGCUGAAAACU |
| 361 | 2364 | UAUUGUUGCUGAAAACUCU |
| 362 | 2365 | AUUGUUGCUGAAAACUCUA |
| 363 | 2366 | UUGUUGCUGAAAACUCUAU |
| 364 | 2370 | UGCUGAAAACUCUAUGUCC |
| 365 | 2503 | AAAUGUAGCUUUUAUGUGA |
| 366 | 2562 | AAGCUUUGGUUAUGAAACA |
| 367 | 2563 | AGCUUUGGUUAUGAAACAU |
| 368 | 2620 | UUAAAUGCUUUUUAUCGCU |
| 369 | 2621 | UAAAUGCUUUUUAUCGCUA |
| 370 | 2622 | AAAUGCUUUUUAUCGCUAA |
| 371 | 2623 | AAUGCUUUUUAUCGCUAAA |
| 372 | 2624 | AUGCUUUUUAUCGCUAAAU |
| 373 | 2625 | UGCUUUUUAUCGCUAAAUG |
| 374 | 2629 | UUUUAUCGCUAAAUGACUU |
| 375 | 2630 | UUUAUCGCUAAAUGACUUG |
| 376 | 2632 | UAUCGCUAAAUGACUUGCA |
| 377 | 2633 | AUCGCUAAAUGACUUGCAG |
| 378 | 2635 | CGCUAAAUGACUUGCAGAU |
| 379 | 2639 | AAAUGACUUGCAGAUGAAA |
| 380 | 2679 | UUUAAAUGCUGUGUACAAC |
| 381 | 2682 | AAAUGCUGUGUACAACAAU |
| 382 | 2683 | AAUGCUGUGUACAACAAUG |
| 383 | 2687 | CUGUGUACAACAAUGCUUU |
| 384 | 2690 | UGUACAACAAUGCUUUGAU |
| 385 | 2691 | GUACAACAAUGCUUUGAUA |
| 386 | 2692 | UACAACAAUGCUUUGAUAA |
| 387 | 2833 | UAAUUUUGAAAUGAUUCAU |
| 388 | 2834 | AAUUUUGAAAUGAUUCAUC |
| 389 | 2835 | AUUUUGAAAUGAUUCAUCU |
| 390 | 2838 | UUGAAAUGAUUCAUCUUUC |
| 391 | 2839 | UGAAAUGAUUCAUCUUUCA |
| 392 | 2858 | GAAAUAAAAGUAUGAAUCU |

*‡Each uracil base (U) in any one of the target sequences provided in Table 2 may independently and optionally be replaced with a thymine base (T).*

*Modified RNAi agents (e.g., modified CYP7A1 RNAi agents)*

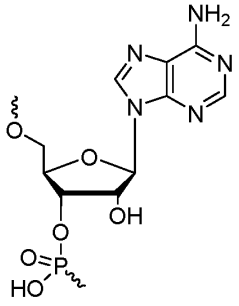
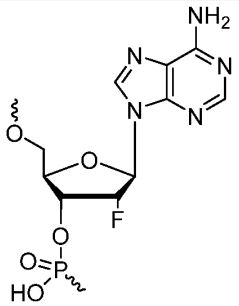
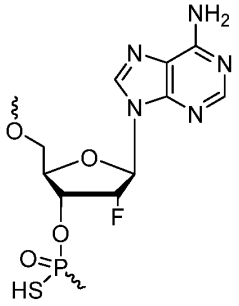
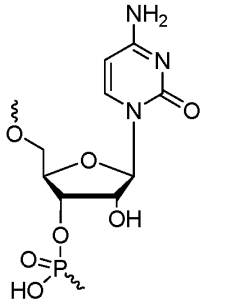
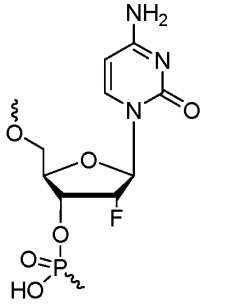
Some aspects of the present disclosure provide modified RNAi agents (e.g., modified CYP7A1 RNAi agents). In some embodiments, a modified RNAi agent (e.g., modified CYP7A1 RNAi agent) comprise modified nucleosides and/or modified internucleoside linkages.

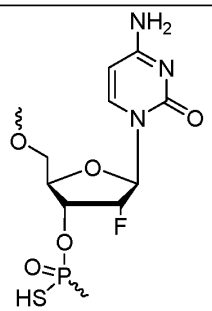
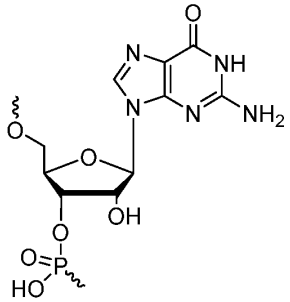
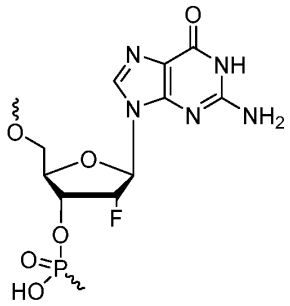
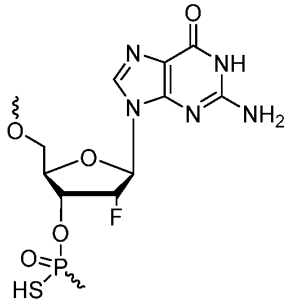
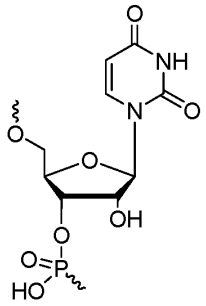
In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, or more) modified nucleosides and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) modified internucleoside linkages. In some embodiments, a modified nucleoside comprises a modification at the 2' position of the sugar (referred to herein as a "2'-modified nucleoside"). In some embodiments, all modified nucleosides in an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) are 2'-modified nucleosides. In some embodiments, the 2'-modified nucleoside is selected from 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside, and combinations thereof. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a mix of two or more different 2'-modified nucleosides (e.g., any combination of 2'-F modified nucleosides, 2'-O-Me modified nucleosides and 2'-MOE modified nucleosides).

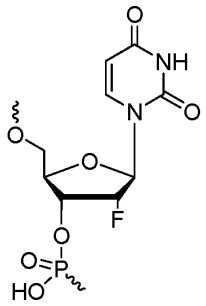
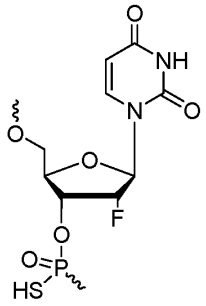
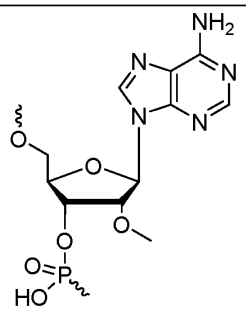
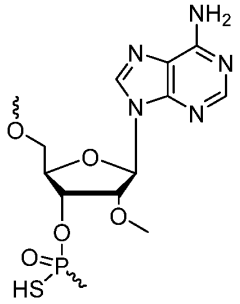
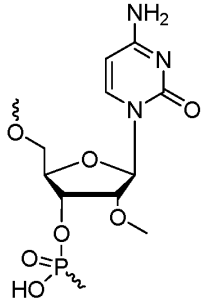
In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) modified internucleoside linkages. Nonlimiting examples of internucleoside linkages include phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, mesyl phosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) phosphorothioate internucleoside linkages.

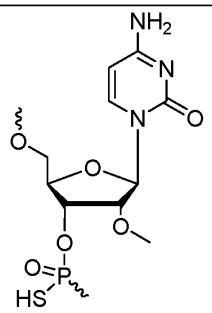
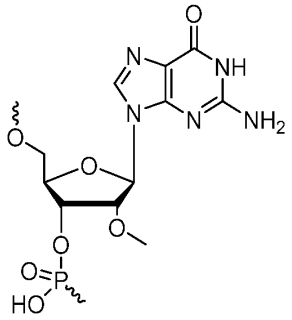
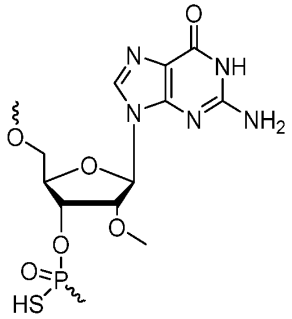
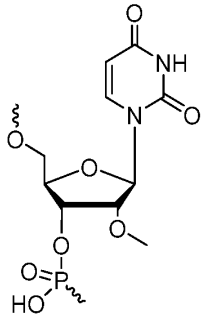
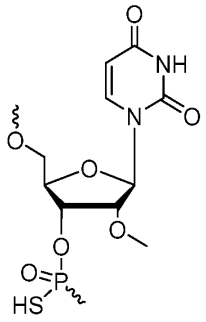
Table 3 lists various exemplary nucleosides with 3'-phosphate or 3'-phosphorothioate and the structures thereof.

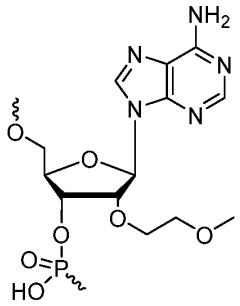
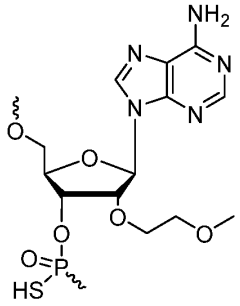
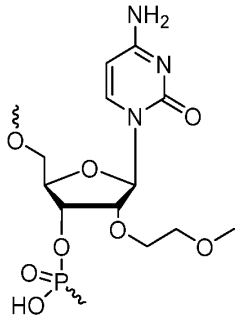
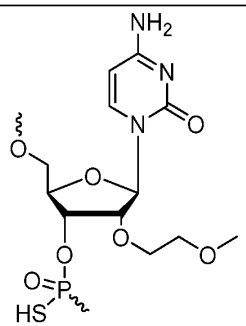
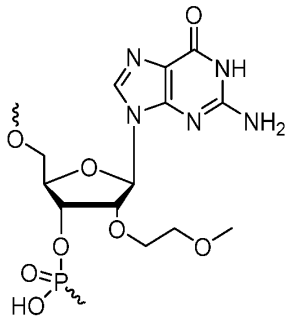
Table 3. Exemplary nucleosides with 3'-phosphate or 3'-phosphorothioate and structures

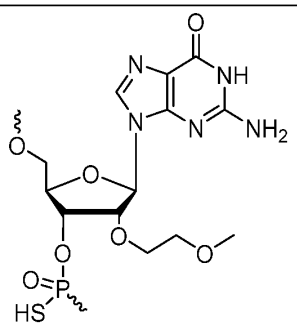
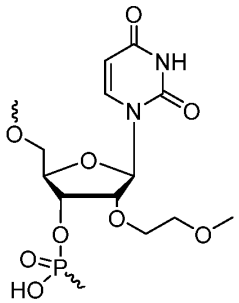
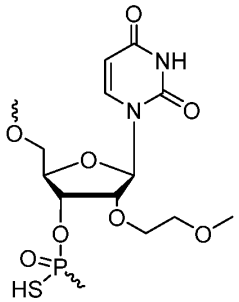
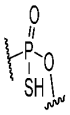
| Abbreviation | Nucleosides or linkage | Structure |
|---|---|---|
| A | Adenosine-3'-phosphate |  |
| fA | 2'-fluoroadenosine-3'-phosphate |  |
| fAs | 2'-fluoroadenosine-3'-phosphorothioate |  |
| C | Cytidine-3'-phosphate |  |
| fC | 2'-fluorocytidine-3'-phosphate |  |

| | | |
|---|---|---|
| fCs | 2'-fluorocytidine-3'-phosphorothioate |  |
| G | Guanosine-3'-phosphate |  |
| fG | 2'-fluoroguanosine-3'-phosphate |  |
| fGs | 2'-fluoroguanosine-3'-phosphorothioate |  |
| U | Uridine-3'-phosphate |  |

| | | |
|---|---|---|
| fU | 2'-fluorouridine-3'-phosphate |  |
| fUs | 2'-fluorouridine-3'-phosphorothioate |  |
| mA | 2'-*O*-methyladenosine-3'-phosphate |  |
| mAs | 2'-*O*-methyladenosine-3'-phosphorothioate |  |
| mC | 2'-*O*-methylcytidine-3'-phosphate |  |

| | | |
|---|---|---|
| mCs | 2'-*O*-methylcytidine-3'-phosphorothioate |  |
| mG | 2'-*O*-methylguanosine-3'-phosphate |  |
| mGs | 2'-*O*-methylguanosine-3'-phosphorothioate |  |
| mU | 2'-*O*-methyluridine-3'-phosphate |  |
| mUs | 2'-*O*-methyluridine-3'-phosphorothioate |  |

| | | |
|---|---|---|
| | 2'-*O*-methoxyethyladenosine-3'-phosphate |  |
| | 2'-*O*-methoxyethyladenosine-3'-phosphorothioate |  |
| | 2'-*O*-methoxyethylcytidine-3'-phosphate |  |
| | 2'-*O*-methoxyethylcytidine-3'-phosphorothioate |  |
| | 2'-*O*-methoxyethylguanosine-3'-phosphate |  |

| | | |
|---|---|---|
| | 2'-*O*-methoxyethylguanosine-3'-phosphorothioate |  |
| | 2'-*O*-methoxyethylluridine-3'-phosphate |  |
| | 2'-*O*-methoxyethyluridine-3'-phosphorothioate |  |
| s | Phosphorothioate linkage |  |

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises an antisense strand comprising one or more modified nucleosides (e.g., one or more 2'-modified nucleosides). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises an antisense strand comprising a mix of two or more different 2'-modified nucleosides (e.g., any combination of 2'-F modified nucleosides, 2'-O-Me modified nucleosides, and 2'-MOE modified nucleosides). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand comprising one or more modified nucleosides (e.g., one or more 2'-modified nucleosides). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand comprising

a mix of two or more different 2'-modified nucleosides (e.g., any combination of 2'-F modified nucleosides, 2'-O-Me modified nucleosides, and 2'-MOE modified nucleosides).

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises an antisense strand and a sense strand, wherein each nucleoside of the antisense strand is a modified nucleoside (e.g., 2'-modified nucleoside) and each nucleoside of the sense strand is a modified nucleoside (e.g., 2'-modified nucleoside). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises an antisense strand and a sense strand, wherein each nucleoside of the antisense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside, a 2'-O-Me modified nucleoside, and a 2'-MOE modified nucleoside, and each nucleoside of the sense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside, a 2'-O-Me modified nucleoside, and a 2'-MOE modified nucleoside. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises an antisense strand and a sense strand, wherein each nucleoside of the antisense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside, and each nucleoside of the sense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at two or more (e.g., 2, 3, 4, 5, or 6) of positions 8, 9, 10, 11, 12, and 13 (counting 5'→3') of the sense strand are nucleosides with the same 2' chemistry in the sugar moiety, e.g., unmodified (i.e., 2'-hydroxy nucleoside), 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside, or 2'-deoxy nucleoside. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein each of the nucleosides at positions 8-12, 8-13, 9-12, or 9-13 (counting 5'→3') of the sense strand are nucleosides with the same 2' chemistry in the sugar moiety, e.g., unmodified (i.e., 2'-hydroxy nucleoside), 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside, or 2'-deoxy nucleoside. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein each of the nucleosides at positions 8-12, 8-13, 9-12, or 9-13 (counting 5'→3') of the sense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein each of the nucleosides at positions 8-12, 8-13, 9-12, or 9-13 (counting 5'→3') of the sense strand are unmodified nucleosides (i.e., 2'-hydroxy nucleosides). The ranges referred to includes the nucleosides at both ends of the range. For example, nucleosides at positions 9-12 includes nucleosides at positions 9, 10, 11, and 12.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at two or more (e.g., 2, 3, 4, 5, or 6) of positions 8, 9, 10, 11, 12, and 13 (counting 5'→3') of the sense strand are consecutive nucleosides with the same 2' chemistry in the sugar moiety, e.g., unmodified nucleosides (i.e., 2'-hydroxy nucleosides), 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides and the nucleosides immediately upstream and downstream to the two or more consecutive nucleosides with the same 2' chemistry in the sugar moiety are nucleosides with different 2' chemistry in the sugar moiety.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5'→3') of the sense strand are nucleosides with the same 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside, or 2'-deoxy nucleoside and the nucleosides at positions 8 or 13 (counting 5'→3') are nucleosides with different 2' chemistry in the sugar moiety including, e.g., unmodified nucleosides, i.e., 2'-hydroxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and the nucleosides at positions 8 and 13 (counting 5'→3') have a different 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleoside, 2'-O-Me modified nucleoside, 2'-MOE modified nucleoside, or 2'-deoxy nucleoside.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 8 and 13 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE

modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides positions 2 and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides positions at 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, and one or more of positions 5, 6, and 8 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides and the nucleosides at other positions are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides and all nucleosides that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more positions 1, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more positions 1, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 4, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 4, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the nucleosides at positions 1, 4, 6, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the nucleosides at positions 1, 4, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'- deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the nucleosides at positions 1, 4, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, the nucleosides at positions 1, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, and 23 (counting 5'→3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, 2'-deoxy nucleosides, or 2'-hydroxy nucleosides. In some embodiments, one or more nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides. In some embodiments, all nucleosides of the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises one or more (e.g., 1, 2, or 3) phosphorothioate internucleoside linkages in at least one strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises one or more (e.g., 1, 2, or 3) phosphorothioate internucleoside linkages in the sense strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises one or more (e.g., 1, 2, 3, 4, or 5) phosphorothioate internucleoside linkages in the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises two phosphorothioate internucleoside linkages in the sense strand and four phosphorothioate internucleoside linkages in the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises two phosphorothioate internucleoside linkages in the sense strand (e.g., the first two internucleoside linkages from 5'→3') and four phosphorothioate internucleoside linkages in the antisense strand (e.g., the first two internucleoside linkages and the last two internucleoside linkages from 5'→3'). It is to be understood that, in any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein, the rest of internucleoside linkages, unless otherwise specified, are all phosphodiester internucleoside linkages.

In some embodiments, provided is a population of two or more of the same modified RNAi agents as described herein, wherein all of the phosphorothioate internucleoside linkages are stereorandom. In some embodiments, provided is a chirally enriched population of such modified RNAi agents as described herein, wherein the chirally enriched population is enriched for modified RNAi agents comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents comprising at least one particular phosphorothioate internucleoside linkage having the (*Sp*) or (*Rp*) configuration. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents comprising at least one particular phosphorothioate internucleoside linkage having the (*Sp*) configuration. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents comprising at least one particular phosphorothioate internucleoside linkage having the (*Rp*) configuration. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents having the (*Rp*) configuration at one particular phosphorothioate internucleoside linkage and the (*Sp*) configuration at each of the remaining phosphorothioate internucleoside linkages. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents having the (*Sp*) configuration at one particular phosphorothioate internucleoside linkage and the (*Rp*) configuration at each of the remaining phosphorothioate internucleoside linkages. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents having at least 3 contiguous phosphorothioate internucleoside linkages in the (*Sp*), (*Sp*), and (*Rp*) configurations, in the 5' to 3' direction. In some embodiments, the chirally enriched population of modified RNAi agents is enriched for modified RNAi agents having at least 4 contiguous phosphorothioate internucleoside linkages in the (*Sp*), (*Sp*), (*Sp*), and (*Rp*) configurations, in the 5' to 3' direction.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two

and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages. An example of such a modified RNAi agent is provided in FIG. 1 – MOD1 ("M1").

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages. An example of such a modified RNAi agent is provided in FIG. 1 – MOD2 ("M2").

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages. An example of such a modified RNAi agent is provided in FIG. 1 – MOD3 ("M3").

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages. An example of such a modified RNAi agent is provided in FIG. 1 – MOD4 ("M4").

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and

comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 777-1190, and a sense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 9-776, wherein:

(i) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages;

(ii) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages;

(iii) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages; or

(iv) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190 and further comprises a sense strand that is substantially complementary to the antisense strand and

comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190, and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190, and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190, and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified

nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 1161-1190 and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 404, 419, 430, 439, 443, 464, 465, 473, 492, 493, 510, 514, 516, 517, 550, 553, 564, 566, 582, 591, 595, 597, 598, 604, 618, 623, 642, 648, 652, and 664, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 1161, 1162, 1165, 1166, 1169, 1173, 1174, 1180, 1181, 1184, 1188, and 1190 and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 404, 419, 439, 443, 464, 473, 493, 517, 566, 597, 598, 604, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 1161, 1162, 1165, 1166, 1169, 1173, 1174, 1180, 1181, 1184, 1188, and 1190 and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 404, 419, 439, 443, 464, 473, 493, 517, 566, 597, 598,

604, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 1161, 1162, 1165, 1166, 1169, 1173, 1174, 1180, 1181, 1184, 1188, and 1190 and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 404, 419, 439, 443, 464, 473, 493, 517, 566, 597, 598, 604, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that comprises the nucleobase sequence of any one of SEQ ID NOs: 1161, 1162, 1165, 1166, 1169, 1173, 1174, 1180, 1181, 1184, 1188, and 1190 and further comprises a sense strand that is substantially complementary to the antisense strand and comprises the nucleobase sequence of any one of SEQ ID NOs: 404, 419, 439, 443, 464, 473, 493, 517, 566, 597, 598, 604, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 419 and a structure of (5' → 3')

[mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] (SEQ ID NO: 1219), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1161 and a structure of (5' → 3'):

(i)

[mUs][fUs][fU][mG][fC][fU][fC][fU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] (SEQ ID NO: 1989);

(ii)

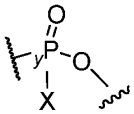
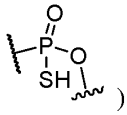
[mUs][fUs][fU][mG][mC][mU][fC][mU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] (SEQ ID NO: 1990);

(iii)

[mUs][fUs][fU][mG][fC][mU][fC][fU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] (SEQ ID NO: 1991); or

(iv)

[mUs][fUs][fU][mG][fC][fU][fC][mU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] (SEQ ID NO: 1992)

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 517 and a structure of (5' → 3')

[mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] (SEQ ID NO: 1329), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1162 and a structure of (5' → 3'):

(i)

[mUs][fUs][fG][mU][fC][fA][fU][fU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] (SEQ ID NO: 1993);

(ii)

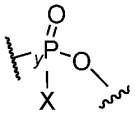
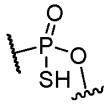
[mUs][fUs][fG][mU][mC][mA][fU][mU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] (SEQ ID NO: 1994);

(iii)

[mUs][fUs][fG][mU][fC][mA][fU][fU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] (SEQ ID NO: 1995); or

(iv)

[mUs][fUs][fG][mU][fC][fA][fU][mU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] (SEQ ID NO: 1996);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 597 and a structure of (5' → 3')

[mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] (SEQ ID NO: 1417), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1165 and a structure of (5' → 3'):

(i)

[mUs][fUs][fA][mA][fC][fU][fG][fU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] (SEQ ID NO: 1999);

(ii)

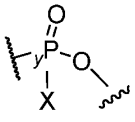
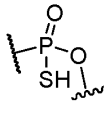
[mUs][fUs][fA][mA][mC][mU][fG][mU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] (SEQ ID NO: 2000);

(iii)

[mUs][fUs][fA][mA][fC][mU][fG][fU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] (SEQ ID NO: 2001); or

(iv)

[mUs][fUs][fA][mA][fC][fU][fG][mU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] (SEQ ID NO: 2002);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 566 and a structure of (5' → 3')

[mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] (SEQ ID NO: 1382), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1166 and a structure of (5' → 3'):

(i)

[mUs][fAs][fU][mA][fC][fU][fG][fG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] (SEQ ID NO: 2003);

(ii)

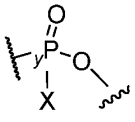
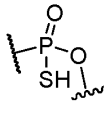
[mUs][fAs][fU][mA][mC][mU][fG][mG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] (SEQ ID NO: 2004);

(iii)

[mUs][fAs][fU][mA][fC][mU][fG][fG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] (SEQ ID NO: 2005); or

(iv)

[mUs][fAs][fU][mA][fC][fU][fG][mG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] (SEQ ID NO: 2006);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 439 and a structure of (5' → 3')

[mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] (SEQ ID NO: 1241), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1169 and a structure of (5' → 3'):

(i)

[mUs][fGs][fA][mU][fU][fU][fG][fU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] (SEQ ID NO: 2009);

(ii)

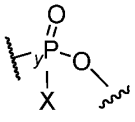
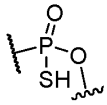
[mUs][fGs][fA][mU][mU][mU][fG][mU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] (SEQ ID NO: 2010);

(iii)

[mUs][fGs][fA][mU][fU][mU][fG][fU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] (SEQ ID NO: 2011); or

(iv)

[mUs][fGs][fA][mU][fU][fU][fG][mU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] (SEQ ID NO: 2012);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure of (5' → 3')

[mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure of (5' → 3'):

(i)

[mUs][fCs][fU][mA][fA][fG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2017);

(ii)

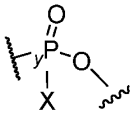
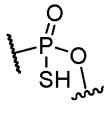
[mUs][fCs][fU][mA][mA][mG][fU][mG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2018);

(iii)

[mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016); or

(iv)

[mUs][fCs][fU][mA][fA][fG][fU][mG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2019);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure of (5' → 3')

[mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418), and

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure of (5' → 3'):

(i)

[mUs][fUs][fU][mA][fA][fC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2021);

(ii)

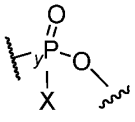
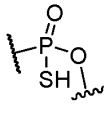
[mUs][fCs][fU][mA][mA][mG][fU][mG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2018);

(iii)

[mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020); or

(iv)

[mUs][fUs][fU][mA][fA][fC][fU][mG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2023);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure of (5' → 3')

[mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202), and

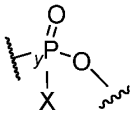
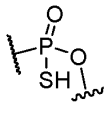
an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure of (5' → 3'):

(i)
[mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029);

(ii)
[mUs][fCs][fA][mA][mG][mA][fA][mU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2030);

(iii)
[mUs][fCs][fA][mA][fG][mA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2031); or

(iv)
[mUs][fCs][fA][mA][fG][fA][fA][mU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2032);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:
a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure of (5' → 3')
[mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267), and

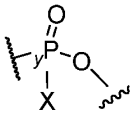
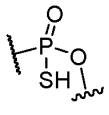
an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure of (5' → 3'):

(i)
[mUs][fUs][fU][mC][fC][fG][fU][fG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2034);

(ii)
[mUs][fUs][fU][mC][mC][mG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2035);

(iii)
[mUs][fUs][fU][mC][fC][mG][fU][fG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2036); or

(iv)
[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:
a sense strand comprising the nucleobase sequence of SEQ ID NO: 443 and a structure of (5' → 3')
[mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] (SEQ ID NO: 1246), and

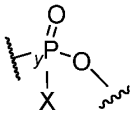
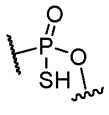
an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1184 and a structure of (5' → 3'):

(i)
[mUs][fGs][fU][mA][fU][fG][fA][fC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] (SEQ ID NO: 2039);

(ii)
[mUs][fGs][fU][mA][mU][mG][fA][mC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] (SEQ ID NO: 2040);

(iii)
[mUs][fGs][fU][mA][fU][mG][fA][fC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] (SEQ ID NO: 2041); or

(iv)
[mUs][fGs][fU][mA][fU][fG][fA][mC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] (SEQ ID NO: 2042);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:
a sense strand comprising the nucleobase sequence of SEQ ID NO: 493 and a structure of (5' → 3')
[mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] (SEQ ID NO: 1301), and

| sense numbering | 5' | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 3' |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MOD1 | sense | | | mN | mN | mN | mN | mN | mN | mN | mN | fN | fN | fN | fN | mN | mN | mN | mN | mN | mN | mN | mN | mN | MOD1 |
| | antisense | mN | mN | mN | mN | mN | mN | mN | mN | mN | fN | mN | mN | mN | fN | mN | fN | fN | mN | fN | mN | fN | fN | mN | |
| MOD2 | sense | | | mN | mN | mN | mN | mN | mN | mN | mN | fN | fN | fN | fN | mN | mN | mN | mN | mN | mN | mN | mN | mN | MOD2 |
| | antisense | mN | mN | mN | mN | mN | mN | mN | mN | mN | fN | mN | mN | mN | fN | mN | mN | fN | fN | fN | mN | fN | fN | mN | |
| MOD3 | sense | | | mN | mN | mN | mN | mN | mN | mN | mN | fN | fN | fN | fN | mN | mN | mN | mN | mN | mN | mN | mN | mN | MOD3 |
| | antisense | mN | mN | mN | mN | mN | mN | mN | mN | mN | fN | mN | mN | mN | fN | mN | fN | fN | fN | fN | mN | fN | fN | mN | |
| MOD4 | sense | | | mN | mN | mN | mN | mN | mN | mN | mN | fN | fN | fN | fN | mN | mN | mN | mN | mN | mN | mN | mN | mN | MOD4 |
| | antisense | mN | mN | mN | mN | mN | mN | mN | mN | mN | fN | mN | mN | mN | fN | mN | mN | fN | mN | mN | mN | fN | fN | mN | |
| antisense numbering | 3' | 23 | 22 | 21 | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | 5' |

FIG. 1

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1190 and a structure of (5' → 3'):

(i)

[mUs][fAs][fU][mG][fA][fU][fA][fC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] (SEQ ID NO: 2051);

(ii)

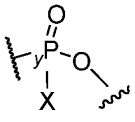
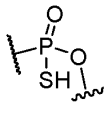
[mUs][fAs][fU][mG][mA][mU][fA][mC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] (SEQ ID NO: 2052);

(iii)

[mUs][fAs][fU][mG][fA][mU][fA][fC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] (SEQ ID NO: 2053); or

(iv)

[mUs][fAs][fU][mG][fA][fU][fA][mC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] (SEQ ID NO: 2054);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand indicates a phosphorothioate linkage between the 3'-terminal nucleoside of the sense strand and a conjugated moiety (e.g., a targeting moiety described herein). In some embodiments, the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 16, 17, 18, 20, and 21 (counting 5'→3') of the sense strand are nucleosides with a first 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides, and the nucleosides at positions 9, 10, 11, 12, 13, and 19 (counting 5'→3') of the sense strand are nucleosides with a

second 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides, wherein the first 2' chemistry in the sugar moiety and the second 2' chemistry in the sugar moiety are different. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 16, 17, 18, 20, and 21 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and the nucleosides at positions 9, 10, 11, 12, 13, and 19 (counting 5'→3') of the sense strand are nucleosides with a different 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12, 13, and 19 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides , and the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 16, 17, 18, 20, and 21 (counting 5'→3') of the sense strand are nucleosides with a different 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 16, 17, 18, 20, and 21 (counting 5'→3') of the sense strand are 2'-O-Me modified nucleosides and the nucleosides at positions 9, 10, 11, 12, 13, and 19 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 4, 11, 12, 16, 18, 21, 22, and 23 (counting 5'→3') of the antisense strand are nucleosides with a first 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides, and the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 19, and 20 (counting 5'→3') of the antisense strand are nucleosides with a second 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides, wherein the first 2' chemistry in the sugar moiety and the second 2' chemistry in the sugar moiety are different. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 4, 11, 12, 16, 18, 21, 22, and 23 (counting 5'→3') of the antisense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 19, and 20 (counting 5'→3') of the antisense strand are nucleosides with a different 2' chemistry in

the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleoside. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 19, and 20 (counting 5'→3') of the antisense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and the nucleosides at positions 1, 4, 11, 12, 16, 18, 21, 22, and 23 (counting 5'→3') of the antisense strand are nucleosides with a different 2' chemistry in the sugar moiety, e.g., 2'-F modified nucleosides, 2'-O-Me modified nucleosides, 2'-MOE modified nucleosides, or 2'-deoxy nucleosides.

In some embodiment, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 4, 11, 12, 16, 18, 21, 22, and 23 (counting 5'→3') of the antisense strand are the 2'-O-Me modified nucleosides, and the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 19, and 20 (counting 5'→3') of the antisense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 777-1160, and a sense strand that is 18-25 nucleosides (e.g., 18, 19, 20, 21, 22, 23, 24, or 25 nucleosides) in length and comprises at least 15 (e.g., 16, 17, 18, 19, 20, 21, 22, or 23) consecutive nucleobases of any one of SEQ ID NOs: 9-776, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 14, 15, 16, 17, 18, 20, and 21 (counting 5'→3') of the sense strand are 2'-O-Me modified nucleosides and the nucleosides at positions 9, 10, 11, 12, 13, and 19 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and wherein the nucleosides at positions 1, 4, 11, 12, 16, 18, 21, 22, and 23 (counting 5'→3') of the antisense strand are the 2'-O-Me modified nucleosides, and the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 17, 19, and 20 (counting 5'→3') of the antisense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides.

In some embodiments, a CYP7A1 RNAi agent described herein comprises an antisense strand comprising the nucleobase sequence of any one of SEQ ID NOs: 777-1160, and a sense strand comprising the nucleobase sequence of any one of SEQ ID NOs: 9-776, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5'→3') of the sense strand are 2'-O-Me modified nucleosides and the nucleosides at positions 9, 10, 11, and 12 (counting 5'→3') of the sense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides, and wherein the nucleosides at positions 1, 4, 11, 12, 18, 19, 20, 21, 22, and 23

(counting 5'→3') of the antisense strand are the 2'-O-Me modified nucleosides, and the nucleosides at positions 2, 3, 5, 6, 7, 8, 9, 10, 13, 14, 15, 16, and 17 (counting 5'→3') of the antisense strand are unmodified nucleosides, i.e., 2'-hydroxy nucleosides.

*Additional modified CYP7A1 RNAi agents*

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprising nucleobase sequences (e.g., nucleobase sequences of the antisense strand and the sense strand) of the siRNAs listed in Table 5A and Table 7A can include any of the chemical modifications (e.g., one or more modified nucleosides and/or one or more modified internucleoside linkages) described below. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein can include any of the chemical modifications as disclosed in Hu et al. Therapeutic siRNA: State of the Art. *Sig Transduct Target Ther* 5, 101 (2020), the entire contents of which is herein incorporated by reference.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises any of the chemical modifications and/or modification patterns as disclosed in US10233448, US11504391, US9290760, US9796974, US9399775 US9796974, US11015198, US10676742, US11661604, US11655473, and WO2022204430. The disclosures in these references related to chemical modifications of oligonucleotides (e.g., RNAi agents) are incorporated herein by reference.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises one or more chemical modifications and/or modification patterns as described in US10233448, US11504391, US9290760, US9796974, US9399775, US9796974, and US11015198. For example, in some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein one or both of the sense strand and the antisense strand comprise chemical modifications on nucleosides of a portion or the entire strand that form an alternating motif. The term "alternating motif" refers to a type of modification pattern formed by one or more repeats of a sequence (e.g., a sequence of two, three, four, five, six, or more nucleosides), wherein each repeat of the sequence comprises a chemical modification on each nucleoside forming a pattern (e.g., an alternating pattern). For example, if A, B and C each represent one type of modified nucleoside, the alternating motif can be "ABABABABABAB...," "AABBAABBAABB...," "AABAABAABAAB...," "AAABAAABAAAB...," "AAABBBAAABBB...," or "ABCABCABCABC...," *etc.*

The type of modifications contained in an alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on a nucleoside, an

alternating motif, e.g., modifications on every other nucleoside, may be the same throughout the entire alternating motif (e.g., ABABABAB… throughout), or may be different in one portion of the motif than another portion (e.g., ABABABABACACACAC). In some embodiments, each of the sense strand or antisense strand can comprise several possibilities of alternating patterns such as "ABABAB…," "ACACAC…," "BDBDBD…," or "CDCDCD…," *etc.*, within an alternating motif.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein may comprise, on one or both of the sense strand and the antisense strand, one or more alternating motifs. In some embodiments, the type of modifications contained in one or more alternating motif of the sense strand may be the same or different from the one or more alternating motifs of the antisense strand.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprises a sense strand and an antisense strand, wherein the sense strand comprises a different alternating motif relative to the alternating motif on the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, each of which comprises an alternating motif composed of the same types of modifications on nucleosides, but each nucleoside on the antisense strand comprises a different modification than the nucleoside it base pairs with on the sense strand. In some embodiments, in an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein, the sense strand when paired with the antisense strand to form a duplex region, the alternating motif in the sense strand may start with "ABABAB" from 5' → 3' of the sense strand and the alternating motif in the antisense strand may start with "ABABAB" from 5' → 3' of the antisense strand within the duplex region. In some embodiments, the alternating motif in the sense strand may start with "AABBAABB" from 5' → 3' of the sense strand and the alternating motif in the antisense strand may start with "AABBAABB" from 5' → 3' of the antisense strand within the duplex region. In some embodiments, in an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein, the sense strand when paired with the antisense strand to form a duplex region, the alternating motif in the sense strand may start with "ABABAB" from 5' → 3' of the sense strand and the alternating motif in the antisense strand may start with "BABABA" from 5' → 3' of the antisense strand within the duplex region. In some embodiments, the alternating motif in the sense strand may start with "AABBAABB" from 5' → 3' of the sense strand and the alternating motif in the antisense strand may start with "BBAABBAA" from 5' → 3' of the antisense strand within the duplex region.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand or

the antisense strand comprises at least two different types of modified nucleosides (e.g., 2'-O-Me and 2'-F modified nucleosides). In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein each of the sense strand and the antisense strand comprises at least two different types of modified nucleosides (e.g., 2'-O-Me and 2'-F modified nucleosides) on each strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein each nucleoside of the sense strand and the antisense strand is a modified nucleoside, and wherein each of the sense strand and the antisense strand comprises an alternating motif of 2'-F modified nucleosides and 2'-O-Me modified nucleosides.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand comprises an alternating motif of 2'-O-Me modified nucleosides and 2'-F modified nucleosides and the antisense strand comprises an alternating motif of 2'-O-Me modified nucleosides and 2'-F modified nucleosides, wherein a 2'-O-Me modified nucleoside on the sense strand base pairs with a 2'-F modified nucleoside on the antisense strand and vice versa. In some embodiments, position 1 (counting 5' → 3') of the sense strand is a 2'-F modified nucleoside, and the corresponding position of the antisense strand is a 2'- O-Me modified nucleoside. In some embodiments, position 1 (counting 5' → 3') of the sense strand is a 2'-O-Me modified nucleoside, and the corresponding position of the antisense strand is a 2'-F modified nucleoside. In some embodiments, an RNAi agent (e.g., CYP7A1 RNA agent) further comprises one or more phosphorothioate internucleoside linkages. In some embodiments, the first two internucleoside linkages (from 5'→ 3') and the last two internucleoside linkages (from 5'→ 3') of the sense strand are internucleoside linkages and/or (e.g., and) the last two internucleoside linkages (from 5'→ 3') of the antisense strand are internucleoside linkages. In some embodiments, the sense strand and the antisense strand are both 19 nucleosides in length and for a duplex of 19 base pairs in length.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein one or both of the sense strand and the antisense strand comprises one or more motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications). In some embodiments, at least one of the one or more motifs of three nucleosides is located on the sense and/or antisense strand at positions corresponding to or near the site on a target mRNA where cleavage occurs (these positions on the sense and/or antisense strand are collectively referred to herein as the "cleavage site"). In some embodiments, at least one of the one or more motifs of

three nucleosides is located at or near the cleavage site on the antisense strand (e.g., positions 10, 11, and 12 (counting 5' → 3') of the antisense strand). For an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprising a duplex region of 19-23 nucleosides in length, the positions on the antisense strand corresponding to the cleavage site is typically around positions 10, 11, and 12 (counting 5'→ 3'). Thus, the three identical sugar modifications (e.g., identical 2'-modifications) may occur at positions 9, 10, 11; positions 10, 11, 12; positions 11, 12, 13; positions 12, 13, 14; or positions 13, 14, 15 of the antisense strand (counting 5'→ 3' or counting from the first paired nucleoside within the duplex region from the 5'- end of the antisense strand). The positions on the antisense strand corresponding to the cleavage site may also change according to the length of the duplex region of the RNAi agent from the 5'-end.

In some embodiments, when the sense strand and antisense strand of an RNAi agent comprise one or more motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at or near the cleavage site on at least one strand of an RNAi agent, gene silencing activity of the RNAi agent is observed.

In some embodiments, the sense strand of an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprises at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications), wherein at least one of the motifs occurs at or near the cleavage site on the sense strand.

In some embodiments, the antisense strand of the RNAi agent comprises at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications), wherein at least one of the motifs occurs at or near the cleavage site on the antisense strand.

In some embodiments, the rest of the nucleosides in the sense strand and antisense strand of the RNAi agent are modified nucleosides. In some embodiments, the rest of the nucleosides in the sense strand and antisense strand of the RNAi agent are modified nucleosides with a different modification than the modification on the motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications).

In some embodiments, the sense strand of an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprises at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at or near the cleavage site on the sense strand and the antisense strand comprises at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at or near the cleavage site on the antisense strand. When the sense strand and the antisense strand form a duplex region, the sense strand and the antisense strand can be so aligned that one motif of the three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) on

the sense strand and one motif of the three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) on the antisense strand have at least one nucleoside overlap, *i.e.*, at least one of the three consecutive nucleosides of the motif on the sense strand forms a base pair with at least one of the three consecutive nucleosides of the motif on the antisense strand. Alternatively, at least two nucleosides may overlap, or all three nucleosides may overlap. In some embodiments, the overlapping nucleoside on the sense strand comprises a different sugar modification than the nucleoside it overlaps with on the antisense strand.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein is an RNAi agent comprising a duplex region of 19 nucleosides in length, wherein the duplex region is formed by a sense strand and an antisense strand, wherein the nucleosides at three consecutive positions of the sense strand are 2'-F modified nucleosides and the nucleosides at three consecutive positions of the antisense strand are 2'-O-Me modified nucleosides. In some embodiments, the nucleosides at positions 7, 8, and 9 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) is an RNAi agent comprising a duplex region of 20 nucleosides in length, wherein the duplex region is formed by a sense strand and an antisense strand, wherein the nucleosides at three consecutive positions of the sense strand are 2'-F modified nucleosides and the nucleosides at three consecutive positions of the antisense strand are 2'-O-Me modified nucleosides. In some embodiments, the nucleosides at positions 8, 9, and 10 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein is an RNAi agent comprising a duplex region of 21 nucleosides in length, wherein the duplex region is formed by a sense strand and an antisense strand, wherein nucleosides at three consecutive positions of the sense strand are 2'-F modified nucleosides and the nucleosides at three consecutive positions of the antisense strand are 2'-O-Me modified nucleosides. In some embodiments, the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprises a sense strand and an antisense strand wherein the sense strand is 21 nucleosides in length and the antisense strand is 23 nucleosides in length, wherein the sense strand comprises at

least one motif of three consecutive nucleosides with identical sugar modifications (e.g., 2'-F modified nucleosides) and the antisense strand comprises at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., 2'-O-Me modified nucleosides). In some embodiments, the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides. In some embodiments, the rest of the nucleosides in the sense strand and the antisense strand of the RNAi agent are modified nucleosides (e.g., 2' modified nucleosides). In some embodiments, the rest of the nucleosides in the sense strand and antisense strand are a mix of 2'-F modified nucleosides and 2'-O-Me modified nucleosides.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein one or both of the sense strand and the antisense strand comprise the at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at or near the cleavage site and comprise one or more alternating motifs. In some embodiments, the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides, and the rest of the nucleosides in the sense strand and the antisense strand comprise alternating motifs of 2'-O-Me modified nucleosides and 2'-F modified nucleosides.

In some embodiments, when the motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) is present on any of the strands, the modification of the nucleoside next to the motif is a different modification than the modification of the motif. For example, in some embodiments, the portion of the sequence containing the motif is "…$N_a$YYY$N_b$…," wherein "Y" represents the modification of the motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications), and "$N_a$" and "$N_b$" represent a modification to the nucleoside 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.

In some embodiments, an RNAi agent comprising a sense strand and an antisense strand each or both comprising one or more motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) and alternating motifs described herein comprise two blunt ends, one blunt end and one end with an overhang, or two ends each with overhangs. In some embodiments, one end of the RNAi agent is blunt, while the other end comprises a two nucleoside overhang (e.g., formed by a sense strand of 21 nucleosides in length

and an antisense strand of 23 nucleosides in length). In some embodiments, the two nucleoside overhang is at the 3'-end of the antisense strand.

In some embodiments, a two nucleoside overhang is at the 3'-end of the antisense strand, and the antisense strand comprises two phosphorothioate internucleoside linkages between the terminal three nucleosides, wherein two of the three nucleosides are the overhang nucleosides, and the third nucleoside is a paired nucleoside next to the overhang nucleoside. In some embodiments, the RNAi agent further comprises two phosphorothioate internucleoside linkages of the sense strand (e.g., first two internucleoside linkages from 5'→ 3') and two phosphorothioate internucleoside linkages in the antisense strand (e.g., first two internucleoside linkages from 5'→ 3').

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 1, 3, 5, 7, 9, 10, 11, 13, 15, 17, 19, and 21 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 6, 8, 10, 14, 16, 18, 20, and 22 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 5, 7, 9, 11, 12, 13, 15, 17, 19, 21, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, and wherein the last two internucleoside linkages of the antisense strand (counting 5' → 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent) described herein comprises a sense strand and an antisense strand, wherein one or both of the sense strand and the antisense strand comprise more than one (e.g., 2, 3, or more) motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications). The first motif may occur at or near the cleavage site on the sense strand or antisense strand and the other motif(s) may occur at another portion of the strand that is separated from (e.g., by at least one or more nucleosides) the first motif or may be adjacent to the first motif. When the other motif(s) are adjacent to the first motif, the chemistries are different from that of the first motif. When the motifs are separated by one or more nucleosides, the chemistries can be the same or different. In some embodiments, when two other motifs are present, each motif may occur at one end relative to the first motif which is at or near cleavage site or on either side of the first motif.

Like the sense strand, in some embodiments, the antisense strand of the RNAi agent may contain more than one motif (e.g., 2, 3, or more) of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications), with at least one of the motifs occurring at or near the cleavage site on the strand. In some embodiments, the antisense strand comprises one or more motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) in an alignment with the motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) that are present on the sense strand.

In some embodiments, a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two nucleosides at the 3'-end, 5'-end, or both ends of the strand.

In some embodiments, a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleosides 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 RNAi agent each comprise at least one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications), the motifs may fall on the same end of the duplex region, and have an overlap of one, two, or three nucleosides.

When the sense strand and the antisense strand of the RNAi agent each comprise at least two motifs of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-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, comprising an overlap of one, two, or three nucleosides; two modifications each from one strand fall on the other end of the duplex region, comprising an overlap of one, two or three nucleosides; two modifications one strand fall on each side of the lead motif, comprising an overlap of one, two or three nucleosides in the duplex region.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at the 5' end or 3' end of the sense strand and/or the antisense strand. In some embodiments, the antisense strand comprises a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at the 3' end of the strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the

sense strand and antisense strand each comprise a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at or near the cleavage site, wherein the RNAi agents further comprise a motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications) at the 3' end of the sense strand, and wherein the sense strand and antisense strand each comprise an alternating motif comprising chemical modifications in an alternating pattern. In some embodiments, the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 11, 12, and 13 (counting 5'→ 3') of the antisense strand are 2'-O-Me modified nucleosides and the rest of the nucleosides in the sense strand and the antisense strand comprise one or more alternating motifs.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 1, 3, 5, 7, 9, 10, 11, 13, 15, 17, 19, and 21 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 2, 4, 6, 8, 12, 14, 16, 18, and 20 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 5, 7, 9, 11, 12, 13, 15, 17, 19, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, the sense strand comprises modified nucleosides that form a modification pattern represented by formula (IV):

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' (IV)

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 nucleosides, each sequence comprising at least two differently modified nucleosides;

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

each $n_p$ and $n_q$ independently represent an overhang nucleoside;

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

XXX, YYY, and ZZZ each independently represent one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications). Preferably YYY is all 2'-F modified nucleosides.

In some embodiments, the $N_a$ or $N_b$ comprises an alternating pattern of modified nucleosides.

In some embodiments, the YYY motif occurs at or near the cleavage site on the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleosides in length, the YYY motif can occur at or the near 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) on the sense strand, (counting 5' → 3') or counting from the first paired nucleoside 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' $n_p$-$N_a$-YYY-$N_b$-ZZZ-$N_a$-$n_q$ 3' (IVb);

5' $n_p$-$N_a$-XXX-$N_b$-YYY-$N_a$-$n_q$ 3' (IVc); or

5' $n_p$-$N_a$-XXX-$N_b$-YYY-$N_b$-ZZZ-$N_a$-$n_q$ 3' (IVd).

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

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

When the sense strand is represented as formula (IVd), each $N_b$ independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleosides. Preferably, $N_b$ 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 nucleosides.

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

In some 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' (IVa).

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

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

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' (V)

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 nucleosides, each sequence comprising at least two differently modified nucleosides;

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

each $n_{p'}$ and $n_{q'}$ independently represent an overhang nucleoside;

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 consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications).

In some embodiments, the $N_a$' or $N_b$' comprises an alternating pattern of modified nucleosides.

The Y'Y'Y' motif occurs at or near the cleavage site on the antisense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleosides 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 nucleoside, from the 5'-end; or optionally, the count starting at the first paired nucleoside within the duplex region, from the 5'-end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In some embodiments, Y'Y'Y' motif is all 2'-O-Me modified nucleosides.

In some 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' (Vb);

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' (Vd).

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 nucleosides. Each $N_a$' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides.

When the antisense strand is represented as formula (Vc), $N_b$' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified

nucleosides. Each $N_a$' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides.

When the antisense strand is represented as formula (Vd), 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 nucleosides. Each $N_a$' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides. Preferably, $N_b$ is 0, 1, 2, 3, 4, 5, or 6.

In some 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' (IVa).

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

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

In some embodiments, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nucleosides, the count starting from the first nucleoside from the 5'-end, or optionally, the count starting at the first paired nucleoside within the duplex region, from the 5'- end; and Y represents a 2'-F modified nucleoside. The sense strand may additionally contain XXX motif or ZZZ motifs at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-O-Me modified nucleoside or a 2'-F modified nucleoside.

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 nucleoside from the 5'-end, or optionally, the count starting at the first paired nucleoside within the duplex region, from the 5'-end; and Y′ represents 2'-O-Me modification. The antisense strand may additionally contain X′X′X′ motif or Z′Z′Z′ motifs at the opposite end of the duplex region; and X′X′X′ and Z′Z′Z′ each independently represents a 2'-O-Me modified nucleoside or a 2'-F modified nucleoside.

The sense strand represented by any one of the above formulas (IVa), (IVb), (IVc), and (IVd) forms a duplex with an antisense strand being represented by any one of formulas (Va), (Vb), (Vc), and (Vd), respectively.

Accordingly, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein may comprise a sense strand and an antisense strand, each strand comprising 14 to 30 nucleosides, the RNAi agent represented by formula (VI):

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'

(VI)

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 nucleosides, each sequence comprising at least two differently modified nucleosides;

each $N_b$ and $N_b$' independently represents an oligonucleotide sequence comprising 0-10 modified nucleosides;

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

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three consecutive nucleosides with identical sugar modifications (e.g., identical 2'-modifications).

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 a RNAi agent 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'

(VIa)

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'

(VIb)

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'

(VIc)

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'

(VId)

When the RNAi agent is represented by formula (VIa), each $N_a$ independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides.

When the RNAi agent is represented by formula (VIb), each $N_b$ independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleosides. Each $N_a$ independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides.

When the RNAi agent is represented as formula (VIc), 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 nucleosides. Each $N_a$ independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides.

When the RNAi agent is represented as formula (VId), 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 nucleosides. Each $N_a$, $N_a$' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleosides. Each of $N_a$, $N_a$', $N_b$, and $N_b$' independently comprises an alternating pattern of modified nucleosides.

Each of X, Y, and Z in formulas (VI), (VIa), (VIb), (VIc), and (VId) may be the same or different from each other.

When the RNAi agent is represented by formula (VI), (VIa), (VIb), (VIc), and (VId), at least one of the Y nucleosides may form a base pair with one of the Y′ nucleosides. Alternatively, at least two of the Y nucleosides form base pairs with the corresponding Y′ nucleosides; or all three of the Y nucleosides all form base pairs with the corresponding Y′ nucleosides.

When the RNAi agent is represented by formula (VIb) or (VId), at least one of the Z nucleosides may form a base pair with one of the Z′ nucleosides. Alternatively, at least two of the Z nucleosides form base pairs with the corresponding Z′ nucleosides; or all three of the Z nucleosides all form base pairs with the corresponding Z′ nucleosides.

When the RNAi agent is represented as formula (VIc) or (VId), at least one of the X nucleosides may form a base pair with one of the X′ nucleosides. Alternatively, at least two of the X nucleosides form base pairs with the corresponding X′ nucleosides; or all three of the X nucleosides all form base pairs with the corresponding X′ nucleosides.

In some embodiments, the modification on the Y nucleoside is different than the modification on the Y' nucleoside, the modification on the Z nucleoside is different than the modification on the Z' nucleoside, or the modification on the X nucleoside is different than the modification on the X' nucleoside.

In some embodiments, when the RNAi agent is represented by formula (VId), $N_a$ modified nucleosides are 2′-O-Me modified nucleosides or 2′-F modified nucleosides. In some embodiments, when the RNAi agent is represented by formula (VId), the $N_a$ modified nucleosides are 2′-O-Me or 2′-F modified nucleosides and $n_p'$ >0 and at least one $n_p'$ is linked to a neighboring nucleoside *via* a phosphorothioate linkage. In some embodiments, when the RNAi agent is represented by formula (VId), the $N_a$ modified nucleosides are 2′-O-Me or 2′-F modified nucleosides, $n_p'$ >0 and at least one $n_p'$ is linked to a neighboring nucleoside *via* phosphorothioate linkage. In some embodiments, when the RNAi agent is represented by formula (VId), the $N_a$ modified nucleosides are 2′-O-Me or 2′-F modifications, $n_p'$ >0 and at least one $n_p'$ is linked to a neighboring nucleoside *via* phosphorothioate linkage, and the sense strand comprises at least one phosphorothioate linkage.

In some embodiments, when the RNAi agent is represented by formula (VIa), the $N_a$ modified nucleosides are 2′-O-Me or 2′-F modifications, $n_p'$ >0 and at least one $n_p'$ is linked to a neighboring nucleoside *via* phosphorothioate linkage, and the sense strand comprises at least one phosphorothioate linkage.

In some embodiments, the RNAi agent is a multimer containing at least two duplexes represented by formula (VI), (VIa), (VIb), (VIc), and (VId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. 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 RNAi agent is a multimer containing three, four, five, six, or more duplexes represented by formula (VI), (VIa), (VIb), (VIc), and (VId), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. 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 RNAi agents represented by at least one of formulas (VI), (VIa), (VIb), (VIc), and (VId) are linked to each other at the 5' end, and one or both of the 3' ends.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises ten or fewer nucleosides with a 2'-F modification. For example, the RNAi agent may contain 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 or 0 nucleosides with a 2'-F modification. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises ten 2'-F modified nucleosides, e.g., four 2'-F modified nucleosides in the sense strand and six 2'-F modified nucleosides in the antisense strand. In some embodiments, any one of the

RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises six 2'-F modified nucleosides, e.g., four 2'-F modified nucleosides in the sense strand and two 2'-F modified nucleosides in the antisense strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises eight 2'-F modified nucleosides, *e.g.*, four 2'-F modified nucleosides in the sense strand and four 2'-F modified nucleosides in the antisense strand. In some embodiments, the rest of the nucleosides in the sense strand and the antisense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7, 9, 10, and 11 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 6, 8, 9, 14, and 16 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 4, 5, 7, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages and wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7, 9, 10, and 11 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 6, 14, and 16 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 4, 5, 7, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages and wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein can be optimized for RNA interference by incorporating thermally destabilizing modifications at one or more positions selected from positions 2, 3, 4, 5, 6, 7, 8, and 9 (counting 5'→ 3') of the 5'-end of the antisense strand to reduce or inhibit off-target gene silencing. 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 nucleoside positions (counting 5'→ 3') of the antisense strand. Such thermally destabilizing modifications are described, for example, in US10233448 and US11504391. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises thermally destabilizing modifications as described in US10233448 and US11504391. In some embodiments, the thermally destabilizing modification is an abasic modification, mismatch with the opposing nucleoside in the duplex, or a sugar modification (e.g., a 2′-deoxy nucleoside or acyclic nucleoside e.g., unlocked nucleic acids (UNA) or glycerol nucleic acid (GNA)). In some embodiments, the thermally destabilizing modification is a GNA. In some embodiments, the antisense strand comprises at least one (e.g., one, two, three, four, five or more) GNA within the first 9 nucleoside positions (counting 5'→ 3') of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7, 9, 10, and 11 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 6, 8, 9, 14, and 16 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 4, 5, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the nucleoside at position 7 of the antisense strand is a glycol modified nucleoside (GNA), and wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages and wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleoside at the 3'-end of the sense strand is a deoxy nucleoside (e.g., deoxy-thymine (dT)) or the nucleoside at

the 3'-end of the antisense strand is a deoxy nucleoside (e.g., dT). For example, in some embodiments, there is a short sequence of dT nucleosides, for example, two dT nucleosides on the 3'-end of the sense, antisense strand, or both strands. In some embodiments there is one dT nucleoside at the 3'-end of the sense, antisense strand, or both strands. In some embodiments, the dT nucleoside is an inverted dT nucleoside.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7 and 9 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, and wherein the nucleoside at position 11 (counting 5' → 3') is a deoxy nucleoside (*e.g.* dT), wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 5, 6, 8, 10, 12, 14, 16, and 18 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 7, 9, 11, 13, 15, 17, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 3, 5, 7, 9, 10, 11, 13, 16, and 18 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 4, 6, 8, 12, 14, 15, 17, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 25 nucleosides in length, wherein the nucleosides at positions 2, 3, 5, 8, 10, 14, 16, and 18 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 4, 6, 7, 9, 11, 12, 13, 15, 17, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the nucleosides at position 24 and 25 (counting 5' → 3') are deoxy nucleosides (*e.g.* dT), wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the internucleoside linkages between positions 21 and 22 and between positions 22 and 23 of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein

the RNAi agent has a four nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 1, 3, 5, 7, 9, 10, 11, 13, 17, 19, and 21 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 2, 4, 6, 8, 12, 14, 15, 16, 18, and 20 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 6, 7, 8, 10, 14, 16, 18, 20, and 22 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 5, 9, 11, 12, 13, 15, 17, 19, 21, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7, 9, 11, 13, and 15 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, and 20 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 5, 7, 9, 11, 13, 15, 17, 19, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 10 and 11 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-

O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 6, 8, 10, 14, 16, 18, and 20 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 5, 7, 9, 11, 12, 13, 15, 17, 19, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 1, 3, 5, 7, 9, 10, 11, and 13 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 2, 4, 6, 8, 12, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 4, 8, 10, 14, 16, and 20 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 5, 6, 7, 9, 11, 12, 13, 15, 17, 18, 19, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 21 nucleosides in length, wherein the nucleosides at positions 7, 9, 10, and 11 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 8, 12, 13, 14, 15, 16, 17, 18, 19, 20, and 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 23 nucleosides in length, wherein the nucleosides at positions 2, 6, 9, 14, and 16 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 4, 5, 7, 8, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22, and 23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages and wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate

internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the sense strand is 19 nucleosides in length, wherein the nucleosides at positions 5, 7, 8, and 9 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and wherein the nucleosides at positions 1, 2, 3, 4, 6, and 10, 11, 12, 13, 14, 15, 16, 17, 18, and 19 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, wherein the antisense strand is 21 nucleosides in length, wherein the nucleosides at positions 2, 6, 8, 9, 14, and 16 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 1, 3, 4, 5, 7, 10, 11, 12, 13, 15, 17, 18, 19, 20, and 21 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages and wherein the first two and last two internucleoside linkages of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages, and wherein the RNAi agent has a two nucleoside overhang at the 3'-end of the antisense strand, and a blunt end at the 5'-end of the antisense strand.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises the chemical modifications and/or modification patterns as described in US10676742, US11661604, and US11655473. For example, in some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the sense strand comprises a mix of different 2'-modified nucleosides (e.g., 2'-F modified nucleosides and 2'-O-Me modified nucleosides). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at three consecutive positions of the sense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 8 and 12 (counting 5'→ 3') are not 2'-F modified nucleosides, e.g., are 2'-O-Me modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and

the nucleosides at positions 8 and 12 (counting 5'→ 3') are 2'-O-Me modified nucleosides. In some embodiments, the rest of the nucleosides in the sense strand are a mix of 2'-F modified nucleosides and 2'-O-Me modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, and 11 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides and the nucleosides at positions 8 and 12 (counting 5'→ 3') are 2'-O-Me modified nucleosides and the rest of the nucleosides in the sense strand comprise alternating motifs of 2'-O-Me modified nucleosides and 2'-F modified nucleosides.

In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the antisense strand comprises a mix of different 2'-modified nucleosides (e.g., any combination of 2'-F modified nucleosides, 2'-deoxy nucleosides, and 2'-O-Me modified nucleosides). In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at one or more of positions 2, 12, 16, 18, 20, and 21 (counting 5' → 3') of the antisense strand are 2'-deoxy nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 2, 12, 16, 18, 20, and 21 (counting 5' → 3') of the antisense strand are 2'-deoxy nucleosides and the rest of the nucleosides in the antisense strand are not 2'-deoxy nucleosides, e.g., are 2'-O-Me modified nucleosides or 2'-F modified nucleosides. In some embodiments, an RNAi agent (e.g., a CYP7A1 RNAi agent described herein) comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 17, and 19 of the antisense strand are a mix of 2'-F modified nucleosides and 2'-O-Me modified nucleosides. In some embodiments, the nucleosides at positions 3, 4, 5, 6, 7, 8, 9, 10, and 11 of the antisense strand comprise alternating motifs of 2'-O-Me modified nucleosides and 2'-F modified nucleosides.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises one or more (e.g., 1, 2, 3, 4, 5, or 6) phosphorothioate internucleoside linkages in the antisense strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises six phosphorothioate internucleoside linkages in the antisense strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein does not comprise any phosphorothioate internucleoside linkages in the sense strand. In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein further comprises six phosphorothioate internucleoside linkages in the antisense strand (e.g., the first two internucleoside linkages and the last two internucleoside linkages counting 5'→ 3' and the internucleoside linkages between

nucleosides at positions 15-16 and 17-18 counting 5'→ 3') and no phosphorothioate internucleoside linkages in the sense strand. It is to be understood that, in any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein, the rest of the internucleoside linkages, unless otherwise specified, are all phosphodiester internucleoside linkages.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the nucleosides at positions 1, 3, 5, 7, 9, 10, 11, 13, 15, 17, and 19 (counting 5'→ 3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 3, 5, 7, 9, 11, 14, 17, and 19 (counting 5'→ 3') of the antisense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 12, 16, 18, 20, and 21 (counting 5' → 3') of the antisense strand are 2'-deoxy nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides or 2'-deoxy nucleosides are 2'-O-Me modified nucleosides, and wherein the first two and the last two internucleoside linkages of the antisense strand (counting 5'→ 3') and the internucleoside linkages between positions 15-16 and 17-18 of the antisense strand (counting 5'→ 3') are phosphorothioate internucleoside linkages.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein nucleosides at positions 8, 9, 10, and 11 of the sense strand are 2'-F modified nucleosides and nucleosides at positions 2, 3, 4, 5, 7, 10, and 14 of the antisense strand are 2'-F modified nucleosides, wherein the rest of the nucleosides in the sense strand and antisense strand are 2'-O-Me modified nucleosides, wherein the first two or first three internucleoside linkages of the antisense strand (counting 5' → 3') are phosphorothioate internucleoside linkages.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein nucleosides at one or more of positions 3, 5, 8, 9, 10, 11, 13, 15, or 17 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides. In some embodiments, nucleosides at one or more of positions 1, 2, 4, 6, 7, 12, 14, 16, 18, 19, or 20 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides. In some embodiments, nucleosides at one or more of positions 2, 3, 4, 5, 7, 9, 10, 14, 16, or 18 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides. In some embodiments, nucleosides at one or more of positions 1, 6, 8, 11, 12, 13, 15, 17, 19, 20, 21, or 22 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides.

In some embodiments, nucleosides at all of positions 3, 5, 8, 9, 10, 11, 13, 15, or 17 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and the rest of the nucleosides in the sense strand are 2'-O-Me modified nucleosides. In some embodiments, nucleosides at all of positions 2, 3, 4, 5, 7, 9, 10, 14, 16, and 18 (counting 5' → 3') of the

antisense strand are 2'-F modified nucleosides and the rest of the nucleosides in the antisense strand are 2'-O-Me modified nucleosides. In some embodiments, the first internucleoside linkage of the sense strand (counting 5' → 3') is a phosphorothioate internucleoside linkage and the first three and last two internucleoside linkages of the antisense strand (counting 5' → 3') are phosphorothioate internucleoside linkages. In some embodiments, nucleosides at all of positions 3, 5, 8, 9, 10, 11, 13, 15, or 17 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides and all of positions 2, 3, 4, 5, 7, 9, 10, 14, 16, and 18 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides, and the rest of the nucleosides in the sense strand and antisense strand are 2'-O-Me modified nucleosides, wherein the first internucleoside linkage of the sense strand (counting 5' → 3') is a phosphorothioate internucleoside linkage and the first three and last two internucleoside linkages of the antisense strand (counting 5' → 3') are phosphorothioate internucleoside linkages.

In some embodiments, any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein comprises a sense strand and an antisense strand, wherein the antisense strand comprises a phosphorylated nucleoside at the 5' terminus, wherein the phosphorylated nucleoside is selected from uridine and adenosine. In some embodiments, the phosphorylated nucleoside is uridine. In some embodiments, the 5'-nucleoside of the antisense strand comprises a 4'-phosphate analog. In some embodiments, the phosphate analog is oxymethylphosphonate, vinylphosphonate, or malonylphosphonate. In some embodiments, the phosphate analog is a 4'-phosphate analog comprising 5'-methoxyphosphonate-4'-oxy.

*Conjugated CYP7A1 RNAi agents*

In some embodiments, any one of the RNAi agents described herein is conjugated (e.g., covalently linked) to another chemical moiety (e.g., a targeting moiety for, e.g., improved delivery, cellular uptake and distribution). In some embodiments, any one of the RNAi agents described herein is conjugated (e.g., covalently linked) to a targeting moiety via a linker. Any of the linkers described herein may be used. In some embodiments, the targeting moiety is conjugated (e.g., covalently linked) to the 5′ end of the antisense strand of an RNAi agent. In some embodiments, the targeting moiety is conjugated (e.g., covalently linked) to the 3′ end of the antisense strand of an RNAi agent. In some embodiments, the targeting moiety is conjugated (e.g., covalently linked) to the 5′ end of the sense strand of an RNAi agent. In some embodiments, the targeting moiety is conjugated (e.g., covalently linked) to the 3′ end of the sense strand of an RNAi agent.

Any suitable targeting moiety in the field of RNA interference may be conjugated (e.g., covalently linked) to any one of the RNAi agents described herein. Non-limiting examples of

targeting moieties include carbohydrates (e.g., monosaccharides (such as GalNAc)), disaccharides, trisaccharides, tetrasaccharides, polysaccharides), folate, mannose-6P, clusters of sugars such as GalNAc cluster, mannose cluster, galactose cluster, an aptamer, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, asialoglycoprotein receptor ligands, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL and HDL ligands.

In some embodiments, the targeting moiety is an antibody or antigen binding fragment thereof that specifically binds to a cell surface protein, e.g., a cell surface receptor that promotes receptor mediated endocytosis and internalization of the RNAi agents described herein. Cell surface proteins that may be targeted by the targeting moiety include, without limitation: asialoglycoprotein receptors, CD63, MHC-I, Kremen-1, Kremen-2, LRP5, LRP6, LRP8, transferrin receptor, LDL-receptor, LDL-related protein 1 receptor, ASGRl, ASGR2, amyloid precursor protein-like protein-2 (APLP2), apelin receptor (APL R), PRLR (prolactin receptor), MAL (Myelin And Lymphocyte protein, a.k.a. VIP 17), IGF2R, vacuolar-type H+ ATPase, diphtheria toxin receptor, folate receptor, glutamate receptors, glutathione receptor, leptin receptors, scavenger receptors, SCARAl-5, SCARBl-3, CD36, CDH16 (Cadheri-16), CLDN16 (Claudn-16), KL (Klotho), PTH1R (parathyroid hormone receptor), SLC22A13 (Solute carrier family 22 member 13), SLC5A2 (Sodium/glucose cotransporter 2), UMOD (Uromodulin), BMPRIA (Bone morphogenetic protein receptor 1A), m-cadherin, CD9, MuSK (muscle-specific kinase), LGR4/GPR48 (G protein-coupled receptor 48), cholinergic receptor (nicotinic) alpha 1, CDH15 (Cadheri-15), ITGA7 (Integrin alpha-7), CACNG1 (L-type calcium channel subunit gamma- 1), CACNAls (L-type calcium channel subunit alpha- 15), CACNG6 (L-type calcium channel subunit gamma-6), SCN1B (Sodium channel subunit beta-1), CHRNA1 (ACh receptor subunit alpha), CHRND (ACh receptor subunit delta), LRRC14B (Leucine-rich repeat-containing protein 14B), and POPDC3 (Popeye domain-containing protein 3). In some embodiments, antibodies or antigen binding fragments that may be used as a targeting moiety according to the present disclosure is a Fab fragment, a Fab', a F(ab')2 fragment, a Fv fragment, a scFv, a VHH, or a diabody. In some embodiments, the antibody or antigen binding fragment thereof is covalently linked directly or indirectly (e.g., via a linker) to an RNAi agent described herein.

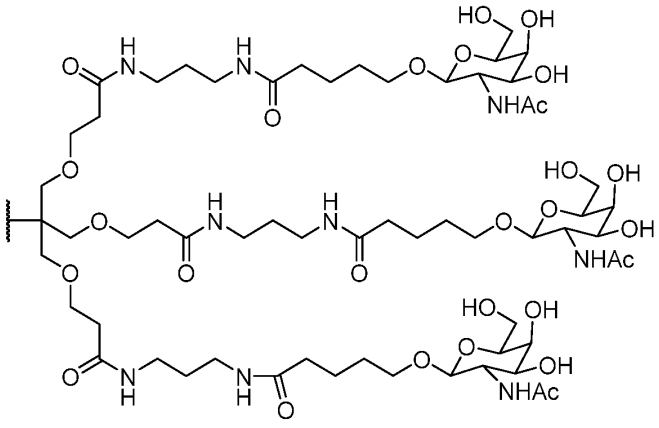
In some embodiments, the targeting moiety is a hepatospecific targeting moiety (i.e., it directs the RNAi agent composition to liver cells). In some embodiments, the targeting moiety comprises a monosaccharide. In some embodiments, the monosaccharide is an N-acetylgalactosamine (GalNAc). In some embodiments, the targeting moiety comprises one or more (e.g, 1, 2, 3, 4, or more) GalNAc. In some embodiments, the one or more GalNAc in the targeting moiety are linked via phosphorothioate linkages. In some embodiments, the GalNAc

targeting moiety serves as a ligand that targets the RNAi agent to particular cells. In some embodiments, the GalNAc targeting moiety targets the RNAi agent to liver cells, e.g., by serving as a ligand for the asialoglycoprotein receptor of liver cells (e.g., hepatocytes). GalNAc targeting moieties, which comprise one or more GalNAc, have been described, for example, in the following references:

| Reference No. | Application No. | Filing Date |
|---|---|---|
| US 8,106,022 | US 12/328,528 | Dec. 4, 2008 |
| US 10,246,709 | US 15/452,423 | Mar. 7, 2017 |
| US 5,994,517 | US 08/755,062 | Nov. 22, 1996 |
| US 6,906,182 | US 09/998,497 | Nov. 30, 2001 |
| US 10,294,474 | US 15/452,324 | Mar. 7, 2017 |
| US 2017305956 | US 15/621,395 | Jun. 13, 2017 |
| US 10,233,448 | US 15/504,855 | Feb. 17, 2017 |
| US 2016122761 | US 14/898,873 | Jun. 23, 2014 |
| US 10,808,246 | US 14/901,945 | Dec. 29, 2015 |
| WO 2022/159158A1 | PCT/US2021/057016 | Oct. 28, 2021 |

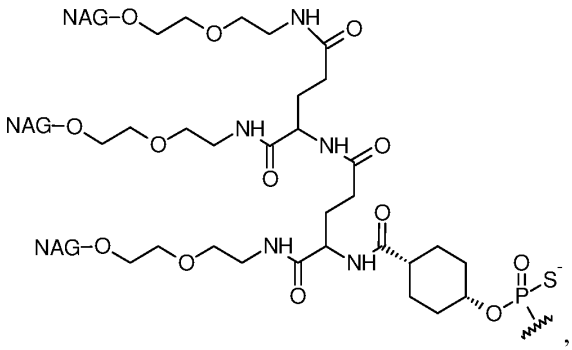
The disclosures in these references related to GalNAc are hereby incorporated herein by reference.

In some embodiments, a targeting moiety comprises the structure below:



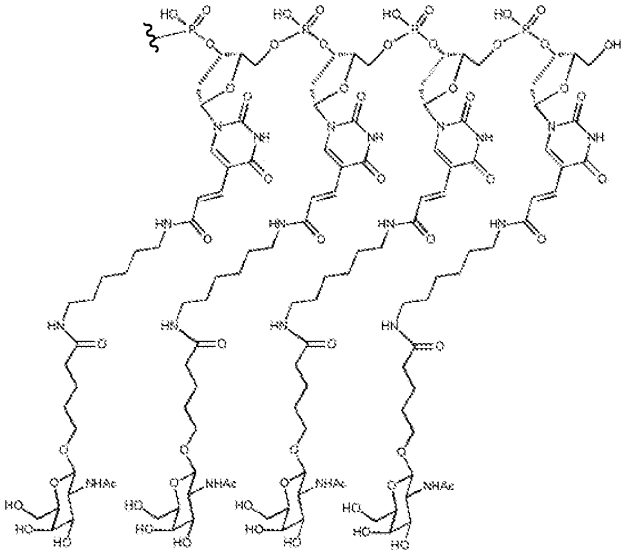
, or a pharmaceutically acceptable salt thereof.

In some embodiments a targeting moiety comprises the structure below:



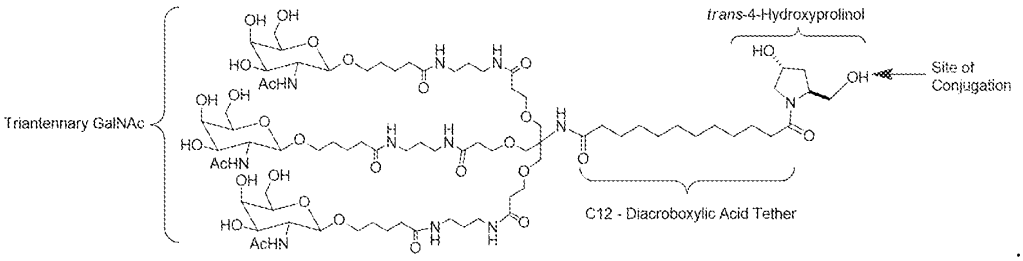

, or a pharmaceutically acceptable salt thereof.

In some embodiments, a targeting moiety comprises the structure below:



, or a pharmaceutically acceptable salt thereof.

In some embodiments, an RNAi agent is conjugated to the targeting moiety via a tether, also referred to as a linker. A non-limiting example of a tether includes a linear aliphatic group comprising one or more groups selected from alkyl, substituted alkyl, ether, thioether, disulfide, amide and polyethylene glycol (PEG) groups in any combination. In some embodiments, a targeting moiety covalently linked to a tether shown below:



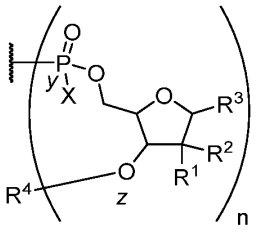

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Additional carbohydrate conjugates and linkers suitable for use in the present invention

include those described in US Patent Nos. 9,127,276, 9,145,558, and 10,683,499. Disclosures in US Patent Nos. 9,127,276 (US Patent Application No. 14/267,842 filed May 1, 2014), 9,145,558 (US Patent Application No. 14/633,491 filed February 27, 2015), and 10,683,499 (US Patent Application No. 15/687,306 filed August 25, 2017) regarding carbohydrate conjugates and linkers are incorporated herein by reference.

Some aspects of the present disclosure further provide additional targeting moieties comprising one or more GalNAc and structures that may be conjugated to any one of the CYP7A1 RNAi agents described herein. In some embodiments, a targeting moiety comprising one or more GalNAc is conjugated (attached) to a CYP7A1 RNAi agent.

In some embodiments, the targeting moiety comprises a structure of Formula (**I**):

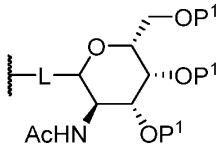


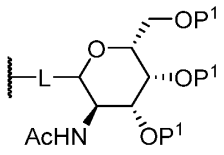
(**I**)

or a pharmaceutically acceptable salt thereof;

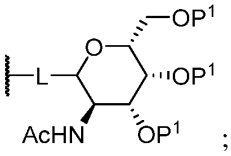
wherein:

each X is independently $-N(R^A)_2$, $-SR^A$, or $-OR^A$;

each $R^1$ is independently hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, $-OR^A$, or a group of formula: ;

each $R^2$ is independently hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, $-OR^A$, or a group of formula: ;

each $R^3$ is independently hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, $-OR^A$, or a group of formula:

;

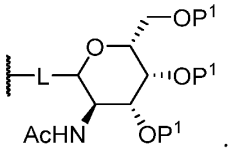
$R^4$ is hydrogen or substituted or unsubstituted alkyl;

each $P^1$ is independently hydrogen or an oxygen protecting group;

each L is independently a bond, substituted or unsubstituted aliphatic, substituted or unsubstituted substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbocyclylene, substituted or unsubstituted arylene, -O-, -N($R^A$)-, -S-, -C(=O)-, -C(=O)O-, -C(=O)N$R^A$-, -N$R^A$C(=O)-, -N$R^A$C(=O)$R^A$-, -C(=O)$R^A$-, -N$R^A$C(=O)O-, -N$R^A$C(=O)N($R^A$)-, -OC(=O)-, -OC(=O)O-, -OC(=O)N($R^A$)-, -S(O)$_2$N$R^A$-, -N$R^A$S(O)$_2$-, or a combination thereof;

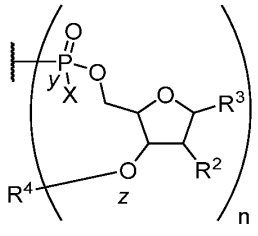
each $R^A$ is independently hydrogen, substituted or unsubstituted acyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, a nitrogen protecting group when attached to a nitrogen atom, an oxygen protecting group when attached to an oxygen atom, or a sulfur protecting group when attached to a sulfur atom, or two $R^A$ groups are joined to form a substituted or unsubstituted heterocyclyl ring, or a substituted or unsubstituted heteroaryl ring; and

n is 1-20;

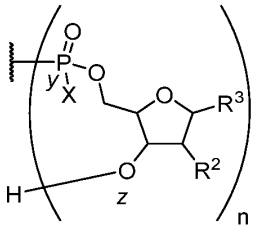
wherein the phosphorous labeled *y* is conjugated to the 3' end of the sense strand; and wherein when n is greater than 1, with the exception of the phosphorous directly attached to an RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit; provided that each repeat unit of Formula (**I**) in the targeting moiety comprises one group of formula: 

.

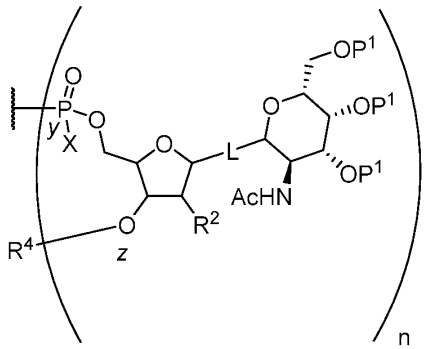
In some embodiments, the targeting moiety comprises a structure of Formula:



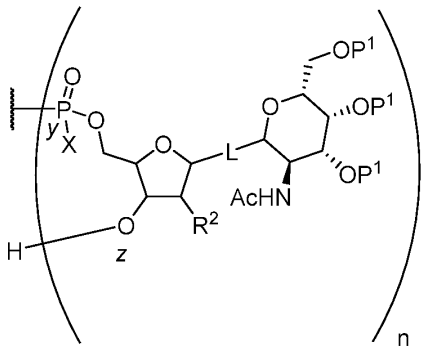
(**I-a**);



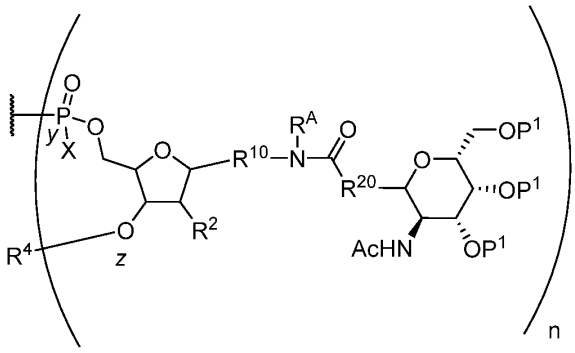
(**I-a-1**);



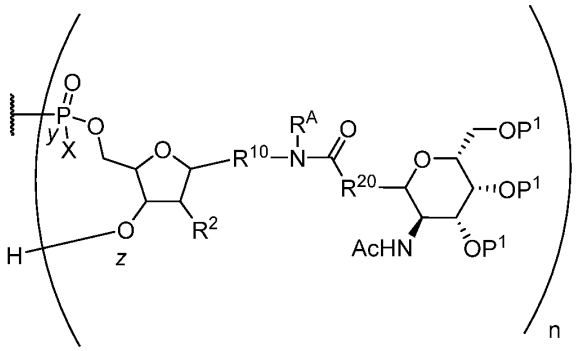
(**I-b**);



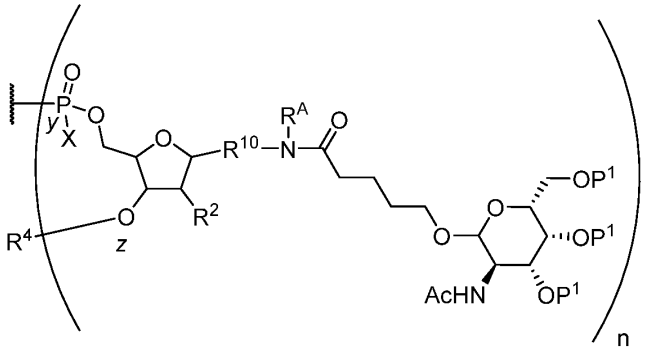
(**I-b-1**);



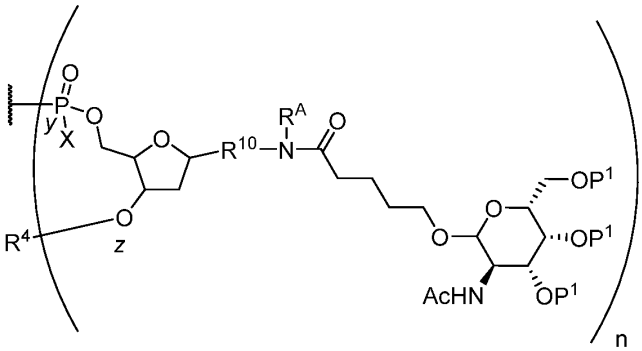
(**I-c**);



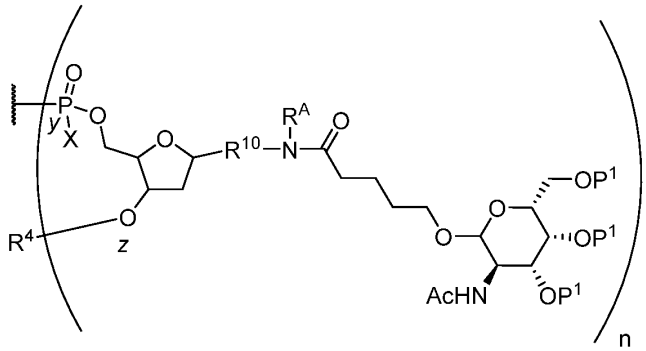
(**I-c-1**);



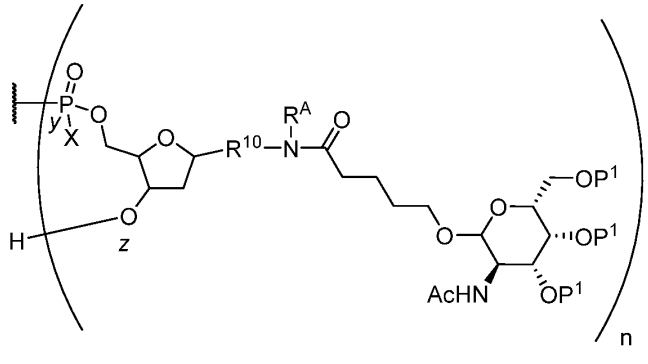
(**I-d**);



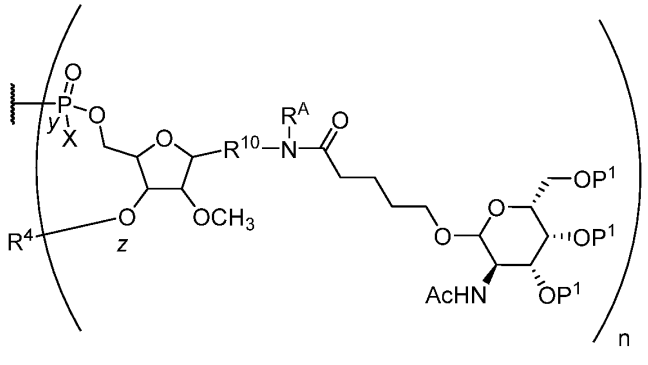
(**I-d-1**);



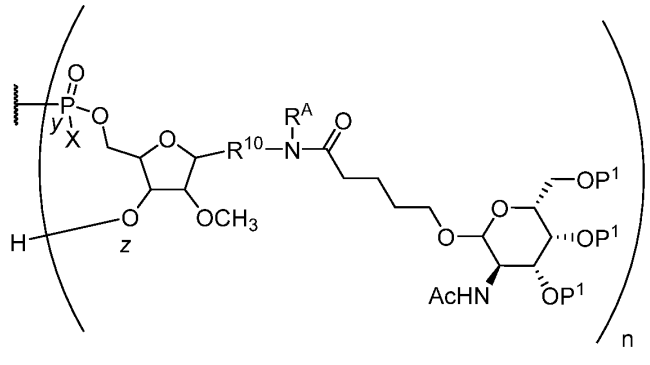
(**I-e**);



(**I-e-1**);



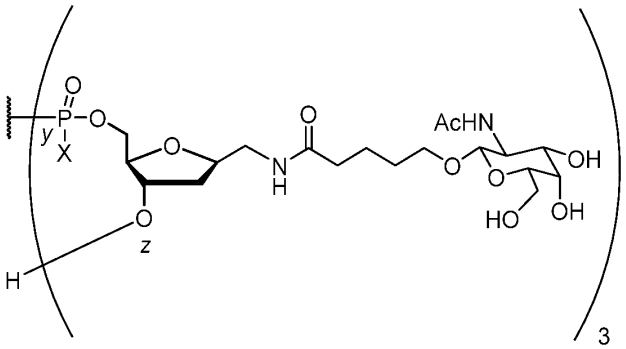
(**I-f**); or



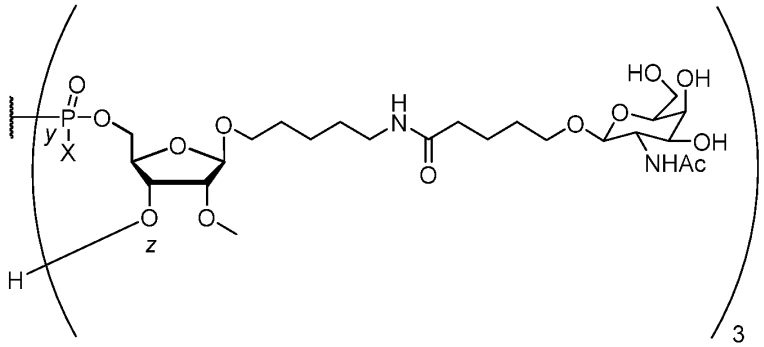
(**I-f-1**);

or a pharmaceutically acceptable salt thereof; wherein $R^{10}$ is substituted or unsubstituted $C_{1-8}$ alkylene having 0-3 carbon atoms replaced with -O-; and $R^{20}$ is substituted or unsubstituted $C_{1-8}$ alkylene having 0-3 carbon atoms replaced with -O-; and $P^1$, $R^2$, $R^3$, $R^4$, $R^A$, n, and X are as defined for Formula (**I**).

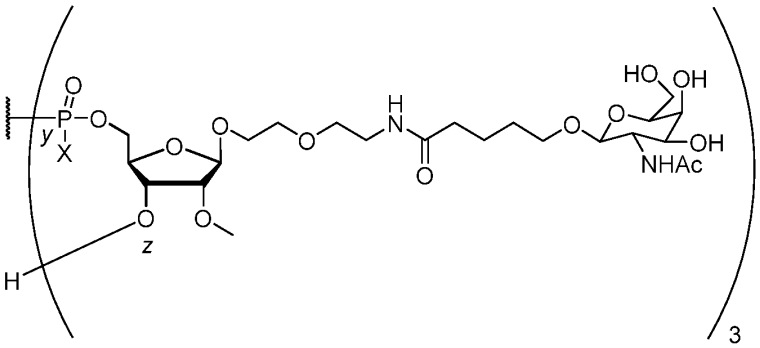
In some embodiments, the targeting moiety comprises a structure of formula:



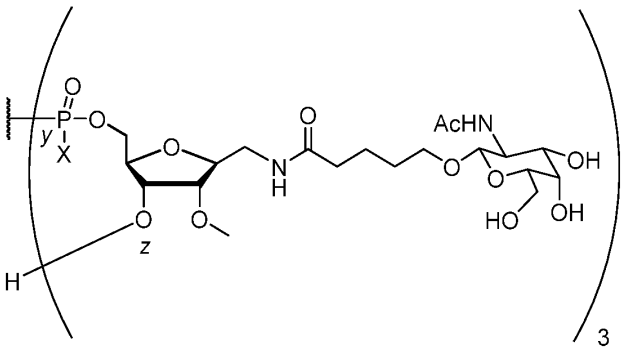
(**I-g**);



(**I-h**);



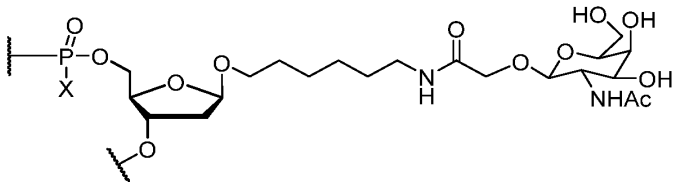
(**I-i**); or



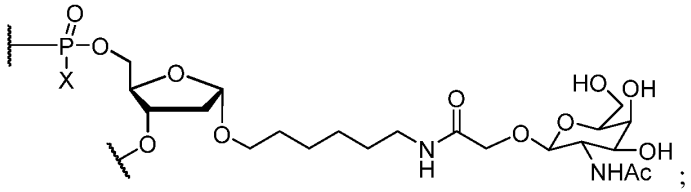
(**I-j**);

or a pharmaceutically acceptable salt thereof, wherein X is as defined for Formula (**I**).

In some embodiments, the targeting moiety does not comprise a structure of:



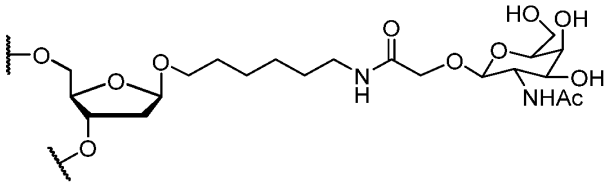
; or



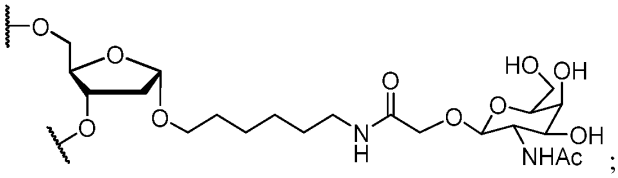
;

or a salt thereof, wherein X is -$OR^A$ or -$SR^A$ (e.g., where $R^A$ is H).

In some embodiments, the targeting moiety does not comprise a structure of:



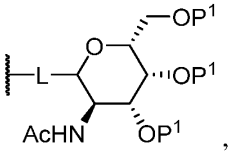
; or



;

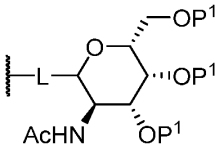
or a salt thereof.

In some embodiments, in a targeting moiety comprising a structure of Formula (I), when



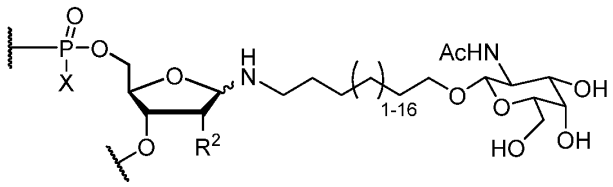
$R^1$ and $R^2$ are hydrogen, and $R^3$ is a group of formula , L does not attach to the 1' position of the ribose by an oxygen atom.

In some embodiments, in a targeting moiety comprising a structure of Formula (I), when



$R^3$ is a group of formula , L does not attach to the 1' position of the ribose by a nitrogen atom.

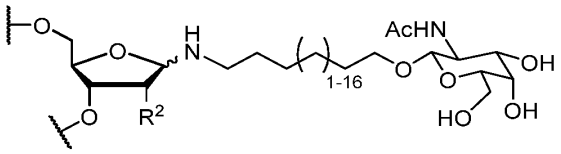
In some embodiments, the targeting moiety does not comprise a structure of formula:



,

or a salt thereof, wherein X is -$OR^A$ or -$SR^A$ (e.g., where $R^A$ is H); and $R^2$ is hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or -$OR^A$. In some embodiments, the targeting moiety does not comprise a group of the above formula, wherein X is -$OR^A$ or -$SR^A$ (e.g., where $R^A$ is H); and $R^2$ is hydrogen, fluoro, -$OCH_3$, -OTBS, or -$OCH_2CH_2OCH_3$.

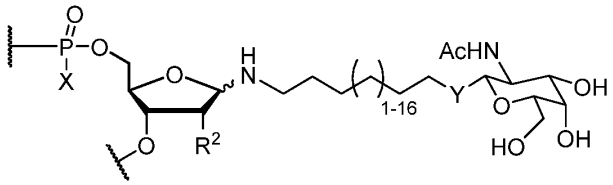
In some embodiments, the targeting moiety does not comprise a structure of formula:



,

or a salt thereof, wherein $R^2$ is hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or -$OR^A$. In some embodiments, the targeting moiety does not comprise a group of the above formula, wherein $R^2$ is hydrogen, fluoro, -$OCH_3$, -OTBS, or -$OCH_2CH_2OCH_3$.

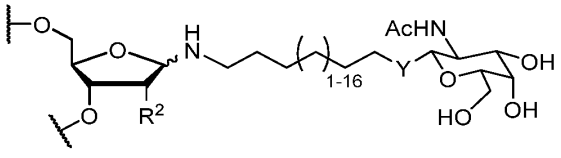
In some embodiments, the targeting moiety does not comprise a group of formula:



,

or a salt thereof, wherein X is -$OR^A$ or -$SR^A$ (e.g., where $R^A$ is H); Y is -O-, -S-, -$CH_2$-, -NH(C=O)-, -(C=O)NH-, or -NH(C=O)O-; and $R^2$ is hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or -$OR^A$. In some embodiments, the targeting moiety does not comprise a group of the above formula, wherein X is -$OR^A$ or -$SR^A$ (e.g., where $R^A$ is H); Y is -O-, -S-, -$CH_2$-, -NH(C=O)-, -(C=O)NH-, or -NH(C=O)O-; and $R^2$ is hydrogen, fluoro, -$OCH_3$, -OTBS, or -$OCH_2CH_2OCH_3$.

In some embodiments, the targeting moiety does not comprise a structure of formula:

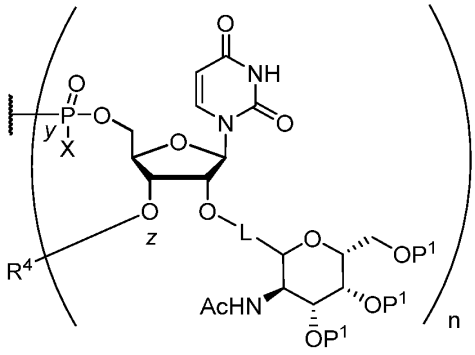


,

or a salt thereof, wherein Y is -O-, -S-, -$CH_2$-, -NH(C=O)-, -(C=O)NH-, or -NH(C=O)O-; and

$R^2$ is hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or $-OR^A$. In some embodiments, the targeting moiety does not comprise a group of the above formula, wherein Y is -O-, -S-, $-CH_2-$, -NH(C=O)-, -(C=O)NH-, or -NH(C=O)O-; and $R^2$ is hydrogen, fluoro, $-OCH_3$, -OTBS, or $-OCH_2CH_2OCH_3$.

In some embodiments, the targeting moiety comprises a structure of Formula (**II**):



(**II**)

or a pharmaceutically acceptable salt thereof;

wherein:

$R^4$ is hydrogen or substituted or unsubstituted alkyl;

each X is independently $-N(R^A)_2$, $-SR^A$, or $-OR^A$;

each $P^1$ is independently hydrogen or an oxygen protecting group;

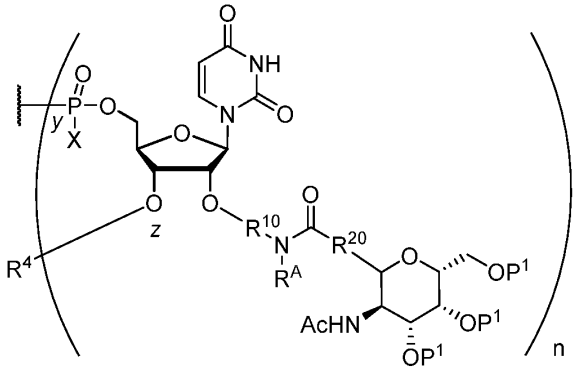
each L is independently a bond, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted arylene, -O-, $-N(R^A)-$, -S-, -C(=O)-, $-C(=O)O-$, $-C(=O)NR^A-$, $-NR^AC(=O)-$, $-NR^AC(=O)R^A-$, $-C(=O)R^A-$, $-NR^AC(=O)O-$, $-NR^AC(=O)N(R^A)-$, -OC(=O)-, -OC(=O)O-, $-OC(=O)N(R^A)-$, $-S(O)_2NR^A-$, $-NR^AS(O)_2-$, or a combination thereof;

each $R^A$ is independently hydrogen, substituted or unsubstituted acyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, a nitrogen protecting group when attached to a nitrogen atom, an oxygen protecting group when attached to an oxygen atom, or a sulfur protecting group when attached to a sulfur atom, or two $R^A$ groups are joined to form a substituted or unsubstituted heterocyclyl ring, or a substituted or unsubstituted heteroaryl ring; and

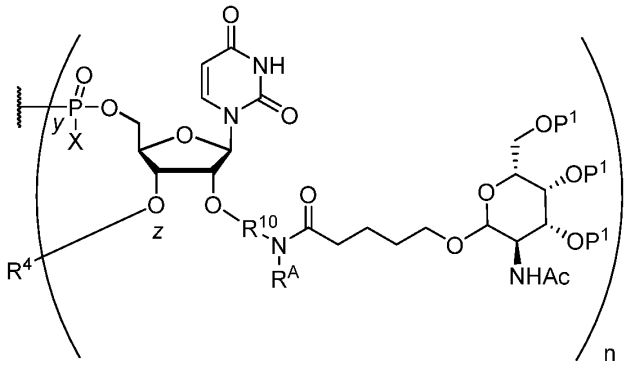
n is 1-20;

wherein the phosphorous labeled *y* is conjugated to the 3' end of the sense strand; and wherein when n is greater than 1, with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit.

In some embodiments, the targeting moiety comprises a structure of Formula:



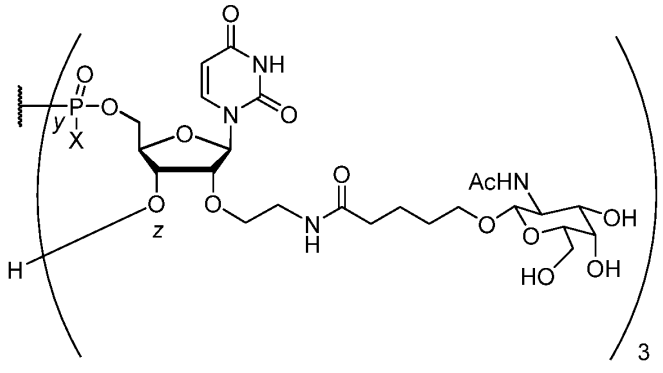
(**II-a**); or



(**II-b**);

or a pharmaceutically acceptable salt thereof; wherein $R^{10}$ is substituted or unsubstituted $C_{1-8}$ alkylene having 0-3 carbon atoms replaced with -O-; and $R^{20}$ is substituted or unsubstituted $C_{1-8}$ alkylene having 0-3 carbon atoms replaced with -O-; and $P^1$, $R^4$, $R^A$, n, and X are as defined for Formula (**II**).

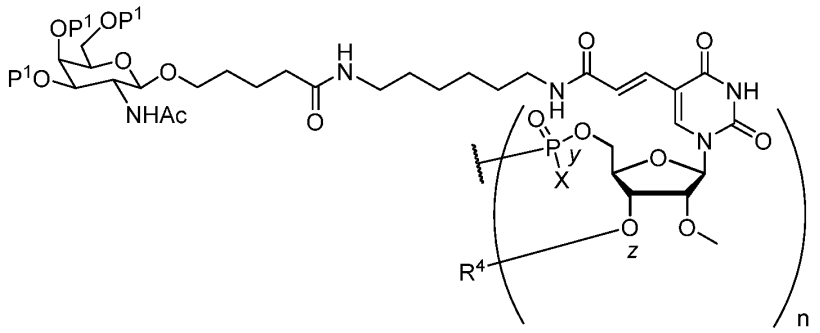
In some embodiments, the targeting moiety comprises a structure of formula:



(**II-c**);

or a pharmaceutically acceptable salt thereof, wherein X is as defined for Formula (**II**).

In some embodiments, the targeting moiety comprises a structure of Formula (**III**):



(**III**)

or a pharmaceutically acceptable salt thereof,

wherein:

$R^4$ is hydrogen or substituted or unsubstituted alkyl;

each X is independently $-N(R^A)_2$, $-SR^A$, or $-OR^A$;

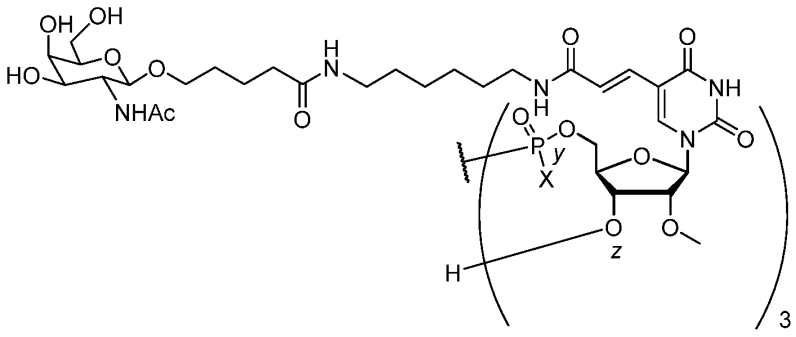
each $P^1$ is independently hydrogen or an oxygen protecting group;

each $R^A$ is independently hydrogen, substituted or unsubstituted acyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, an oxygen protecting group when attached to an oxygen atom, or a sulfur protecting group when attached to a sulfur atom; and

n is 1-20;

wherein the phosphorous labeled *y* is conjugated to the 3' end of the sense strand; and wherein when n is greater than 1, with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit.

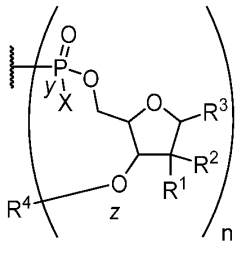
In some embodiments, the targeting moiety comprises a structure of formula:



(**III-a**);

or a pharmaceutically acceptable salt thereof, wherein X is as defined for Formula (**III**).

In some embodiments, the targeting moiety comprises a structure of Formula (**IV**):



(**IV**),

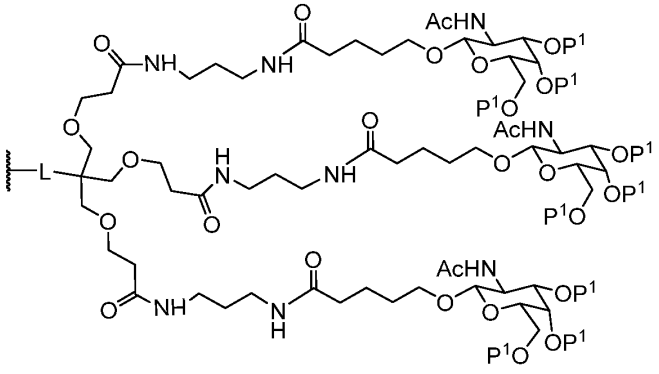
wherein:

each X is independently $-N(R^A)_2$, $-SR^A$, or $-OR^A$;

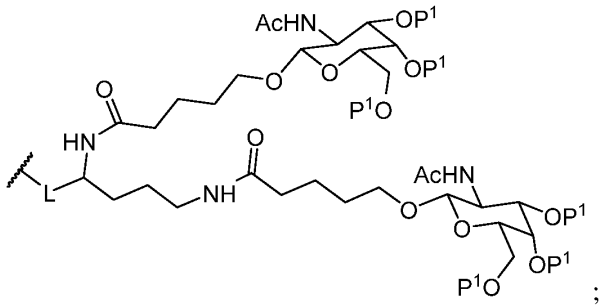
each $R^1$ is independently hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or $-OR^A$;

each $R^2$ is independently hydrogen, halogen, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, or $-OR^A$;

each $R^3$ is independently of formula:



or



;

$R^4$ is hydrogen or substituted or unsubstituted alkyl;

each $P^1$ is independently hydrogen or an oxygen protecting group;

each L is independently a bond, substituted or unsubstituted aliphatic, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heteroalkenyl, substituted or unsubstituted heteroalkynyl, substituted or unsubstituted carbocyclylene, substituted or unsubstituted arylene, -O-, $-N(R^A)-$, -S-, -C(=O)-, -C(=O)O-, $-C(=O)NR^A-$, $-NR^AC(=O)-$, $-NR^AC(=O)R^A-$, $-C(=O)R^A-$,

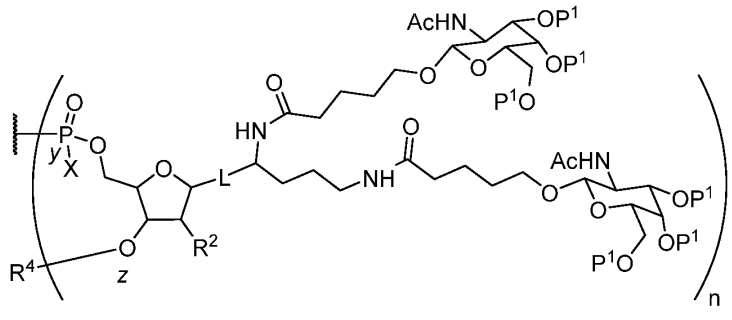
-$NR^AC(=O)O$-, -$NR^AC(=O)N(R^A)$-, -$OC(=O)$-, -$OC(=O)O$-, -$OC(=O)N(R^A)$-, -$S(O)_2NR^A$-, -$NR^AS(O)_2$-, or a combination thereof;

each $R^A$ is independently hydrogen, substituted or unsubstituted acyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclyl, substituted or unsubstituted heterocyclyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, a nitrogen protecting group when attached to a nitrogen atom, an oxygen protecting group when attached to an oxygen atom, or a sulfur protecting group when attached to a sulfur atom, or two $R^A$ groups are joined to form a substituted or unsubstituted heterocyclyl ring, or a substituted or unsubstituted heteroaryl ring; and

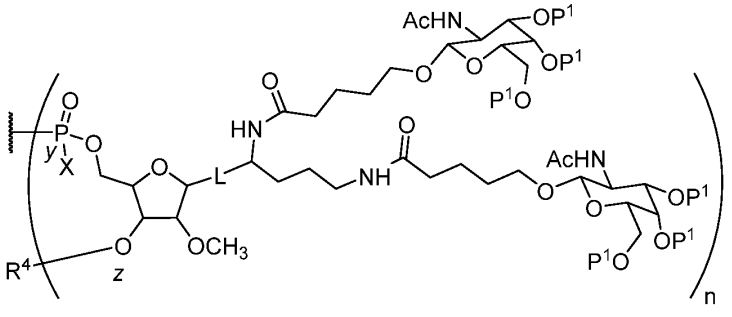
n is 1-20;

wherein the phosphorous labeled *y* is conjugated to the 3' end of the sense strand; and wherein when n is greater than 1, with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit.

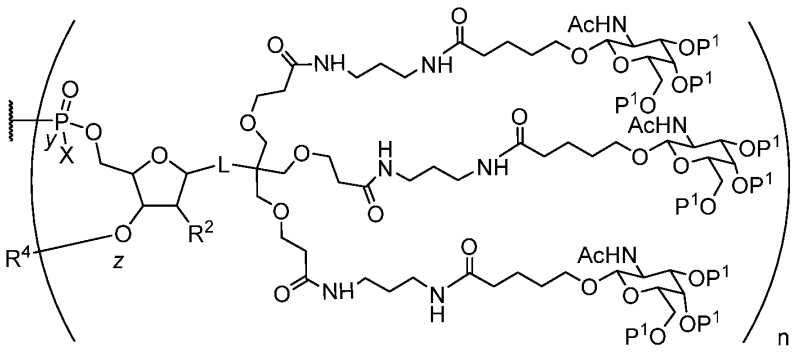
In some embodiments, the targeting moiety comprises a structure of formula:



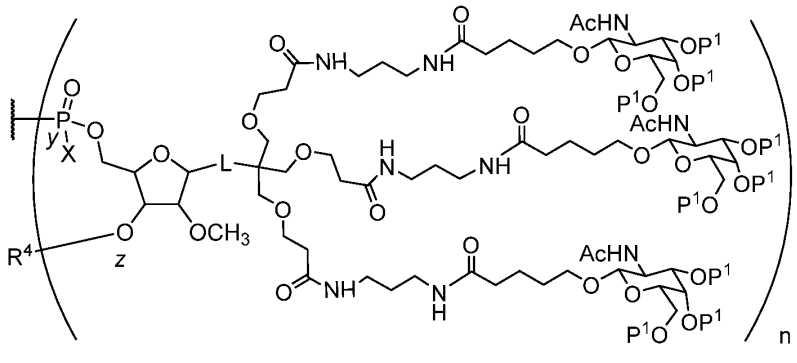

(**IV-a**);




(**IV-b**);



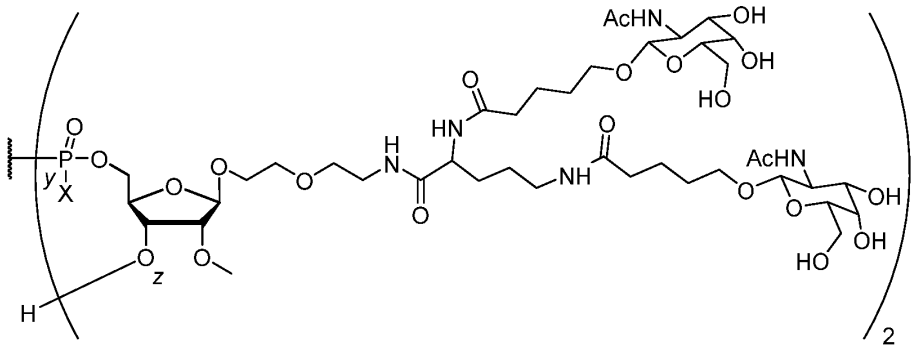
(**IV-c**); or



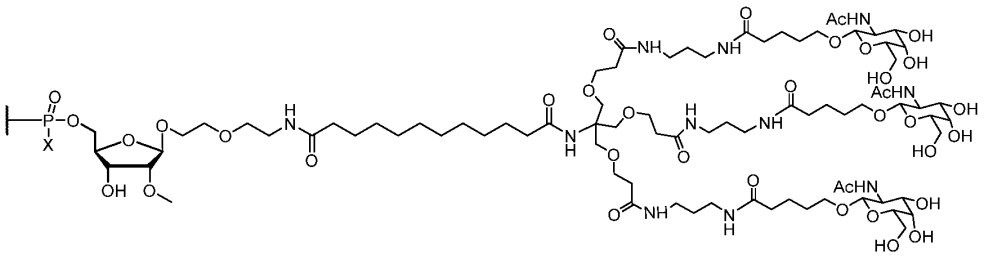
(**IV-d**);

or a pharmaceutically acceptable salt thereof; wherein $R^4$, L, $P^1$, n, and X are as defined for Formula (**IV**). For any one of Formulas (**IV-a**), (**IV-b**), (**IV-c**), and (**IV-d**), when n is greater than 1, with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit, and with the exception of the oxygen directly attached to $R^4$, each instance of oxygen labeled *z* is bound to the phosphorous labeled *y* of the adjacent unit.

In some embodiments, the targeting moiety comprises a structure of formula:



(**IV-e**); or



(**IV-f**);

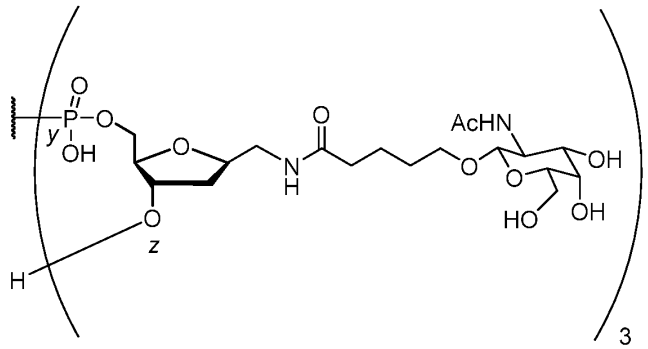
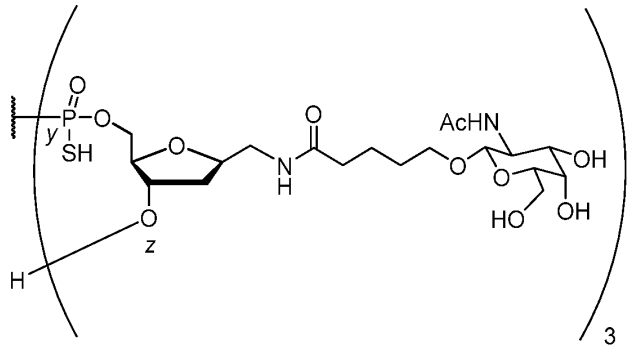
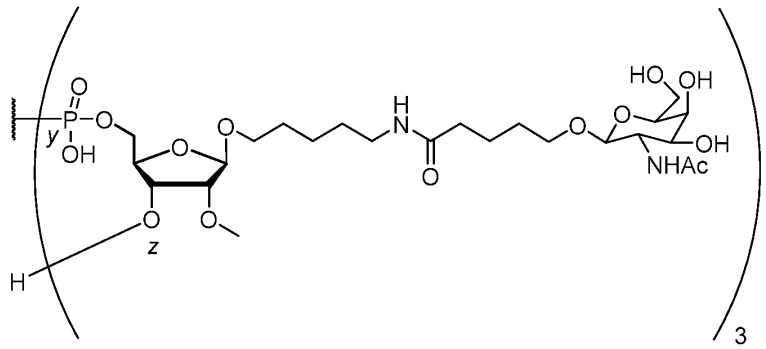
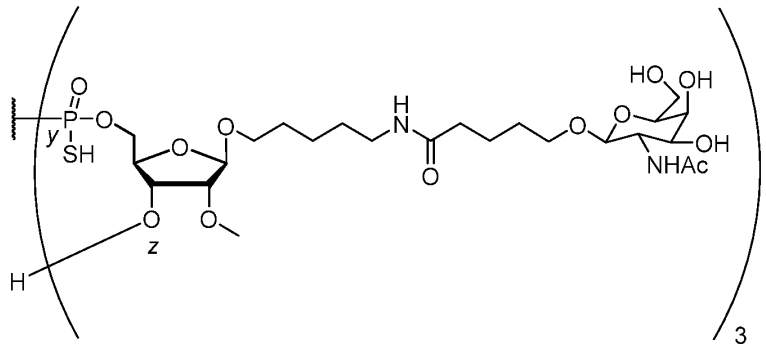
or a pharmaceutically acceptable salt thereof, wherein X is as defined for Formula (**IV**). For Formula (**IV-e**), with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit, and with the exception of the oxygen directly attached to hydrogen, the oxygen labeled *z* is bound to the phosphorous labeled *y* of the adjacent unit.

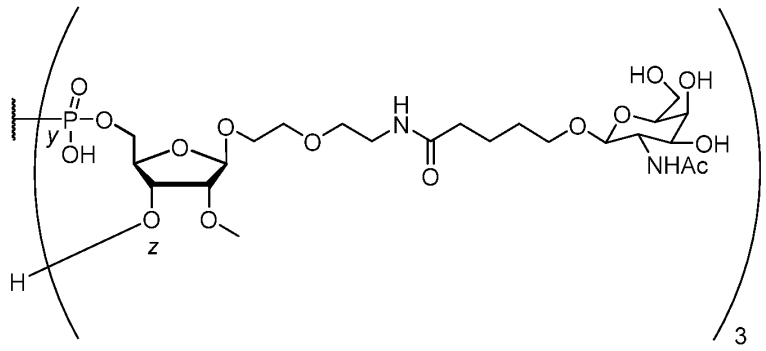
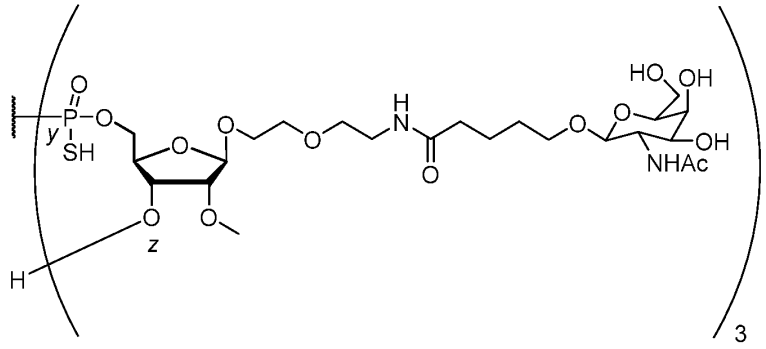
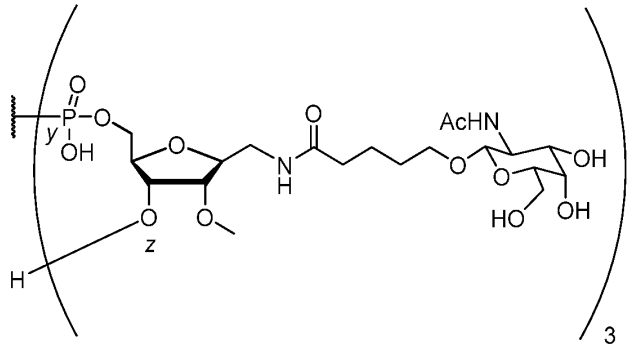
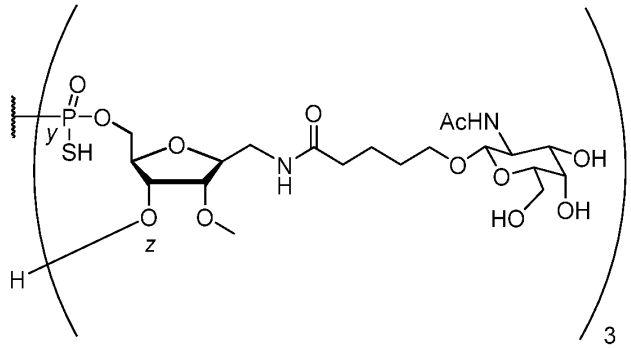
In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 1-6. In some embodiments, in a targeting moiety comprising a structure of Formula ( (**I**), (**II**), (**III**), or (**IV**), n is 1-3. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 2-3. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 1-4. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 2-4. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 1. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 2. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), n is 3. In some embodiments, the targeting moiety is conjugated to the sense strand via the phosphorous labeled *y*. In some embodiments, the targeting moiety is conjugated to the 3' end of the sense strand via the phosphorous labeled *y*.

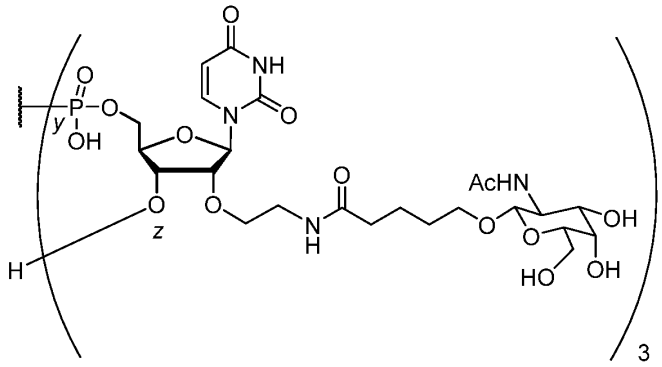
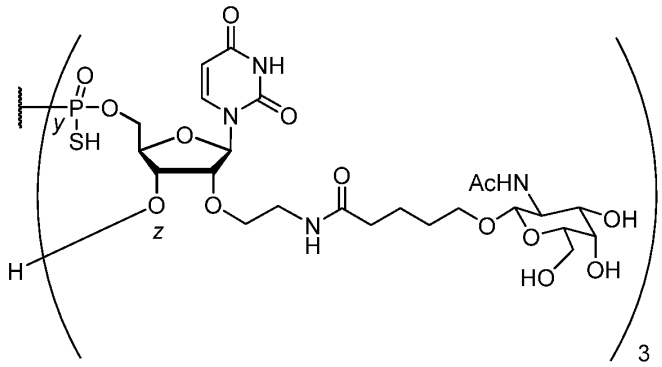
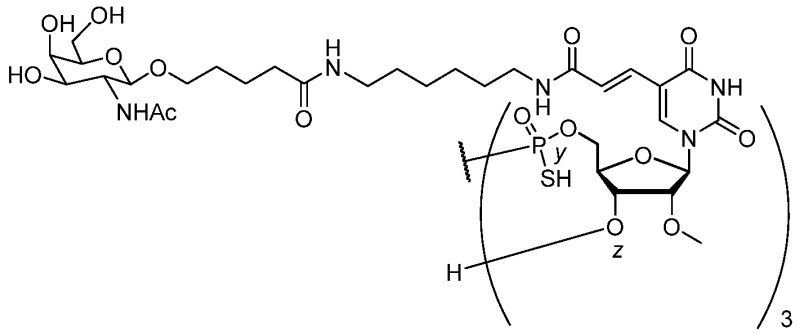
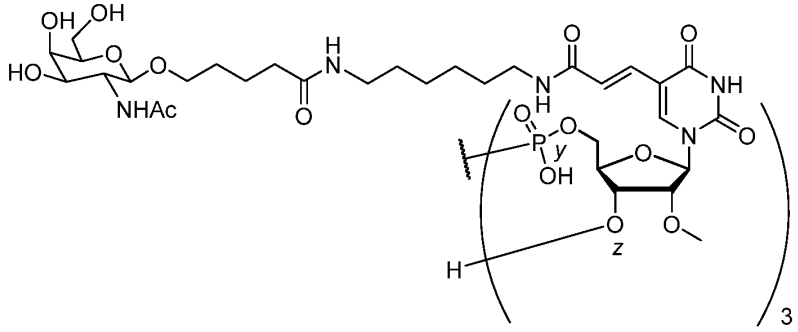
In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), each X is independently $-SR^A$. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), each X is independently $-OR^A$. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), each X is independently -SH or -OH. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), each X is independently -SH. In some embodiments, in a targeting moiety comprising a structure of Formula (**I**), (**II**), (**III**), or (**IV**), each X is independently -OH.

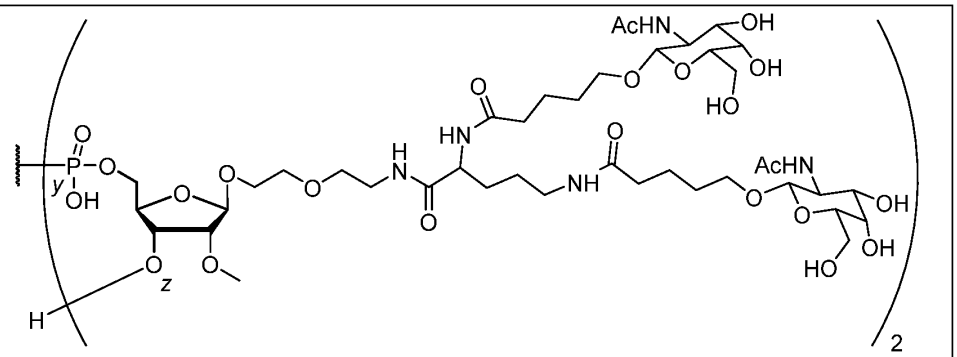
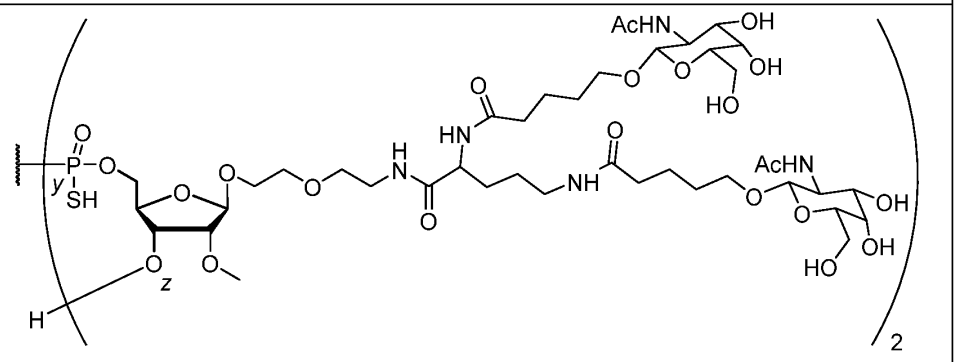
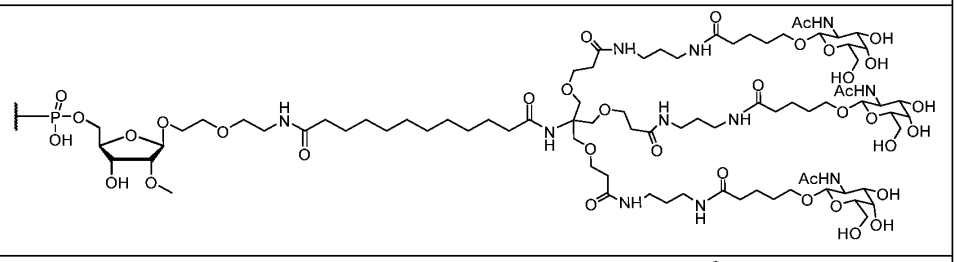
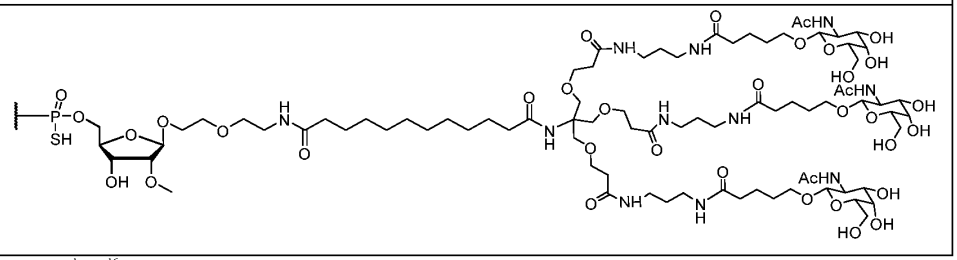
Table 4 lists various exemplary GalNAc targeting moieties. Included are pharmaceutically acceptable salts of the exemplary targeting moieties.

Table 4. Exemplary targeting moieties comprising GalNAc

| Targeting Moiety ID: | Structure |
|---|---|
| ($Z^1$) |  |
| ($Z^2$) |  |
| ($Z^3$) |  |
| ($Z^4$) |  |

| | |
|---|---|
| ($Z^5$) |  |
| ($Z^6$) |  |
| ($Z^7$) |  |
| ($Z^8$) |  |

| | |
|---|---|
| ($Z^9$) |  |
| ($Z^{10}$) |  |
| ($Z^{11}$) |  |
| ($Z^{12}$) |  |

| | |
|---|---|
| ($Z^{13}$) |  |
| ($Z^{14}$) |  |
| ($Z^{15}$) |  |
| ($Z^{16}$) |  |

*For any one of Formulas ($Z^1$)-($Z^{16}$), with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent repeat unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.*

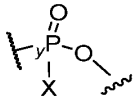
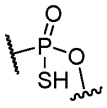
In some embodiments, a targeting moiety is linked to the RNAi agent via a linker. Various linkers may be used to conjugate a targeting moiety to any one of the RNAi agents described herein. The linker facilitates covalent linkage of the agent to a targeting moiety. The linker is conjugated to the 5' or 3' end of an RNAi agent sense strand or antisense strand. In some embodiments, the linker is conjugated to the 5' or 3' end of an RNAi agent sense strand. In some embodiments, a linker is conjugated to the 5' end of an RNAi agent sense strand. In some embodiments, a linker is conjugated to the 3' end of an RNAi agent sense strand. In some

embodiments, a linker is conjugated to the 5' end of an RNAi agent antisense strand. In some embodiments, a linker is conjugated to the 3' end of an RNAi agent antisense strand. Examples of linkers can include, but are not limited to: reactive groups such a primary amines and alkynes, alkyl groups, abasic nucleotides, ribitol (abasic ribose), non-nucleotidic linker, and/or PEG groups. In some embodiments, the linker is a monovalent, bivalent, trivalent, or tetravalent branched linker. In some embodiments, the linker is a phosphorus-containing linker (e.g., phosphate, phosphodiester, phosphorothioate, phosphorodithioate, phosphoroamidate, etc), or other linker. Non-limiting examples of other linkers can include, but are not limited to: reactive groups such a primary amines and alkynes, alkyl groups, abasic nucleotides, ribitol (abasic ribose), and/or PEG groups. In some embodiments, the linker is a phosphorothioate linkage.

In some embodiments, any one of the CYP7A1 RNAi agents described herein is conjugated (e.g., covalently linked) linked to a targeting moiety comprising a structure of any one of Formulae ($Z^1$)-($Z^{16}$). For any one of Formulas ($Z^1$)-($Z^{16}$) referred to herein, with the exception of the phosphorous directly attached to the RNAi agent, each instance of phosphorous labeled *y* is bound to the oxygen labeled *z* of the adjacent repeat unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled *z* is bound to the phosphorous labeled *y* of the adjacent unit. In some embodiments, any one of the CYP7A1 RNAi agents described herein is (e.g., covalently linked) to a targeting moiety comprising a structure of Formula ($Z^6$). In some embodiments, any one of the CYP7A1 RNAi agents described herein is (e.g., covalently linked) at the 3' end (e.g., to the 3' terminal nucleoside) of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$). For any one of Formula ($Z^6$) referred to herein, with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent repeat unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

Unless stated otherwise, use of the symbol  means that any group or groups may be linked thereto that is in accordance with the scope of the inventions described herein. For example, in any one of the structures of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), and ($Z^1$)-($Z^{16}$), the symbol  means the attachment point at which the RNAi agent (e.g., at the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand) is attached to the target moiety.

It is to be understood that in an RNAi agent (e.g., CYP7A1 RNAi agent) that further comprises a targeting moiety conjugated to the 3' terminal nucleoside of the sense strand, the 3'-

terminal nucleoside is linked to the targeting moiety via a phosphorothioate linkage. As such, when the 3'-terminal nucleoside of the sense strand is represented as [mAs] (e.g., the sense strands of the siRNAs provided in Table 9 and elsewhere in the present disclosure), the "s" corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formulae: (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$.

*Examples of RNAi agents conjugated to targeting moieties*

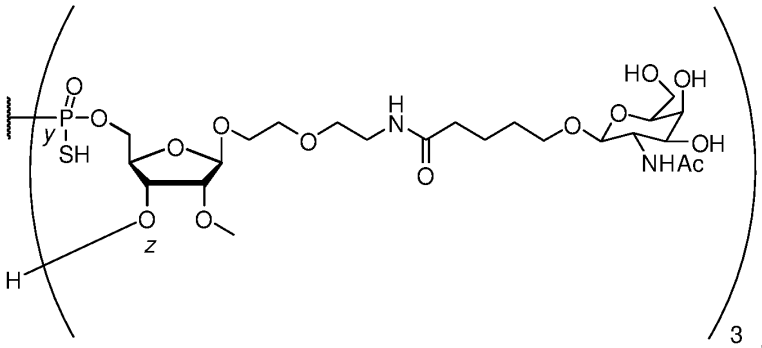
Aspects of the present disclosure provide conjugates comprising any one of the RNAi agents described herein conjugated to any one of the targeting moieties described herein (e.g., comprising one or more GalNAc, such as targeting moieties comprising a structure of Formula (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$). In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is conjugated to a targeting moiety (e.g., comprising one or more GalNAc, such as targeting moieties of Formula (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$) via a linker described herein. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is conjugated to a targeting moiety via a monovalent, bivalent, trivalent, or tetravalent branched linker. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is conjugated to a targeting moiety via a phosphorus-containing linker (e.g., a phosphorothioate linkage).

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to any one of the targeting moieties described herein (e.g., comprising one or more GalNAc, such as targeting moieties of Formula (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or $(Z^1)$-$(Z^{16})$) via a linker (e.g., a phosphorothioate linkage), wherein the CYP7A1 RNAi agent comprises a sense strand and an

antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity comprising a region of complementarity of at least 15 nucleosides to a target sequence in CYP7A1 mRNA, (e.g., a target sequence listed in Table 2), wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to any of the targeting moieties described herein (e.g., comprising one or more GalNAc, such as targeting moieties of Formula (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or ($Z^1$)-($Z^{16}$)) via a linker (e.g., a phosphorothioate linkage), wherein the CYP7A1 RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleotides to a CYP7A1 target sequence set forth in nucleosides 113-133, 221-241, 249-269, 290-310, 301-321, 475-495, 476-496, 504-524, 593-613, 600-620, 671-691, 779-799, 839-859, 842-862, 1003-1023, 1009-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1227, 1215-1235, 1225-1245, 1226-1246, 1235-1255, 1289-1309, 1296-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1, wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

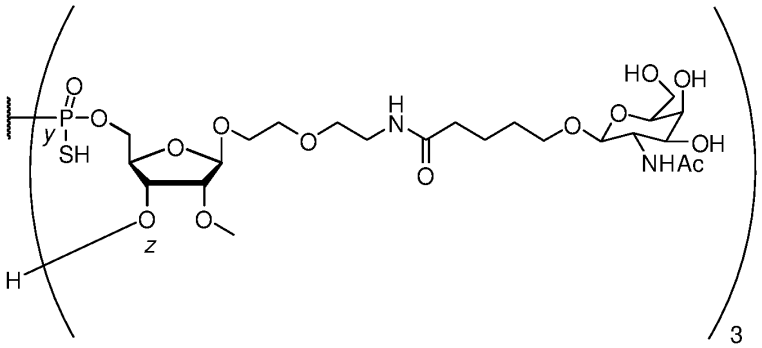
In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to a targeting moiety comprising a structure of any one of Formula ($Z^1$)-($Z^{16}$) (e.g., Formula ($Z^6$)), wherein the CYP7A1 RNAi agent comprises an antisense strand comprising the nucleobase sequence of any one of SEQ ID Nos: 777-1190 and a sense strand substantially complementary to the antisense strand and comprising the nucleobase sequence of any one of SEQ ID Nos: 393-776, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand. In some embodiments, the targeting moiety comprises a structure of Formula ($Z^6$).



3 ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

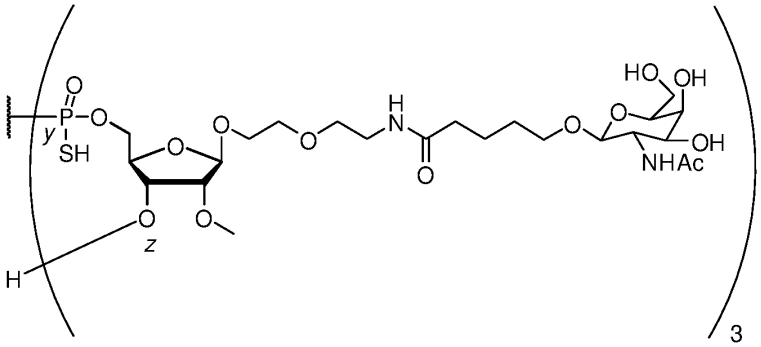
In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to a targeting moiety comprising a structure of any one of Formula ($Z^1$)-($Z^{16}$) (e.g., Formula ($Z^6$)), wherein the CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand. In some embodiments, the targeting moiety comprises a structure of Formula ($Z^6$).



3 ($Z^6$), or a pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked

to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to a targeting moiety, wherein the CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, and wherein the targeting moiety comprises a structure of Formula ($Z^6$):

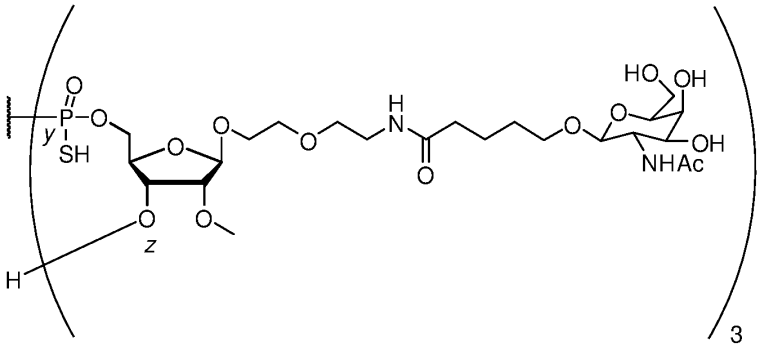



($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the ✂ indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the

adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to a targeting moiety, wherein the CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, and wherein the targeting moiety comprises a structure of Formula ($Z^6$):

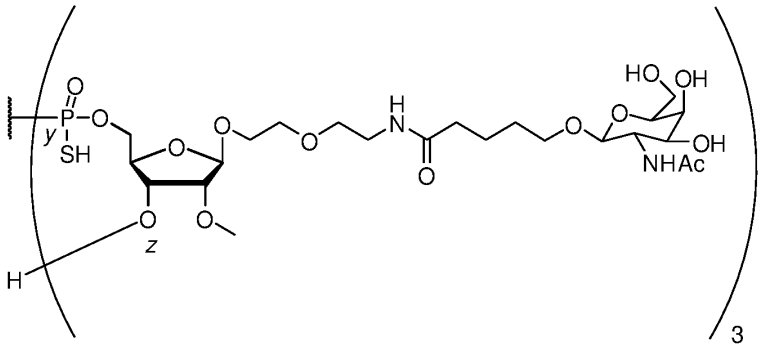



($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the wavy line indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g.,

covalently linked) to a targeting moiety, wherein the CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, and wherein the targeting moiety comprises a structure of Formula ($Z^6$):

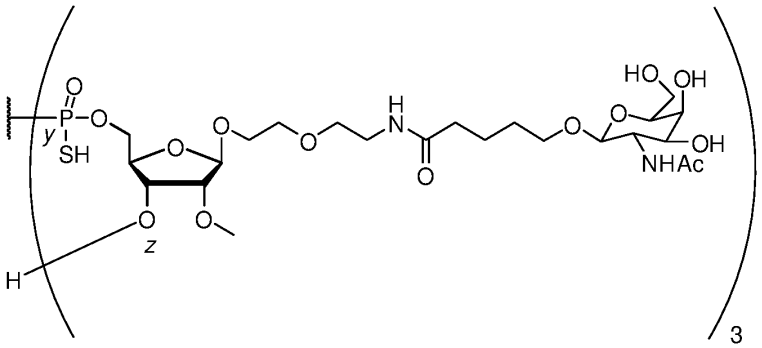



($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the wavy line indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

In some embodiments, a CYP7A1 RNAi agent described herein is conjugated (e.g., covalently linked) to a targeting moiety, wherein the CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72',

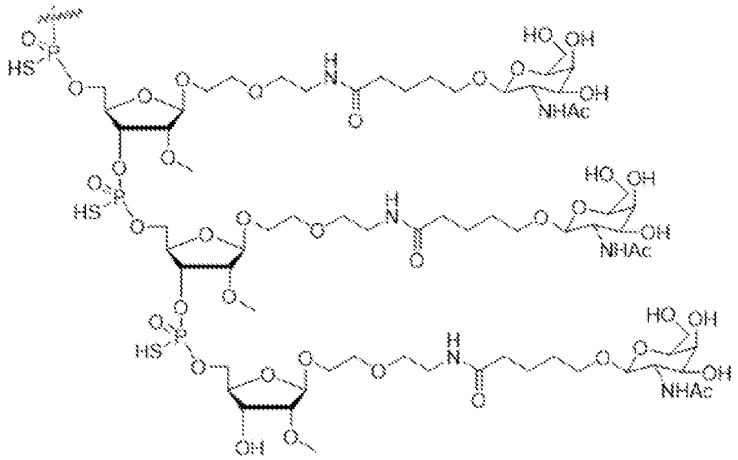
siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, and wherein the targeting moiety comprises a structure of Formula ($Z^6$):




($Z^6$),

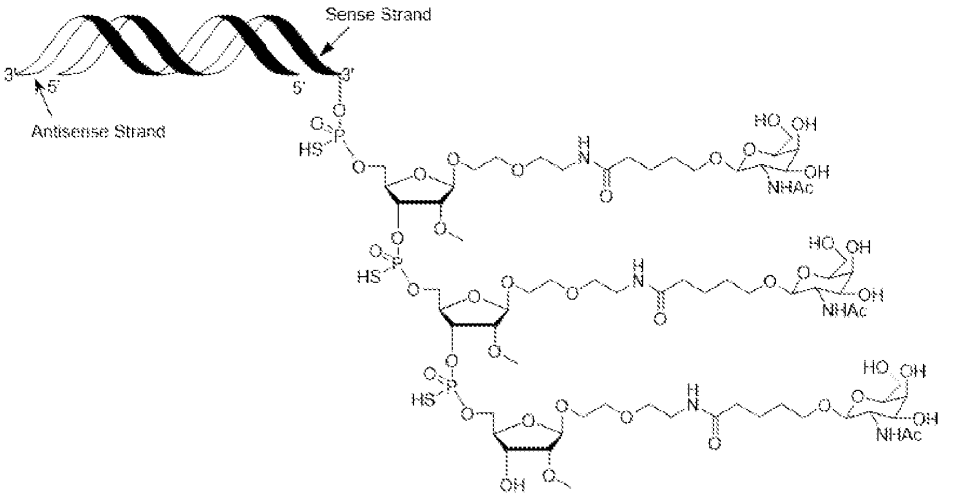
or a pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand. For Formula ($Z^6$), with the exception of the phosphorous directly attached to the sense strand, each instance of phosphorous labeled y is bound to the oxygen labeled z of the adjacent unit, and with the exception of the oxygen directly attached to hydrogen, each instance of oxygen labeled z is bound to the phosphorous labeled y of the adjacent unit.

In some embodiments, any one of the CYP7A1 RNAi agents described herein is conjugated (e.g., covalently linked) to a targeting moiety, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, wherein the targeting moiety comprising a structure of formula:



, or a

pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand.In some embodiments, a conjugate described herein comprises a CYP7A1 RNAi agent conjugated (e.g., covalently linked) to a targeting moiety, wherein the targeting moiety is conjugated (e.g., covalently linked) to the 3' terminal nucleoside of the sense strand, wherein the conjugate comprises a structure of:




,

or a pharmaceutically acceptable salt thereof,

wherein a CYP7A1 RNAi agent comprises nucleobase sequences (e.g., nucleobase sequences of the sense strand and antisense strand) of an siRNA selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205',

siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272', and wherein:

(i) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages;

(ii) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages;

(iii) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages; or

(iv) the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

In some embodiments, a CYP7A1 RNAi agent described herein comprises:

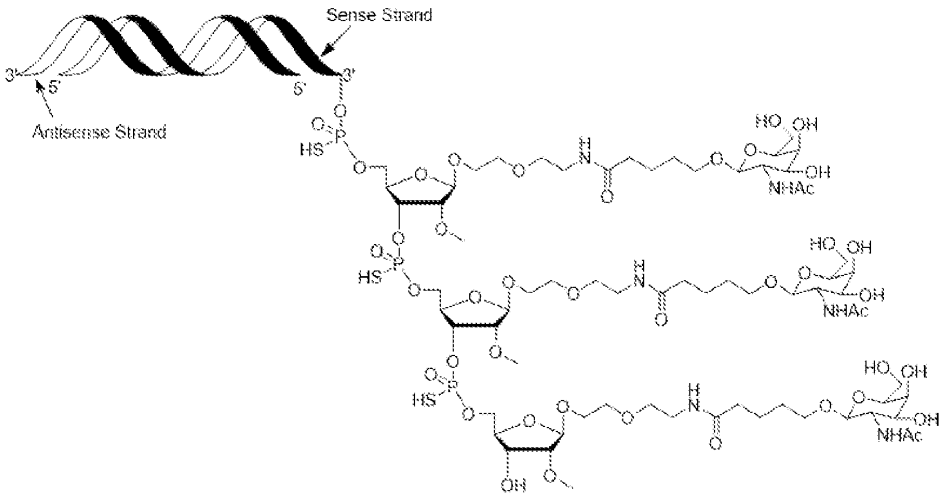
an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure (5' → 3') of

[mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016); and

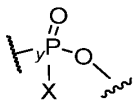
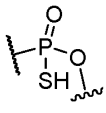
a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure (5' → 3') of
[mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425);

wherein mA, mC, mG and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a targeting moiety comprising a structure of Formula ($Z^6$), wherein the conjugated CYP7A1 RNAi agent comprises a structure as shown below:




,

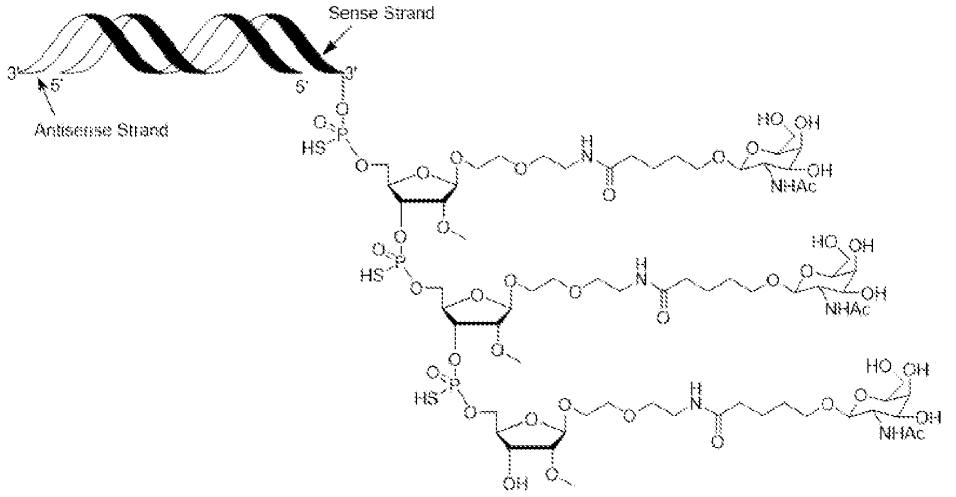
or a pharmaceutically acceptable salt thereof. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

In some embodiments, a CYP7A1 RNAi agent described herein comprises:
an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure (5' → 3') of
[mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020); and

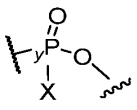
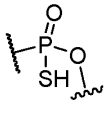
a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure (5' → 3') of
[mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418),

wherein mA, mC, mG and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a targeting moiety comprising a structure of Formula ($Z^6$), wherein the conjugated CYP7A1 RNAi agent comprises a structure as shown below:




,

or a pharmaceutically acceptable salt thereof. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

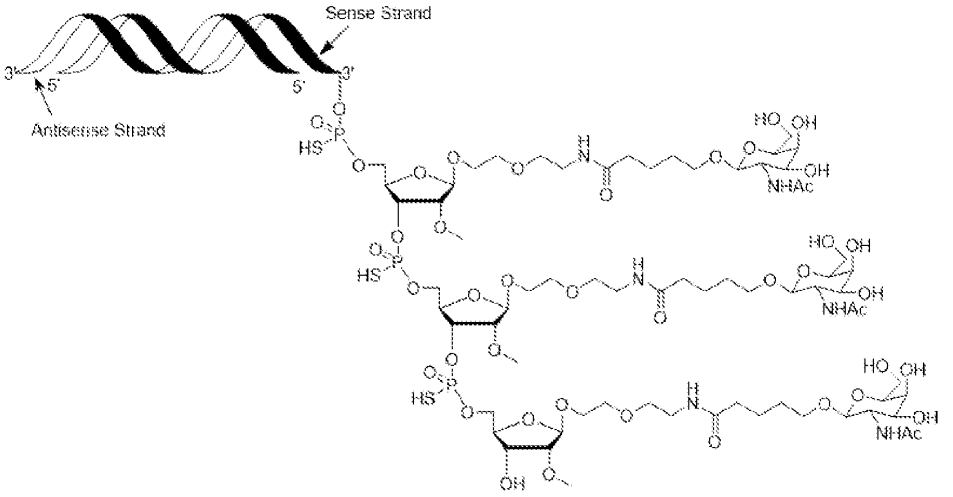
In some embodiments, a CYP7A1 RNAi agent described herein comprises:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure (5' → 3') of
[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033); and

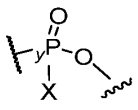
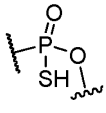
a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure (5' → 3') of [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267),

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a targeting moiety comprising a structure of Formula ($Z^6$), wherein the conjugated CYP7A1 RNAi agent comprises a structure as shown below:




,

or a pharmaceutically acceptable salt thereof. It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage (e.g., , wherein X is -SH, i.e., ) of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

In some embodiments, an RNAi agent comprising a targeting moiety described herein (e.g., a targeting moiety of Formulae (I), (I-a), (I-a-1), (I-b), (I-b-1), (I-c), (I-c-1), (I-d), (I-d-1), (I-e), (I-e-1), (I-f), (I-f-1), (I-g), (I-h), (I-i), (I-j), (II), (II-a), (II-b), (II-c), (III), (III-a), (IV), (IV-a), (IV-b), (IV-c), (IV-d), (IV-e), (IV-f), or ($Z^1$)-($Z^{16}$)) may be synthesized via including GalNAc-containing phosphoramidite during solids phase synthesis of the sense strand such that

the GalNAc-containing repeat units of the targeting moiety is directly added to the 5' or 3' end of the sense strand during synthesis.

**Pharmaceutical Compositions**

Pharmaceutical compositions comprising one or more RNAi agents (e.g., CYP7A1 RNAi agents), either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers are provided. The pharmaceutical compositions comprising RNAi agents (e.g., CYP7A1 RNAi agents) provided herein are for use in, but not limited to, diagnosing, detecting, or monitoring a disease, in preventing, treating, managing, or ameliorating a disease or one or more symptoms thereof, and/or in research. In some embodiments, a pharmaceutical composition may further comprise any other suitable therapeutic agent for treatment of a subject, e.g., a human subject having a CYP7A1 disease or CYP7A1-associated disease. In some embodiments, the other therapeutic agents may enhance or supplement the effectiveness of the complexes described herein. Non-limiting examples of other therapeutic agents include another RNAi agent, a small molecule drug, an antibody, an antibody fragment, peptide and/or aptamer. In some embodiments, the other therapeutic agents may function to treat a different symptom or disease than the CYP7A1 RNAi agents described herein. The formulation of pharmaceutical compositions, either alone or in combination with prophylactic agents, therapeutic agents, and/or pharmaceutically acceptable carriers, are known to one skilled in the art.

An aspect of the disclosure includes pharmaceutical compositions for inhibiting expression of a gene encoding CYP7A1 comprising any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is in an unbuffered solution. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is formulated in water or in an aqueous solution (e.g., water with pH adjustments). In some embodiments, the unbuffered solution is saline. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is formulated in a buffer solution such as a phosphate-buffered saline solution, liposome, micellar structure, and capsid. In some embodiments, an RNAi agent (e.g., CYP7A1 RNAi agent) is formulated in a basic buffered aqueous solution (e.g., PBS). In some embodiments, formulations as disclosed herein comprise an excipient. In some embodiments, an excipient confers to a composition improved stability, improved absorption, improved solubility and/or (e.g., and) therapeutic enhancement of the active ingredient. In some embodiments, an excipient is a buffering agent (e.g., sodium citrate, sodium phosphate, a tris base, or sodium hydroxide) or a vehicle (e.g., a buffered solution, petrolatum, dimethyl sulfoxide, or mineral oil). Any one of the described RNAi agents (e.g., CYP7A1 RNAi agents),

when added to pharmaceutically acceptable excipients, can be packaged into kits, containers, packs, or dispensers. The pharmaceutical compositions described herein can be packaged in pre-filled syringes or vials.

In some embodiments, the delivery vehicle can be used to deliver a CYP7A1 RNAi agent to a cell or tissue. A delivery vehicle is a compound that improves delivery of a CYP7A1 RNAi agent to a cell or tissue. A delivery vehicle can include, or consist of, but is not limited to: a polymer, such as an amphipathic polymer, a membrane active polymer, a peptide, a melittin peptide, a melittin-like peptide (MLP), a lipid, a reversibly modified polymer or peptide, or a reversibly modified membrane active polyamine. In some embodiments, any one of the CYP7A1 RNAi agents or pharmaceutical compositions described herein can be combined with lipids, nanoparticles, polymers, liposomes, micelles, DPCs or other delivery systems available in the art. The CYP7A1 RNAi agents can also be chemically conjugated to targeting moiety, lipids (including, but not limited to cholesterol and cholesteryl derivatives), nanoparticles, polymers, liposomes, micelles, DPCs (see, for example US2004171044, US8137695, US8313772, US8501930, US8932572, US8933047, US2016015824, US9750819, and US9561286, each of which is incorporated herein by reference), or other delivery systems available in the art.

In some embodiments, a pharmaceutical composition is formulated to be compatible with its intended route of administration. Non-limiting examples of routes of administration include intravenous, intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal routes. In some embodiments, the route of administration is subcutaneous.

**Kits**

An aspect of the disclosure includes kits comprising any one of the RNAi agents (e.g., CYP7A1 RNAi agents) described herein or a pharmaceutical composition described herein. In some embodiments, the kit further comprises instructions for administration. In some embodiments, the kit is for treating a CYP7A1 disease or a CYP7A1-associated disease described herein. In some embodiments, the kit further comprises an additional agent described herein.

**Methods**

Some aspects of the present disclosure provide methods for inhibiting or reducing expression level of CYP7A1 (e.g., mRNA level and/or protein level) in a cell. In some embodiments, a method for inhibiting or reducing CYP7A1 expression comprises contacting the cell with any one of the CYP7A1 RNAi agents described herein or any one of the

pharmaceutical compositions described herein, thereby inhibiting or reducing expression level (e.g., mRNA level and/or protein level) of the CYP7A1 in the cell. In some embodiments, contacting the cell with any one of the CYP7A1 RNAi agents described herein inhibits the expression level (e.g., mRNA level and/or protein level) of CYP7A1 by at least 40%, 50%, 60%, 70%, 80%, 90%, or 95%, relative to the CYP7A1 level in cells not contacted with a CYP7A1 RNAi agent. In some embodiments, the cell is in vitro (e.g., in a cell culture). In some embodiments, the cell is in vivo (e.g., in a subject).

The disclosure also provides methods of decreasing or reducing the expression level of CYP7A1 (e.g., mRNA level and/or protein level) in a subject compared to baseline pre-treatment levels. In some embodiments, methods comprise administering to the subject an effective amount any one of the CYP7A1 RNAi agents described herein or any one of the pharmaceutical compositions described herein. The expression level (e.g., mRNA level and/or protein level) in the subject is reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject. In some embodiments, the above described methods further comprise determining the level of CYP7A1 (e.g., mRNA level and/or protein level) in a sample(s) from the subject (e.g., CYP7A1 level in a blood or serum sample(s)). The level of CYP7A1 in a sample may be measured by general methods known in the art.

The Examples disclosed herein set forth generally known methods for assessing inhibition of CYP7A1 expression and reduction in CYP7A1 expression levels. In some embodiments, a CYP7A1 RNAi agent described herein is administered to a subject at an effective concentration sufficient to inhibit activity or expression of CYP7A1 by at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% relative to a control, e.g., baseline level of gene expression prior to treatment. Some aspects of the disclosure provide methods of maintaining bile acid homeostasis by preventing overproduction of bile acid in the liver, comprising administering to the subject an effective amount any one of the CYP7A1 RNAi agents described herein or any one of the pharmaceutical compositions described herein.

The disclosure also provides methods for reducing inflammation of the bile ducts in a subject, comprising administering to the subject an effective amount any one of the CYP7A1 RNAi agents described herein or any one of the pharmaceutical compositions described herein. In some embodiments, administering to the subject an effective amount any one of the CYP7A1 RNAi agents described herein or any one of the pharmaceutical compositions described herein, reduces inflammation of the bile ducts in a subject by at least 40%, 50%, 60%, 70%, 80%, 90%, or 95% relative to the baseline level of inflammation.

In some embodiments, in any one of the methods described herein, a subject is non-human primate, or rodent. In some embodiments, a subject is a human. In some embodiments, a

subject is a patient, e.g., a human patient that has or is suspected of having a disease. In some embodiments, the subject is a human patient who has or is suspected of having a CYP7A1 disease or CYP7A1-associated disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a liver disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a cholestatic liver disease such as primary sclerosing cholangitis (PSC), familial intrahepatic cholestasis (PFIC, including Type 1, PFIC1, Type 2, PFIC2, and Type 3, PFIC3), primary biliary cholangitis (PBC), Alagille syndrome, and biliary atresia, and other liver diseases such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD).

An aspect of the disclosure includes a method of treating a subject having a CYP7A1 disease or CYP7A1-associated disease. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of any one of the CYP7A1 RNAi agents described herein or a pharmaceutical composition described herein. In some embodiments the method results in treating the subject having the CYP7A1 disease or CYP7A1-associated disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a liver disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a cholestatic liver disease such as primary sclerosing cholangitis (PSC), familial intrahepatic cholestasis (PFIC, including Type 1, PFIC1, Type 2, PFIC2, and Type 3, PFIC3), primary biliary cholangitis (PBC), Alagille syndrome, and biliary atresia, and other liver diseases such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD).

An aspect of the disclosure includes treating at least one symptom in a subject having a CYP7A1 disease or CYP7A1-associated disease. In some embodiments, the method comprises administering to the subject an effective amount of any one of the CYP7A1 RNAi agents described herein, or the pharmaceutical composition described herein, thereby treating at least one symptom in the subject having the CYP7A1 disease or CYP7A1- associated disease.

In some embodiments, the CYP7A1 disease or the CYP7A1-associated disease symptoms to be treated by the administration of an effective amount of any one of the CYP7A1 RNAi agents described herein, or the pharmaceutical composition described herein, include, but are not limited to, biochemical symptoms, cellular symptoms, histological symptoms, physiological symptoms and/or medical symptoms. In some embodiments, the CYP7A1 disease or the CYP7A1-associated disease symptoms to be treated by the administration of an effective amount of any one of the CYP7A1 RNAi agents described herein, or the pharmaceutical composition described herein are selected from the group consisting of increased liver enzymes (ALP, ALT, AST, GGT), liver inflammation and scarring (fibrosis), increased liver stiffness,

bile duct strictures , cirrhosis, liver failure, need for liver transplantation, portal hypertension, variceal bleeding, jaundice, and pruritus.

In some embodiments, any one of the CYP7A1 RNAi agents or any one of the pharmaceutical compositions described herein are effective in treating CYP7A1 disease and CYP7A1-associated disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a liver disease. In some embodiments, the CYP7A1 disease or CYP7A1-associated disease is a cholestatic liver disease such as primary sclerosing cholangitis (PSC), familial intrahepatic cholestasis (PFIC, including Type 1, PFIC1, Type 2, PFIC2, and Type 3, PFIC3), primary biliary cholangitis (PBC), Alagille syndrome, and biliary atresia, and other liver diseases such as nonalcoholic steatohepatitis (NASH), nonalcoholic fatty liver disease (NAFLD), and alcoholic liver disease (ALD).

In some embodiments, the above described methods further comprise administering an additional agent for the treatment of a CYP7A1 disease or CYP7A1-associated disease.

Having now described some embodiments in detail, practice of the invention will be more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

## EXAMPLES

### Example 1: Identification of active RNAi sequences from preliminary in-vitro screening

A bioinformatics method assuming a canonical 19mer dsRNA was first employed to generate a potential set of 19 mer-containing antisense oligonucleotides targeting various regions of the human CYP7A1 mRNA transcript that were, in certain instances, also complementary to either cynomolgus monkey, rat, and/or mouse CYP7A1 mRNA.

The sequence of the human CYP7A1 mRNA transcript (NM_000780.4), upon which the 19mer-containing antisense oligonucleotides were designed, is set forth in **SEQ ID NO. 1.** Of the potential 19mer antisense oligonucleotide sequences generated, 384 were selected for incorporation into chemically modified RNAi agents that could be prepared and evaluated in an in-vitro assay to assess target gene knockdown in primary human hepatocytes expressing CYP7A1. The CYP7A1 19-mer mRNA target sequences are listed in Table 2.

### Example 2: siRNA Synthesis

Generic 21/23-mer double stranded siRNAs (Table 5A) were designed based on the target sequences in Table 2. siRNAs with modifications as indicated in Table 5B were prepared

by Integrated DNA Technologies Inc. (Iowa, USA) using standard solid phase oligonucleotide synthesis.

Table 5A provides the nucleobase sequences of the siRNAs. It is to be understood that any chemical modification pattern may be applied to the nucleobase sequences of the siRNAs in Table 5A. For example, Table 5B lists the siRNA synthesized for *in vitro* screening, which all have a modification pattern M0 (the nucleosides at positions 9-13 and 19 in the sense strand are ribonucleosides and the rest of the nucleosides in the sense strand are 2'-O-Me modified nucleosides; the nucleosides at positions 2, 3, 5-10, 13-15, 17, 19, and 20 of the antisense strand are ribonucleosides and the rest of the nucleosides in the antisense strand are 2'O-Me modified nucleosides).

Table 5A – Nucleobase sequences of siRNAs used in in vitro screening‡

| **Position** | **siRNA#** | **Sense (5'-3')** | **SEQ ID NO:** | **Antisense (5'-3')** | **SEQ ID NO** |
|---|---|---|---|---|---|
| 34 | siRNA1 | UUCUUCCUCAGAGAUUUUGGA | 393 | UCCAAAAUCUCUGAGGAAGAAUU | 777 |
| 40 | siRNA2 | CUCAGAGAUUUUGGCCUAGAA | 394 | UUCUAGGCCAAAAUCUCUGAGUU | 778 |
| 41 | siRNA3 | UCAGAGAUUUUGGCCUAGAUA | 395 | UAUCUAGGCCAAAAUCUCUGAUU | 779 |
| 42 | siRNA4 | CAGAGAUUUUGGCCUAGAUUA | 396 | UAAUCUAGGCCAAAAUCUCUGUU | 780 |
| 43 | siRNA5 | AGAGAUUUUGGCCUAGAUUUA | 397 | UAAAUCUAGGCCAAAAUCUCUUU | 781 |
| 45 | siRNA6 | AGAUUUUGGCCUAGAUUUGCA | 398 | UGCAAAUCUAGGCCAAAAUCUUU | 782 |
| 105 | siRNA7 | CAGCAUGCUGUUGUCUAUGGA | 399 | UCCAUAGACAACAGCAUGCUGUU | 783 |
| 110 | siRNA8 | UGCUGUUGUCUAUGGCUUAUA | 400 | UAUAAGCCAUAGACAACAGCAUU | 784 |
| 111 | siRNA9 | GCUGUUGUCUAUGGCUUAUUA | 401 | UAAUAAGCCAUAGACAACAGCUU | 785 |
| 112 | siRNA10 | CUGUUGUCUAUGGCUUAUUCA | 402 | UGAAUAAGCCAUAGACAACAGUU | 786 |
| 113 | siRNA11 | UGUUGUCUAUGGCUUAUUCUA | 403 | UAGAAUAAGCCAUAGACAACAUU | 787 |
| 115 | siRNA12 | UUGUCUAUGGCUUAUUCUUGA | 404 | UCAAGAAUAAGCCAUAGACAAUU | 788 |
| 116 | siRNA13 | UGUCUAUGGCUUAUUCUUGGA | 405 | UCCAAGAAUAAGCCAUAGACAUU | 789 |
| 117 | siRNA14 | GUCUAUGGCUUAUUCUUGGAA | 406 | UUCCAAGAAUAAGCCAUAGACUU | 790 |
| 118 | siRNA15 | UCUAUGGCUUAUUCUUGGAAA | 407 | UUUCCAAGAAUAAGCCAUAGAUU | 791 |
| 139 | siRNA16 | UAGGAGAAGGCAAACGGGUGA | 408 | UCACCCGUUUGCCUUCUCCUAUU | 792 |
| 141 | siRNA17 | GGAGAAGGCAAACGGGUGAAA | 409 | UUUCACCCGUUUGCCUUCUCCUU | 793 |
| 142 | siRNA18 | GAGAAGGCAAACGGGUGAACA | 410 | UGUUCACCCGUUUGCCUUCUCUU | 794 |
| 143 | siRNA19 | AGAAGGCAAACGGGUGAACCA | 411 | UGGUUCACCCGUUUGCCUUCUUU | 795 |
| 181 | siRNA20 | AAUUCCAUACCUGGGCUGUGA | 412 | UCACAGCCCAGGUAUGGAAUUUU | 796 |
| 182 | siRNA21 | AUUCCAUACCUGGGCUGUGCA | 413 | UGCACAGCCCAGGUAUGGAAUUU | 797 |
| 183 | siRNA22 | UUCCAUACCUGGGCUGUGCUA | 414 | UAGCACAGCCCAGGUAUGGAAUU | 798 |
| 201 | siRNA23 | CUCUGCAAUUUGGUGCCAAUA | 415 | UAUUGGCACCAAAUUGCAGAGUU | 799 |
| 203 | siRNA24 | CUGCAAUUUGGUGCCAAUCCA | 416 | UGGAUUGGCACCAAAUUGCAGUU | 800 |
| 210 | siRNA25 | UUGGUGCCAAUCCUCUUGAGA | 417 | UCUCAAGAGGAUUGGCACCAAUU | 801 |
| 214 | siRNA26 | UGCCAAUCCUCUUGAGUUCCA | 418 | UGGAACUCAAGAGGAUUGGCAUU | 802 |

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|---|---|---|---|---|---|
| 223 | siRNA27 | UCUUGAGUUCCUCAGAGCAAA | 419 | UUUGCUCUGAGGAACUCAAGAUU | 803 |
| 225 | siRNA28 | UUGAGUUCCUCAGAGCAAAUA | 420 | UAUUUGCUCUGAGGAACUCAAUU | 804 |
| 229 | siRNA29 | GUUCCUCAGAGCAAAUCAAAA | 421 | UUUUGAUUUGCUCUGAGGAACUU | 805 |
| 230 | siRNA30 | UUCCUCAGAGCAAAUCAAAGA | 422 | UCUUUGAUUUGCUCUGAGGAAUU | 806 |
| 236 | siRNA31 | AGAGCAAAUCAAAGGAAACAA | 423 | UUGUUUCCUUUGAUUUGCUCUUU | 807 |
| 238 | siRNA32 | AGCAAAUCAAAGGAAACAUGA | 424 | UCAUGUUUCCUUUGAUUUGCUUU | 808 |
| 242 | siRNA33 | AAUCAAAGGAAACAUGGUCAA | 425 | UUGACCAUGUUUCCUUUGAUUUU | 809 |
| 243 | siRNA34 | AUCAAAGGAAACAUGGUCAUA | 426 | UAUGACCAUGUUUCCUUUGAUUU | 810 |
| 244 | siRNA35 | UCAAAGGAAACAUGGUCAUGA | 427 | UCAUGACCAUGUUUCCUUUGAUU | 811 |
| 245 | siRNA36 | CAAAGGAAACAUGGUCAUGUA | 428 | UACAUGACCAUGUUUCCUUUGUU | 812 |
| 246 | siRNA37 | AAAGGAAACAUGGUCAUGUUA | 429 | UAACAUGACCAUGUUUCCUUUUU | 813 |
| 251 | siRNA38 | AAACAUGGUCAUGUUUUUACA | 430 | UGUAAAAACAUGACCAUGUUUUU | 814 |
| 253 | siRNA39 | ACAUGGUCAUGUUUUUACCUA | 431 | UAGGUAAAAACAUGACCAUGUUU | 815 |
| 254 | siRNA40 | CAUGGUCAUGUUUUUACCUGA | 432 | UCAGGUAAAAACAUGACCAUGUU | 816 |
| 262 | siRNA41 | UGUUUUUACCUGCAAACUAAA | 433 | UUUAGUUUGCAGGUAAAAACAUU | 817 |
| 264 | siRNA42 | UUUUUACCUGCAAACUAAUGA | 434 | UCAUUAGUUUGCAGGUAAAAAUU | 818 |
| 266 | siRNA43 | UUUACCUGCAAACUAAUGGGA | 435 | UCCCAUUAGUUUGCAGGUAAAUU | 819 |
| 267 | siRNA44 | UUACCUGCAAACUAAUGGGAA | 436 | UUCCCAUUAGUUUGCAGGUAAUU | 820 |
| 268 | siRNA45 | UACCUGCAAACUAAUGGGAAA | 437 | UUUCCCAUUAGUUUGCAGGUAUU | 821 |
| 291 | siRNA46 | AUGUCCAUUUCAUCACAAAUA | 438 | UAUUUGUGAUGAAAUGGACAUUU | 822 |
| 292 | siRNA47 | UGUCCAUUUCAUCACAAAUCA | 439 | UGAUUUGUGAUGAAAUGGACAUU | 823 |
| 294 | siRNA48 | UCCAUUUCAUCACAAAUCCCA | 440 | UGGGAUUUGUGAUGAAAUGGAUU | 824 |
| 300 | siRNA49 | UCAUCACAAAUCCCUUGUCAA | 441 | UUGACAAGGGAUUUGUGAUGAUU | 825 |
| 302 | siRNA50 | AUCACAAAUCCCUUGUCAUAA | 442 | UUAUGACAAGGGAUUUGUGAUUU | 826 |
| 303 | siRNA51 | UCACAAAUCCCUUGUCAUACA | 443 | UGUAUGACAAGGGAUUUGUGAUU | 827 |
| 304 | siRNA52 | CACAAAUCCCUUGUCAUACCA | 444 | UGGUAUGACAAGGGAUUUGUGUU | 828 |
| 305 | siRNA53 | ACAAAUCCCUUGUCAUACCAA | 445 | UUGGUAUGACAAGGGAUUUGUUU | 829 |
| 309 | siRNA54 | AUCCCUUGUCAUACCAUAAGA | 446 | UCUUAUGGUAUGACAAGGGAUUU | 830 |
| 310 | siRNA55 | UCCCUUGUCAUACCAUAAGGA | 447 | UCCUUAUGGUAUGACAAGGGAUU | 831 |
| 311 | siRNA56 | CCCUUGUCAUACCAUAAGGUA | 448 | UACCUUAUGGUAUGACAAGGGUU | 832 |
| 312 | siRNA57 | CCUUGUCAUACCAUAAGGUGA | 449 | UCACCUUAUGGUAUGACAAGGUU | 833 |
| 313 | siRNA58 | CUUGUCAUACCAUAAGGUGUA | 450 | UACACCUUAUGGUAUGACAAGUU | 834 |
| 315 | siRNA59 | UGUCAUACCAUAAGGUGUUGA | 451 | UCAACACCUUAUGGUAUGACAUU | 835 |
| 319 | siRNA60 | AUACCAUAAGGUGUUGUGCCA | 452 | UGGCACAACACCUUAUGGUAUUU | 836 |
| 360 | siRNA61 | AAAAAUUUCACUUUGCUACUA | 453 | UAGUAGCAAAGUGAAAUUUUUUU | 837 |
| 363 | siRNA62 | AAUUUCACUUUGCUACUUCUA | 454 | UAGAAGUAGCAAAGUGAAAUUUU | 838 |
| 372 | siRNA63 | UUGCUACUUCUGCGAAGGCAA | 455 | UUGCCUUCGCAGAAGUAGCAAUU | 839 |
| 375 | siRNA64 | CUACUUCUGCGAAGGCAUUUA | 456 | UAAAUGCCUUCGCAGAAGUAGUU | 840 |
| 377 | siRNA65 | ACUUCUGCGAAGGCAUUUGGA | 457 | UCCAAAUGCCUUCGCAGAAGUUU | 841 |
| 390 | siRNA66 | CAUUUGGGCACAGAAGCAUUA | 458 | UAAUGCUUCUGUGCCCAAAUGUU | 842 |
| 392 | siRNA67 | UUUGGGCACAGAAGCAUUGAA | 459 | UUCAAUGCUUCUGUGCCCAAAUU | 843 |
| 394 | siRNA68 | UGGGCACAGAAGCAUUGACCA | 460 | UGGUCAAUGCUUCUGUGCCCAUU | 844 |
| 474 | siRNA69 | AUGCCUUGAAUUCCCUCACGA | 461 | UCGUGAGGGAAUUCAAGGCAUUU | 845 |
| 475 | siRNA70 | UGCCUUGAAUUCCCUCACGGA | 462 | UCCGUGAGGGAAUUCAAGGCAUU | 846 |

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|---|---|---|---|---|---|
| 476 | siRNA71 | GCCUUGAAUUCCCUCACGGAA | 463 | UUCCGUGAGGGAAUUCAAGGCUU | 847 |
| 477 | siRNA72 | CCUUGAAUUCCCUCACGGAAA | 464 | UUUCCGUGAGGGAAUUCAAGGUU | 848 |
| 478 | siRNA73 | CUUGAAUUCCCUCACGGAAAA | 465 | UUUUCCGUGAGGGAAUUCAAGUU | 849 |
| 482 | siRNA74 | AAUUCCCUCACGGAAAGCAUA | 466 | UAUGCUUUCCGUGAGGGAAUUUU | 850 |
| 484 | siRNA75 | UUCCCUCACGGAAAGCAUGAA | 467 | UUCAUGCUUUCCGUGAGGGAAUU | 851 |
| 486 | siRNA76 | CCCUCACGGAAAGCAUGAUGA | 468 | UCAUCAUGCUUUCCGUGAGGGUU | 852 |
| 488 | siRNA77 | CUCACGGAAAGCAUGAUGGAA | 469 | UUCCAUCAUGCUUUCCGUGAGUU | 853 |
| 501 | siRNA78 | UGAUGGAAAACCUCCAACGUA | 470 | UACGUUGGAGGUUUUCCAUCAUU | 854 |
| 503 | siRNA79 | AUGGAAAACCUCCAACGUAUA | 471 | UAUACGUUGGAGGUUUUCCAUUU | 855 |
| 505 | siRNA80 | GGAAAACCUCCAACGUAUCAA | 472 | UUGAUACGUUGGAGGUUUUCCUU | 856 |
| 506 | siRNA81 | GAAAACCUCCAACGUAUCAUA | 473 | UAUGAUACGUUGGAGGUUUUCUU | 857 |
| 507 | siRNA82 | AAAACCUCCAACGUAUCAUGA | 474 | UCAUGAUACGUUGGAGGUUUUUU | 858 |
| 509 | siRNA83 | AACCUCCAACGUAUCAUGAGA | 475 | UCUCAUGAUACGUUGGAGGUUUU | 859 |
| 510 | siRNA84 | ACCUCCAACGUAUCAUGAGAA | 476 | UUCUCAUGAUACGUUGGAGGUUU | 860 |
| 511 | siRNA85 | CCUCCAACGUAUCAUGAGACA | 477 | UGUCUCAUGAUACGUUGGAGGUU | 861 |
| 512 | siRNA86 | CUCCAACGUAUCAUGAGACCA | 478 | UGGUCUCAUGAUACGUUGGAGUU | 862 |
| 513 | siRNA87 | UCCAACGUAUCAUGAGACCUA | 479 | UAGGUCUCAUGAUACGUUGGAUU | 863 |
| 514 | siRNA88 | CCAACGUAUCAUGAGACCUCA | 480 | UGAGGUCUCAUGAUACGUUGGUU | 864 |
| 539 | siRNA89 | UCCUCUAACUCAAAGACCGCA | 481 | UGCGGUCUUUGAGUUAGAGGAUU | 865 |
| 552 | siRNA90 | AGACCGCUGCCUGGGUGACAA | 482 | UUGUCACCCAGGCAGCGGUCUUU | 866 |
| 562 | siRNA91 | CUGGGUGACAGAAGGGAUGUA | 483 | UACAUCCCUUCUGUCACCCAGUU | 867 |
| 563 | siRNA92 | UGGGUGACAGAAGGGAUGUAA | 484 | UUACAUCCCUUCUGUCACCCAUU | 868 |
| 564 | siRNA93 | GGGUGACAGAAGGGAUGUAUA | 485 | UAUACAUCCCUUCUGUCACCCUU | 869 |
| 585 | siRNA94 | CUUUCUGCUACCGAGUGAUGA | 486 | UCAUCACUCGGUAGCAGAAAGUU | 870 |
| 587 | siRNA95 | UUCUGCUACCGAGUGAUGUUA | 487 | UAACAUCACUCGGUAGCAGAAUU | 871 |
| 588 | siRNA96 | UCUGCUACCGAGUGAUGUUUA | 488 | UAAACAUCACUCGGUAGCAGAUU | 872 |
| 589 | siRNA97 | CUGCUACCGAGUGAUGUUUGA | 489 | UCAAACAUCACUCGGUAGCAGUU | 873 |
| 593 | siRNA98 | UACCGAGUGAUGUUUGAAGCA | 490 | UGCUUCAAACAUCACUCGGUAUU | 874 |
| 594 | siRNA99 | ACCGAGUGAUGUUUGAAGCUA | 491 | UAGCUUCAAACAUCACUCGGUUU | 875 |
| 595 | siRNA100 | CCGAGUGAUGUUUGAAGCUGA | 492 | UCAGCUUCAAACAUCACUCGGUU | 876 |
| 602 | siRNA101 | AUGUUUGAAGCUGGGUAUUUA | 493 | UAAAUACCCAGCUUCAAACAUUU | 877 |
| 605 | siRNA102 | UUUGAAGCUGGGUAUUUAACA | 494 | UGUUAAAUACCCAGCUUCAAAUU | 878 |
| 606 | siRNA103 | UUGAAGCUGGGUAUUUAACUA | 495 | UAGUUAAAUACCCAGCUUCAAUU | 879 |
| 607 | siRNA104 | UGAAGCUGGGUAUUUAACUAA | 496 | UUAGUUAAAUACCCAGCUUCAUU | 880 |
| 608 | siRNA105 | GAAGCUGGGUAUUUAACUAUA | 497 | UAUAGUUAAAUACCCAGCUUCUU | 881 |
| 609 | siRNA106 | AAGCUGGGUAUUUAACUAUCA | 498 | UGAUAGUUAAAUACCCAGCUUUU | 882 |
| 610 | siRNA107 | AGCUGGGUAUUUAACUAUCUA | 499 | UAGAUAGUUAAAUACCCAGCUUU | 883 |
| 612 | siRNA108 | CUGGGUAUUUAACUAUCUUUA | 500 | UAAAGAUAGUUAAAUACCCAGUU | 884 |
| 613 | siRNA109 | UGGGUAUUUAACUAUCUUUGA | 501 | UCAAAGAUAGUUAAAUACCCAUU | 885 |
| 653 | siRNA110 | GACACACAGAAAGCACAUAUA | 502 | UAUAUGUGCUUUCUGUGUGUCUU | 886 |
| 663 | siRNA111 | AAGCACAUAUUCUAAACAAUA | 503 | UAUUGUUUAGAAUAUGUGCUUUU | 887 |
| 665 | siRNA112 | GCACAUAUUCUAAACAAUCUA | 504 | UAGAUUGUUUAGAAUAUGUGCUU | 888 |
| 666 | siRNA113 | CACAUAUUCUAAACAAUCUUA | 505 | UAAGAUUGUUUAGAAUAUGUGUU | 889 |
| 667 | siRNA114 | ACAUAUUCUAAACAAUCUUGA | 506 | UCAAGAUUGUUUAGAAUAUGUUU | 890 |

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|---|---|---|---|---|---|
| 669 | siRNA115 | AUAUUCUAAACAAUCUUGACA | 507 | UGUCAAGAUUGUUUAGAAUAUUU | 891 |
| 670 | siRNA116 | UAUUCUAAACAAUCUUGACAA | 508 | UUGUCAAGAUUGUUUAGAAUAUU | 892 |
| 671 | siRNA117 | AUUCUAAACAAUCUUGACAAA | 509 | UUUGUCAAGAUUGUUUAGAAUUU | 893 |
| 673 | siRNA118 | UCUAAACAAUCUUGACAACUA | 510 | UAGUUGUCAAGAUUGUUUAGAUU | 894 |
| 679 | siRNA119 | CAAUCUUGACAACUUCAAGCA | 511 | UGCUUGAAGUUGUCAAGAUUGUU | 895 |
| 712 | siRNA120 | CUUUCCAGCCCUGGUAGCAGA | 512 | UCUGCUACCAGGGCUGGAAAGUU | 896 |
| 771 | siRNA121 | GGGAGAAACUGGCAGAGAGCA | 513 | UGCUCUCUGCCAGUUUCUCCCUU | 897 |
| 781 | siRNA122 | GGCAGAGAGCUUGAGGCACGA | 514 | UCGUGCCUCAAGCUCUCUGCCUU | 898 |
| 788 | siRNA123 | AGCUUGAGGCACGAGAACCUA | 515 | UAGGUUCUCGUGCCUCAAGCUUU | 899 |
| 841 | siRNA124 | CCUGCGCAUGUUUCUCAAUGA | 516 | UCAUUGAGAAACAUGCGCAGGUU | 900 |
| 844 | siRNA125 | GCGCAUGUUUCUCAAUGACAA | 517 | UUGUCAUUGAGAAACAUGCGCUU | 901 |
| 846 | siRNA126 | GCAUGUUUCUCAAUGACACUA | 518 | UAGUGUCAUUGAGAAACAUGCUU | 902 |
| 850 | siRNA127 | GUUUCUCAAUGACACUUUGUA | 519 | UACAAAGUGUCAUUGAGAAACUU | 903 |
| 851 | siRNA128 | UUUCUCAAUGACACUUUGUCA | 520 | UGACAAAGUGUCAUUGAGAAAUU | 904 |
| 852 | siRNA129 | UUCUCAAUGACACUUUGUCCA | 521 | UGGACAAAGUGUCAUUGAGAAUU | 905 |
| 861 | siRNA130 | ACACUUUGUCCACCUUUGAUA | 522 | UAUCAAAGGUGGACAAAGUGUUU | 906 |
| 862 | siRNA131 | CACUUUGUCCACCUUUGAUGA | 523 | UCAUCAAAGGUGGACAAAGUGUU | 907 |
| 901 | siRNA132 | ACACCUCGUGGUCCUCUGGGA | 524 | UCCCAGAGGACCACGAGGUGUUU | 908 |
| 903 | siRNA133 | ACCUCGUGGUCCUCUGGGCAA | 525 | UUGCCCAGAGGACCACGAGGUUU | 909 |
| 904 | siRNA134 | CCUCGUGGUCCUCUGGGCAUA | 526 | UAUGCCCAGAGGACCACGAGGUU | 910 |
| 905 | siRNA135 | CUCGUGGUCCUCUGGGCAUCA | 527 | UGAUGCCCAGAGGACCACGAGUU | 911 |
| 906 | siRNA136 | UCGUGGUCCUCUGGGCAUCGA | 528 | UCGAUGCCCAGAGGACCACGAUU | 912 |
| 912 | siRNA137 | UCCUCUGGGCAUCGCAAGCAA | 529 | UUGCUUGCGAUGCCCAGAGGAUU | 913 |
| 915 | siRNA138 | UCUGGGCAUCGCAAGCAAACA | 530 | UGUUUGCUUGCGAUGCCCAGAUU | 914 |
| 916 | siRNA139 | CUGGGCAUCGCAAGCAAACAA | 531 | UUGUUUGCUUGCGAUGCCCAGUU | 915 |
| 917 | siRNA140 | UGGGCAUCGCAAGCAAACACA | 532 | UGUGUUUGCUUGCGAUGCCCAUU | 916 |
| 920 | siRNA141 | GCAUCGCAAGCAAACACCAUA | 533 | UAUGGUGUUUGCUUGCGAUGCUU | 917 |
| 921 | siRNA142 | CAUCGCAAGCAAACACCAUUA | 534 | UAAUGGUGUUUGCUUGCGAUGUU | 918 |
| 950 | siRNA143 | UUCUGGAGUUUAUUUCAAAUA | 535 | UAUUUGAAAUAAACUCCAGAAUU | 919 |
| 954 | siRNA144 | GGAGUUUAUUUCAAAUGAUUA | 536 | UAAUCAUUUGAAAUAAACUCCUU | 920 |
| 955 | siRNA145 | GAGUUUAUUUCAAAUGAUUAA | 537 | UUAAUCAUUUGAAAUAAACUCUU | 921 |
| 956 | siRNA146 | AGUUUAUUUCAAAUGAUUAGA | 538 | UCUAAUCAUUUGAAAUAAACUUU | 922 |
| 957 | siRNA147 | GUUUAUUUCAAAUGAUUAGGA | 539 | UCCUAAUCAUUUGAAAUAAACUU | 923 |
| 980 | siRNA148 | CCAGAAGCAAUGAAAGCAGCA | 540 | UGCUGCUUUCAUUGCUUCUGGUU | 924 |
| 982 | siRNA149 | AGAAGCAAUGAAAGCAGCUAA | 541 | UUAGCUGCUUUCAUUGCUUCUUU | 925 |
| 983 | siRNA150 | GAAGCAAUGAAAGCAGCUACA | 542 | UGUAGCUGCUUUCAUUGCUUCUU | 926 |
| 988 | siRNA151 | AAUGAAAGCAGCUACUGAAGA | 543 | UCUUCAGUAGCUGCUUUCAUUUU | 927 |
| 989 | siRNA152 | AUGAAAGCAGCUACUGAAGAA | 544 | UUCUUCAGUAGCUGCUUUCAUUU | 928 |
| 992 | siRNA153 | AAAGCAGCUACUGAAGAAGUA | 545 | UACUUCUUCAGUAGCUGCUUUUU | 929 |
| 993 | siRNA154 | AAGCAGCUACUGAAGAAGUGA | 546 | UCACUUCUUCAGUAGCUGCUUUU | 930 |
| 994 | siRNA155 | AGCAGCUACUGAAGAAGUGAA | 547 | UUCACUUCUUCAGUAGCUGCUUU | 931 |
| 995 | siRNA156 | GCAGCUACUGAAGAAGUGAAA | 548 | UUUCACUUCUUCAGUAGCUGCUU | 932 |
| 996 | siRNA157 | CAGCUACUGAAGAAGUGAAAA | 549 | UUUUCACUUCUUCAGUAGCUGUU | 933 |
| 1005 | siRNA158 | AAGAAGUGAAAAGAACAUUAA | 550 | UUAAUGUUCUUUUCACUUCUUUU | 934 |

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| 1006 | siRNA159 | AGAAGUGAAAAGAACAUUAGA | 551 | UCUAAUGUUCUUUUCACUUCUUU | 935 |
| 1007 | siRNA160 | GAAGUGAAAAGAACAUUAGAA | 552 | UUCUAAUGUUCUUUUCACUUCUU | 936 |
| 1011 | siRNA161 | UGAAAAGAACAUUAGAGAAUA | 553 | UAUUCUCUAAUGUUCUUUUCAUU | 937 |
| 1012 | siRNA162 | GAAAAGAACAUUAGAGAAUGA | 554 | UCAUUCUCUAAUGUUCUUUUCUU | 938 |
| 1013 | siRNA163 | AAAAGAACAUUAGAGAAUGCA | 555 | UGCAUUCUCUAAUGUUCUUUUUU | 939 |
| 1018 | siRNA164 | AACAUUAGAGAAUGCUGGUCA | 556 | UGACCAGCAUUCUCUAAUGUUUU | 940 |
| 1019 | siRNA165 | ACAUUAGAGAAUGCUGGUCAA | 557 | UUGACCAGCAUUCUCUAAUGUUU | 941 |
| 1020 | siRNA166 | CAUUAGAGAAUGCUGGUCAAA | 558 | UUUGACCAGCAUUCUCUAAUGUU | 942 |
| 1021 | siRNA167 | AUUAGAGAAUGCUGGUCAAAA | 559 | UUUUGACCAGCAUUCUCUAAUUU | 943 |
| 1026 | siRNA168 | AGAAUGCUGGUCAAAAAGUCA | 560 | UGACUUUUUGACCAGCAUUCUUU | 944 |
| 1027 | siRNA169 | GAAUGCUGGUCAAAAAGUCAA | 561 | UUGACUUUUUGACCAGCAUUCUU | 945 |
| 1029 | siRNA170 | AUGCUGGUCAAAAAGUCAGCA | 562 | UGCUGACUUUUUGACCAGCAUUU | 946 |
| 1036 | siRNA171 | UCAAAAAGUCAGCUUGGAAGA | 563 | UCUUCCAAGCUGACUUUUUGAUU | 947 |
| 1039 | siRNA172 | AAAAGUCAGCUUGGAAGGCAA | 564 | UUGCCUUCCAAGCUGACUUUUUU | 948 |
| 1042 | siRNA173 | AGUCAGCUUGGAAGGCAAUCA | 565 | UGAUUGCCUUCCAAGCUGACUUU | 949 |
| 1084 | siRNA174 | ACUGAAUGACCUGCCAGUAUA | 566 | UAUACUGGCAGGUCAUUCAGUUU | 950 |
| 1087 | siRNA175 | GAAUGACCUGCCAGUAUUAGA | 567 | UCUAAUACUGGCAGGUCAUUCUU | 951 |
| 1134 | siRNA176 | UUUCCAGUGCCUCCCUCAACA | 568 | UGUUGAGGGAGGCACUGGAAAUU | 952 |
| 1151 | siRNA177 | AACAUCCGGACAGCUAAGGAA | 569 | UUCCUUAGCUGUCCGGAUGUUUU | 953 |
| 1152 | siRNA178 | ACAUCCGGACAGCUAAGGAGA | 570 | UCUCCUUAGCUGUCCGGAUGUUU | 954 |
| 1155 | siRNA179 | UCCGGACAGCUAAGGAGGAUA | 571 | UAUCCUCCUUAGCUGUCCGGAUU | 955 |
| 1158 | siRNA180 | GGACAGCUAAGGAGGAUUUCA | 572 | UGAAAUCCUCCUUAGCUGUCCUU | 956 |
| 1160 | siRNA181 | ACAGCUAAGGAGGAUUUCACA | 573 | UGUGAAAUCCUCCUUAGCUGUUU | 957 |
| 1162 | siRNA182 | AGCUAAGGAGGAUUUCACUUA | 574 | UAAGUGAAAUCCUCCUUAGCUUU | 958 |
| 1165 | siRNA183 | UAAGGAGGAUUUCACUUUGCA | 575 | UGCAAAGUGAAAUCCUCCUUAUU | 959 |
| 1168 | siRNA184 | GGAGGAUUUCACUUUGCACCA | 576 | UGGUGCAAAGUGAAAUCCUCCUU | 960 |
| 1169 | siRNA185 | GAGGAUUUCACUUUGCACCUA | 577 | UAGGUGCAAAGUGAAAUCCUCUU | 961 |
| 1174 | siRNA186 | UUUCACUUUGCACCUUGAGGA | 578 | UCCUCAAGGUGCAAAGUGAAAUU | 962 |
| 1177 | siRNA187 | CACUUUGCACCUUGAGGACGA | 579 | UCGUCCUCAAGGUGCAAAGUGUU | 963 |
| 1178 | siRNA188 | ACUUUGCACCUUGAGGACGGA | 580 | UCCGUCCUCAAGGUGCAAAGUUU | 964 |
| 1189 | siRNA189 | UGAGGACGGUUCCUACAACAA | 581 | UUGUUGUAGGAACCGUCCUCAUU | 965 |
| 1191 | siRNA190 | AGGACGGUUCCUACAACAUCA | 582 | UGAUGUUGUAGGAACCGUCCUUU | 966 |
| 1194 | siRNA191 | ACGGUUCCUACAACAUCCGAA | 583 | UUCGGAUGUUGUAGGAACCGUUU | 967 |
| 1196 | siRNA192 | GGUUCCUACAACAUCCGAAAA | 584 | UUUUCGGAUGUUGUAGGAACCUU | 968 |
| 1200 | siRNA193 | CCUACAACAUCCGAAAAGAUA | 585 | UAUCUUUUCGGAUGUUGUAGGUU | 969 |
| 1201 | siRNA194 | CUACAACAUCCGAAAAGAUGA | 586 | UCAUCUUUUCGGAUGUUGUAGUU | 970 |
| 1203 | siRNA195 | ACAACAUCCGAAAAGAUGACA | 587 | UGUCAUCUUUUCGGAUGUUGUUU | 971 |
| 1204 | siRNA196 | CAACAUCCGAAAAGAUGACAA | 588 | UUGUCAUCUUUUCGGAUGUUGUU | 972 |
| 1205 | siRNA197 | AACAUCCGAAAAGAUGACAUA | 589 | UAUGUCAUCUUUUCGGAUGUUUU | 973 |
| 1207 | siRNA198 | CAUCCGAAAAGAUGACAUCAA | 590 | UUGAUGUCAUCUUUUCGGAUGUU | 974 |
| 1209 | siRNA199 | UCCGAAAAGAUGACAUCAUAA | 591 | UUAUGAUGUCAUCUUUUCGGAUU | 975 |
| 1212 | siRNA200 | GAAAAGAUGACAUCAUAGCUA | 592 | UAGCUAUGAUGUCAUCUUUUCUU | 976 |
| 1214 | siRNA201 | AAAGAUGACAUCAUAGCUCUA | 593 | UAGAGCUAUGAUGUCAUCUUUUU | 977 |
| 1215 | siRNA202 | AAGAUGACAUCAUAGCUCUUA | 594 | UAAGAGCUAUGAUGUCAUCUUUU | 978 |

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| 1217 | siRNA203 | GAUGACAUCAUAGCUCUUUAA | 595 | UUAAAGAGCUAUGAUGUCAUCUU | 979 |
| 1226 | siRNA204 | AUAGCUCUUUACCCACAGUUA | 596 | UAACUGUGGGUAAAGAGCUAUUU | 980 |
| 1227 | siRNA205 | UAGCUCUUUACCCACAGUUAA | 597 | UUAACUGUGGGUAAAGAGCUAUU | 981 |
| 1228 | siRNA206 | AGCUCUUUACCCACAGUUAAA | 598 | UUUAACUGUGGGUAAAGAGCUUU | 982 |
| 1229 | siRNA207 | GCUCUUUACCCACAGUUAAUA | 599 | UAUUAACUGUGGGUAAAGAGCUU | 983 |
| 1230 | siRNA208 | CUCUUUACCCACAGUUAAUGA | 600 | UCAUUAACUGUGGGUAAAGAGUU | 984 |
| 1231 | siRNA209 | UCUUUACCCACAGUUAAUGCA | 601 | UGCAUUAACUGUGGGUAAAGAUU | 985 |
| 1233 | siRNA210 | UUUACCCACAGUUAAUGCACA | 602 | UGUGCAUUAACUGUGGGUAAAUU | 986 |
| 1236 | siRNA211 | ACCCACAGUUAAUGCACUUAA | 603 | UUAAGUGCAUUAACUGUGGGUUU | 987 |
| 1237 | siRNA212 | CCCACAGUUAAUGCACUUAGA | 604 | UCUAAGUGCAUUAACUGUGGGUU | 988 |
| 1238 | siRNA213 | CCACAGUUAAUGCACUUAGAA | 605 | UUCUAAGUGCAUUAACUGUGGUU | 989 |
| 1239 | siRNA214 | CACAGUUAAUGCACUUAGAUA | 606 | UAUCUAAGUGCAUUAACUGUGUU | 990 |
| 1240 | siRNA215 | ACAGUUAAUGCACUUAGAUCA | 607 | UGAUCUAAGUGCAUUAACUGUUU | 991 |
| 1241 | siRNA216 | CAGUUAAUGCACUUAGAUCCA | 608 | UGGAUCUAAGUGCAUUAACUGUU | 992 |
| 1242 | siRNA217 | AGUUAAUGCACUUAGAUCCAA | 609 | UUGGAUCUAAGUGCAUUAACUUU | 993 |
| 1248 | siRNA218 | UGCACUUAGAUCCAGAAAUCA | 610 | UGAUUUCUGGAUCUAAGUGCAUU | 994 |
| 1249 | siRNA219 | GCACUUAGAUCCAGAAAUCUA | 611 | UAGAUUUCUGGAUCUAAGUGCUU | 995 |
| 1261 | siRNA220 | AGAAAUCUACCCAGACCCUUA | 612 | UAAGGGUCUGGGUAGAUUUCUUU | 996 |
| 1283 | siRNA221 | ACUUUUAAAUAUGAUAGGUAA | 613 | UUACCUAUCAUAUUUAAAAGUUU | 997 |
| 1285 | siRNA222 | UUUUAAAUAUGAUAGGUAUCA | 614 | UGAUACCUAUCAUAUUUAAAAUU | 998 |
| 1286 | siRNA223 | UUUAAAUAUGAUAGGUAUCUA | 615 | UAGAUACCUAUCAUAUUUAAAUU | 999 |
| 1288 | siRNA224 | UAAAUAUGAUAGGUAUCUUGA | 616 | UCAAGAUACCUAUCAUAUUUAUU | 1000 |
| 1290 | siRNA225 | AAUAUGAUAGGUAUCUUGAUA | 617 | UAUCAAGAUACCUAUCAUAUUUU | 1001 |
| 1291 | siRNA226 | AUAUGAUAGGUAUCUUGAUGA | 618 | UCAUCAAGAUACCUAUCAUAUUU | 1002 |
| 1292 | siRNA227 | UAUGAUAGGUAUCUUGAUGAA | 619 | UUCAUCAAGAUACCUAUCAUAUU | 1003 |
| 1293 | siRNA228 | AUGAUAGGUAUCUUGAUGAAA | 620 | UUUCAUCAAGAUACCUAUCAUUU | 1004 |
| 1296 | siRNA229 | AUAGGUAUCUUGAUGAAAACA | 621 | UGUUUUCAUCAAGAUACCUAUUU | 1005 |
| 1297 | siRNA230 | UAGGUAUCUUGAUGAAAACGA | 622 | UCGUUUUCAUCAAGAUACCUAUU | 1006 |
| 1298 | siRNA231 | AGGUAUCUUGAUGAAAACGGA | 623 | UCCGUUUUCAUCAAGAUACCUUU | 1007 |
| 1299 | siRNA232 | GGUAUCUUGAUGAAAACGGGA | 624 | UCCCGUUUUCAUCAAGAUACCUU | 1008 |
| 1300 | siRNA233 | GUAUCUUGAUGAAAACGGGAA | 625 | UUCCCGUUUUCAUCAAGAUACUU | 1009 |
| 1301 | siRNA234 | UAUCUUGAUGAAAACGGGAAA | 626 | UUUCCCGUUUUCAUCAAGAUAUU | 1010 |
| 1302 | siRNA235 | AUCUUGAUGAAAACGGGAAGA | 627 | UCUUCCCGUUUUCAUCAAGAUUU | 1011 |
| 1303 | siRNA236 | UCUUGAUGAAAACGGGAAGAA | 628 | UUCUUCCCGUUUUCAUCAAGAUU | 1012 |
| 1306 | siRNA237 | UGAUGAAAACGGGAAGACAAA | 629 | UUUGUCUUCCCGUUUUCAUCAUU | 1013 |
| 1307 | siRNA238 | GAUGAAAACGGGAAGACAAAA | 630 | UUUUGUCUUCCCGUUUUCAUCUU | 1014 |
| 1311 | siRNA239 | AAAACGGGAAGACAAAGACUA | 631 | UAGUCUUUGUCUUCCCGUUUUUU | 1015 |
| 1313 | siRNA240 | AACGGGAAGACAAAGACUACA | 632 | UGUAGUCUUUGUCUUCCCGUUUU | 1016 |
| 1314 | siRNA241 | ACGGGAAGACAAAGACUACCA | 633 | UGGUAGUCUUUGUCUUCCCGUUU | 1017 |
| 1353 | siRNA242 | AGUUAAAGUAUUACUACAUGA | 634 | UCAUGUAGUAAUACUUUAACUUU | 1018 |
| 1354 | siRNA243 | GUUAAAGUAUUACUACAUGCA | 635 | UGCAUGUAGUAAUACUUUAACUU | 1019 |
| 1358 | siRNA244 | AAGUAUUACUACAUGCCCUUA | 636 | UAAGGGCAUGUAGUAAUACUUUU | 1020 |
| 1360 | siRNA245 | GUAUUACUACAUGCCCUUUGA | 637 | UCAAAGGGCAUGUAGUAAUACUU | 1021 |
| 1361 | siRNA246 | UAUUACUACAUGCCCUUUGGA | 638 | UCCAAAGGGCAUGUAGUAAUAUU | 1022 |

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| 1363 | siRNA247 | UUACUACAUGCCCUUUGGAUA | 639 | UAUCCAAAGGGCAUGUAGUAAUU | 1023 |
| 1364 | siRNA248 | UACUACAUGCCCUUUGGAUCA | 640 | UGAUCCAAAGGGCAUGUAGUAUU | 1024 |
| 1382 | siRNA249 | UCGGGAGCUACAAUAUGUCCA | 641 | UGGACAUAUUGUAGCUCCCGAUU | 1025 |
| 1386 | siRNA250 | GAGCUACAAUAUGUCCUGGAA | 642 | UUCCAGGACAUAUUGUAGCUCUU | 1026 |
| 1388 | siRNA251 | GCUACAAUAUGUCCUGGAAGA | 643 | UCUUCCAGGACAUAUUGUAGCUU | 1027 |
| 1389 | siRNA252 | CUACAAUAUGUCCUGGAAGAA | 644 | UUCUUCCAGGACAUAUUGUAGUU | 1028 |
| 1413 | siRNA253 | UCGCUAUCCACGAAAUCAAGA | 645 | UCUUGAUUUCGUGGAUAGCGAUU | 1029 |
| 1415 | siRNA254 | GCUAUCCACGAAAUCAAGCAA | 646 | UUGCUUGAUUUCGUGGAUAGCUU | 1030 |
| 1416 | siRNA255 | CUAUCCACGAAAUCAAGCAAA | 647 | UUUGCUUGAUUUCGUGGAUAGUU | 1031 |
| 1417 | siRNA256 | UAUCCACGAAAUCAAGCAAUA | 648 | UAUUGCUUGAUUUCGUGGAUAUU | 1032 |
| 1424 | siRNA257 | GAAAUCAAGCAAUUUUUGAUA | 649 | UAUCAAAAAUUGCUUGAUUUCUU | 1033 |
| 1425 | siRNA258 | AAAUCAAGCAAUUUUUGAUUA | 650 | UAAUCAAAAAUUGCUUGAUUUUU | 1034 |
| 1431 | siRNA259 | AGCAAUUUUUGAUUCUGAUGA | 651 | UCAUCAGAAUCAAAAAUUGCUUU | 1035 |
| 1433 | siRNA260 | CAAUUUUUGAUUCUGAUGCUA | 652 | UAGCAUCAGAAUCAAAAAUUGUU | 1036 |
| 1506 | siRNA261 | ACCAGUCCCGGGCAGGCUUGA | 653 | UCAAGCCUGCCCGGGACUGGUUU | 1037 |
| 1507 | siRNA262 | CCAGUCCCGGGCAGGCUUGGA | 654 | UCCAAGCCUGCCCGGGACUGGUU | 1038 |
| 1510 | siRNA263 | GUCCCGGGCAGGCUUGGGCAA | 655 | UUGCCCAAGCCUGCCCGGGACUU | 1039 |
| 1511 | siRNA264 | UCCCGGGCAGGCUUGGGCAUA | 656 | UAUGCCCAAGCCUGCCCGGGAUU | 1040 |
| 1513 | siRNA265 | CCGGGCAGGCUUGGGCAUUUA | 657 | UAAAUGCCCAAGCCUGCCCGGUU | 1041 |
| 1518 | siRNA266 | CAGGCUUGGGCAUUUUGCCGA | 658 | UCGGCAAAAUGCCCAAGCCUGUU | 1042 |
| 1555 | siRNA267 | AUUUAAAUAUAAAUUCAAGCA | 659 | UGCUUGAAUUUAUAUUUAAAUUU | 1043 |
| 1556 | siRNA268 | UUUAAAUAUAAAUUCAAGCAA | 660 | UUGCUUGAAUUUAUAUUUAAAUU | 1044 |
| 1557 | siRNA269 | UUAAAUAUAAAUUCAAGCAUA | 661 | UAUGCUUGAAUUUAUAUUUAAUU | 1045 |
| 1558 | siRNA270 | UAAAUAUAAAUUCAAGCAUUA | 662 | UAAUGCUUGAAUUUAUAUUUAUU | 1046 |
| 1559 | siRNA271 | AAAUAUAAAUUCAAGCAUUUA | 663 | UAAAUGCUUGAAUUUAUAUUUUU | 1047 |
| 1561 | siRNA272 | AUAUAAAUUCAAGCAUUUGUA | 664 | UACAAAUGCUUGAAUUUAUAUUU | 1048 |
| 1593 | siRNA273 | GGAAUAAGAGGACACUAGAUA | 665 | UAUCUAGUGUCCUCUUAUUCCUU | 1049 |
| 1594 | siRNA274 | GAAUAAGAGGACACUAGAUGA | 666 | UCAUCUAGUGUCCUCUUAUUCUU | 1050 |
| 1597 | siRNA275 | UAAGAGGACACUAGAUGAUAA | 667 | UUAUCAUCUAGUGUCCUCUUAUU | 1051 |
| 1599 | siRNA276 | AGAGGACACUAGAUGAUAUUA | 668 | UAAUAUCAUCUAGUGUCCUCUUU | 1052 |
| 1601 | siRNA277 | AGGACACUAGAUGAUAUUACA | 669 | UGUAAUAUCAUCUAGUGUCCUUU | 1053 |
| 1605 | siRNA278 | CACUAGAUGAUAUUACAGGAA | 670 | UUCCUGUAAUAUCAUCUAGUGUU | 1054 |
| 1606 | siRNA279 | ACUAGAUGAUAUUACAGGACA | 671 | UGUCCUGUAAUAUCAUCUAGUUU | 1055 |
| 1609 | siRNA280 | AGAUGAUAUUACAGGACUGCA | 672 | UGCAGUCCUGUAAUAUCAUCUUU | 1056 |
| 1610 | siRNA281 | GAUGAUAUUACAGGACUGCAA | 673 | UUGCAGUCCUGUAAUAUCAUCUU | 1057 |
| 1614 | siRNA282 | AUAUUACAGGACUGCAGAACA | 674 | UGUUCUGCAGUCCUGUAAUAUUU | 1058 |
| 1638 | siRNA283 | UCACCACACAGUCCCUUUGGA | 675 | UCCAAAGGGACUGUGUGGGUGAUU | 1059 |
| 1660 | siRNA284 | AAAUGCAUUUAGUGGUGGUAA | 676 | UUACCACCACUAAAUGCAUUUUU | 1060 |
| 1661 | siRNA285 | AAUGCAUUUAGUGGUGGUAGA | 677 | UCUACCACCACUAAAUGCAUUUU | 1061 |
| 1662 | siRNA286 | AUGCAUUUAGUGGUGGUAGAA | 678 | UUCUACCACCACUAAAUGCAUUU | 1062 |
| 1663 | siRNA287 | UGCAUUUAGUGGUGGUAGAAA | 679 | UUUCUACCACCACUAAAUGCAUU | 1063 |
| 1669 | siRNA288 | UAGUGGUGGUAGAAAUGAUUA | 680 | UAAUCAUUUCUACCACCACUAUU | 1064 |
| 1671 | siRNA289 | GUGGUGGUAGAAAUGAUUCAA | 681 | UUGAAUCAUUUCUACCACCACUU | 1065 |
| 1673 | siRNA290 | GGUGGUAGAAAUGAUUCACCA | 682 | UGGUGAAUCAUUUCUACCACCUU | 1066 |

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| 1676 | siRNA291 | GGUAGAAAUGAUUCACCAGGA | 683 | UCCUGGUGAAUCAUUUCUACCUU | 1067 |
| 1683 | siRNA292 | AUGAUUCACCAGGUCCAAUGA | 684 | UCAUUGGACCUGGUGAAUCAUUU | 1068 |
| 1686 | siRNA293 | AUUCACCAGGUCCAAUGUUGA | 685 | UCAACAUUGGACCUGGUGAAUUU | 1069 |
| 1689 | siRNA294 | CACCAGGUCCAAUGUUGUUCA | 686 | UGAACAACAUUGGACCUGGUGUU | 1070 |
| 1690 | siRNA295 | ACCAGGUCCAAUGUUGUUCAA | 687 | UUGAACAACAUUGGACCUGGUUU | 1071 |
| 1692 | siRNA296 | CAGGUCCAAUGUUGUUCACCA | 688 | UGGUGAACAACAUUGGACCUGUU | 1072 |
| 1712 | siRNA297 | AGUGCUUGCUUGUGAAUCUUA | 689 | UAAGAUUCACAAGCAAGCACUUU | 1073 |
| 1713 | siRNA298 | GUGCUUGCUUGUGAAUCUUAA | 690 | UUAAGAUUCACAAGCAAGCACUU | 1074 |
| 1769 | siRNA299 | UCUGCUAGUGAAAAGAACUAA | 691 | UUAGUUCUUUUCACUAGCAGAUU | 1075 |
| 1770 | siRNA300 | CUGCUAGUGAAAAGAACUAGA | 692 | UCUAGUUCUUUUCACUAGCAGUU | 1076 |
| 1824 | siRNA301 | UAAGUCCAUGAAUGUUCAUAA | 693 | UUAUGAACAUUCAUGGACUUAUU | 1077 |
| 1825 | siRNA302 | AAGUCCAUGAAUGUUCAUAUA | 694 | UAUAUGAACAUUCAUGGACUUUU | 1078 |
| 1826 | siRNA303 | AGUCCAUGAAUGUUCAUAUAA | 695 | UUAUAUGAACAUUCAUGGACUUU | 1079 |
| 1827 | siRNA304 | GUCCAUGAAUGUUCAUAUAGA | 696 | UCUAUAUGAACAUUCAUGGACUU | 1080 |
| 1828 | siRNA305 | UCCAUGAAUGUUCAUAUAGCA | 697 | UGCUAUAUGAACAUUCAUGGAUU | 1081 |
| 1829 | siRNA306 | CCAUGAAUGUUCAUAUAGCCA | 698 | UGGCUAUAUGAACAUUCAUGGUU | 1082 |
| 1830 | siRNA307 | CAUGAAUGUUCAUAUAGCCAA | 699 | UUGGCUAUAUGAACAUUCAUGUU | 1083 |
| 1895 | siRNA308 | UUUUUUUCAAAAUGAAGAUAA | 700 | UUAUCUUCAUUUUGAAAAAAAUU | 1084 |
| 2052 | siRNA309 | UGUAUUCUAAUUGGCAGAUUA | 701 | UAAUCUGCCAAUUAGAAUACAUU | 1085 |
| 2053 | siRNA310 | GUAUUCUAAUUGGCAGAUUGA | 702 | UCAAUCUGCCAAUUAGAAUACUU | 1086 |
| 2075 | siRNA311 | UUUUCCUAAGGAAACUGCUUA | 703 | UAAGCAGUUUCCUUAGGAAAAUU | 1087 |
| 2127 | siRNA312 | AAAUGUUCAAAUUCACGUUCA | 704 | UGAACGUGAAUUUGAACAUUUUU | 1088 |
| 2128 | siRNA313 | AAUGUUCAAAUUCACGUUCUA | 705 | UAGAACGUGAAUUUGAACAUUUU | 1089 |
| 2132 | siRNA314 | UUCAAAUUCACGUUCUAGUGA | 706 | UCACUAGAACGUGAAUUUGAAUU | 1090 |
| 2133 | siRNA315 | UCAAAUUCACGUUCUAGUGAA | 707 | UUCACUAGAACGUGAAUUUGAUU | 1091 |
| 2134 | siRNA316 | CAAAUUCACGUUCUAGUGAAA | 708 | UUUCACUAGAACGUGAAUUUGUU | 1092 |
| 2135 | siRNA317 | AAAUUCACGUUCUAGUGAAAA | 709 | UUUUCACUAGAACGUGAAUUUUU | 1093 |
| 2136 | siRNA318 | AAUUCACGUUCUAGUGAAACA | 710 | UGUUUCACUAGAACGUGAAUUUU | 1094 |
| 2137 | siRNA319 | AUUCACGUUCUAGUGAAACUA | 711 | UAGUUUCACUAGAACGUGAAUUU | 1095 |
| 2139 | siRNA320 | UCACGUUCUAGUGAAACUGCA | 712 | UGCAGUUUCACUAGAACGUGAUU | 1096 |
| 2140 | siRNA321 | CACGUUCUAGUGAAACUGCAA | 713 | UUGCAGUUUCACUAGAACGUGUU | 1097 |
| 2144 | siRNA322 | UUCUAGUGAAACUGCAUUAUA | 714 | UAUAAUGCAGUUUCACUAGAAUU | 1098 |
| 2189 | siRNA323 | CGGGUGUGAUCAUAUAUCAUA | 715 | UAUGAUAUAUGAUCACACCCGUU | 1099 |
| 2190 | siRNA324 | GGGUGUGAUCAUAUAUCAUAA | 716 | UUAUGAUAUAUGAUCACACCCUU | 1100 |
| 2191 | siRNA325 | GGUGUGAUCAUAUAUCAUAAA | 717 | UUUAUGAUAUAUGAUCACACCUU | 1101 |
| 2192 | siRNA326 | GUGUGAUCAUAUAUCAUAAAA | 718 | UUUUAUGAUAUAUGAUCACACUU | 1102 |
| 2193 | siRNA327 | UGUGAUCAUAUAUCAUAAAGA | 719 | UCUUUAUGAUAUAUGAUCACAUU | 1103 |
| 2194 | siRNA328 | GUGAUCAUAUAUCAUAAAGGA | 720 | UCCUUUAUGAUAUAUGAUCACUU | 1104 |
| 2195 | siRNA329 | UGAUCAUAUAUCAUAAAGGAA | 721 | UUCCUUUAUGAUAUAUGAUCAUU | 1105 |
| 2199 | siRNA330 | CAUAUAUCAUAAAGGAUAUUA | 722 | UAAUAUCCUUUAUGAUAUAUGUU | 1106 |
| 2209 | siRNA331 | AAAGGAUAUUUCAAAUGAUUA | 723 | UAAUCAUUUGAAAUAUCCUUUUU | 1107 |
| 2210 | siRNA332 | AAGGAUAUUUCAAAUGAUUAA | 724 | UUAAUCAUUUGAAAUAUCCUUUU | 1108 |
| 2211 | siRNA333 | AGGAUAUUUCAAAUGAUUAUA | 725 | UAUAAUCAUUUGAAAUAUCCUUU | 1109 |
| 2215 | siRNA334 | UAUUUCAAAUGAUUAUGAUUA | 726 | UAAUCAUAAUCAUUUGAAAUAUU | 1110 |

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| 2216 | siRNA335 | AUUUCAAAUGAUUAUGAUUAA | 727 | UUAAUCAUAAUCAUUUGAAAUUU | 1111 |
| 2217 | siRNA336 | UUUCAAAUGAUUAUGAUUAGA | 728 | UCUAAUCAUAAUCAUUUGAAAUU | 1112 |
| 2218 | siRNA337 | UUCAAAUGAUUAUGAUUAGUA | 729 | UACUAAUCAUAAUCAUUUGAAUU | 1113 |
| 2220 | siRNA338 | CAAAUGAUUAUGAUUAGUUAA | 730 | UUAACUAAUCAUAAUCAUUUGUU | 1114 |
| 2221 | siRNA339 | AAAUGAUUAUGAUUAGUUAUA | 731 | UAUAACUAAUCAUAAUCAUUUUU | 1115 |
| 2223 | siRNA340 | AUGAUUAUGAUUAGUUAUGUA | 732 | UACAUAACUAAUCAUAAUCAUUU | 1116 |
| 2224 | siRNA341 | UGAUUAUGAUUAGUUAUGUCA | 733 | UGACAUAACUAAUCAUAAUCAUU | 1117 |
| 2225 | siRNA342 | GAUUAUGAUUAGUUAUGUCUA | 734 | UAGACAUAACUAAUCAUAAUCUU | 1118 |
| 2313 | siRNA343 | UUGAUUUCCCAAAAACACUAA | 735 | UUAGUGUUUUUGGGAAAUCAAUU | 1119 |
| 2316 | siRNA344 | AUUUCCCAAAAACACUAAAGA | 736 | UCUUUAGUGUUUUUGGGAAAUUU | 1120 |
| 2317 | siRNA345 | UUUCCCAAAAACACUAAAGGA | 737 | UCCUUUAGUGUUUUUGGGAAAUU | 1121 |
| 2319 | siRNA346 | UCCCAAAAACACUAAAGGUGA | 738 | UCACCUUUAGUGUUUUUGGGAUU | 1122 |
| 2320 | siRNA347 | CCCAAAAACACUAAAGGUGGA | 739 | UCCACCUUUAGUGUUUUUGGGUU | 1123 |
| 2321 | siRNA348 | CCAAAAACACUAAAGGUGGUA | 740 | UACCACCUUUAGUGUUUUUGGUU | 1124 |
| 2353 | siRNA349 | UCAUGUUUUAACUUAUUGUUA | 741 | UAACAAUAAGUUAAAACAUGAUU | 1125 |
| 2355 | siRNA350 | AUGUUUUAACUUAUUGUUGCA | 742 | UGCAACAAUAAGUUAAAACAUUU | 1126 |
| 2356 | siRNA351 | UGUUUUAACUUAUUGUUGCUA | 743 | UAGCAACAAUAAGUUAAAACAUU | 1127 |
| 2362 | siRNA352 | AACUUAUUGUUGCUGAAAACA | 744 | UGUUUUCAGCAACAAUAAGUUUU | 1128 |
| 2364 | siRNA353 | CUUAUUGUUGCUGAAAACUCA | 745 | UGAGUUUUCAGCAACAAUAAGUU | 1129 |
| 2365 | siRNA354 | UUAUUGUUGCUGAAAACUCUA | 746 | UAGAGUUUUCAGCAACAAUAAUU | 1130 |
| 2366 | siRNA355 | UAUUGUUGCUGAAAACUCUAA | 747 | UUAGAGUUUUCAGCAACAAUAUU | 1131 |
| 2370 | siRNA356 | GUUGCUGAAAACUCUAUGUCA | 748 | UGACAUAGAGUUUUCAGCAACUU | 1132 |
| 2503 | siRNA357 | AAAAAUGUAGCUUUUAUGUGA | 749 | UCACAUAAAAGCUACAUUUUUUU | 1133 |
| 2562 | siRNA358 | GGAAGCUUUGGUUAUGAAACA | 750 | UGUUUCAUAACCAAAGCUUCCUU | 1134 |
| 2563 | siRNA359 | GAAGCUUUGGUUAUGAAACAA | 751 | UUGUUUCAUAACCAAAGCUUCUU | 1135 |
| 2620 | siRNA360 | AUUUAAAUGCUUUUUAUCGCA | 752 | UGCGAUAAAAAGCAUUUAAAUUU | 1136 |
| 2621 | siRNA361 | UUUAAAUGCUUUUUAUCGCUA | 753 | UAGCGAUAAAAAGCAUUUAAAUU | 1137 |
| 2622 | siRNA362 | UUAAAUGCUUUUUAUCGCUAA | 754 | UUAGCGAUAAAAAGCAUUUAAUU | 1138 |
| 2623 | siRNA363 | UAAAUGCUUUUUAUCGCUAAA | 755 | UUUAGCGAUAAAAAGCAUUUAUU | 1139 |
| 2624 | siRNA364 | AAAUGCUUUUUAUCGCUAAAA | 756 | UUUUAGCGAUAAAAAGCAUUUUU | 1140 |
| 2625 | siRNA365 | AAUGCUUUUUAUCGCUAAAUA | 757 | UAUUUAGCGAUAAAAAGCAUUUU | 1141 |
| 2629 | siRNA366 | CUUUUUAUCGCUAAAUGACUA | 758 | UAGUCAUUUAGCGAUAAAAAGUU | 1142 |
| 2630 | siRNA367 | UUUUUAUCGCUAAAUGACUUA | 759 | UAAGUCAUUUAGCGAUAAAAAUU | 1143 |
| 2632 | siRNA368 | UUUAUCGCUAAAUGACUUGCA | 760 | UGCAAGUCAUUUAGCGAUAAAUU | 1144 |
| 2633 | siRNA369 | UUAUCGCUAAAUGACUUGCAA | 761 | UUGCAAGUCAUUUAGCGAUAAUU | 1145 |
| 2635 | siRNA370 | AUCGCUAAAUGACUUGCAGAA | 762 | UUCUGCAAGUCAUUUAGCGAUUU | 1146 |
| 2639 | siRNA371 | CUAAAUGACUUGCAGAUGAAA | 763 | UUUCAUCUGCAAGUCAUUUAGUU | 1147 |
| 2679 | siRNA372 | UGUUUAAAUGCUGUGUACAAA | 764 | UUUGUACACAGCAUUUAAACAUU | 1148 |
| 2682 | siRNA373 | UUAAAUGCUGUGUACAACAAA | 765 | UUUGUUGUACACAGCAUUUAAUU | 1149 |
| 2683 | siRNA374 | UAAAUGCUGUGUACAACAAUA | 766 | UAUUGUUGUACACAGCAUUUAUU | 1150 |
| 2687 | siRNA375 | UGCUGUGUACAACAAUGCUUA | 767 | UAAGCAUUGUUGUACACAGCAUU | 1151 |
| 2690 | siRNA376 | UGUGUACAACAAUGCUUUGAA | 768 | UUCAAAGCAUUGUUGUACACAUU | 1152 |
| 2691 | siRNA377 | GUGUACAACAAUGCUUUGAUA | 769 | UAUCAAAGCAUUGUUGUACACUU | 1153 |
| 2692 | siRNA378 | UGUACAACAAUGCUUUGAUAA | 770 | UUAUCAAAGCAUUGUUGUACAUU | 1154 |

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|---|---|---|---|---|---|
| 2833 | siRNA379 | GAUAAUUUUGAAAUGAUUCAA | 771 | UUGAAUCAUUUCAAAAUUAUCUU | 1155 |
| 2834 | siRNA380 | AUAAUUUUGAAAUGAUUCAUA | 772 | UAUGAAUCAUUUCAAAAUUAUUU | 1156 |
| 2835 | siRNA381 | UAAUUUUGAAAUGAUUCAUCA | 773 | UGAUGAAUCAUUUCAAAAUUAUU | 1157 |
| 2838 | siRNA382 | UUUUGAAAUGAUUCAUCUUUA | 774 | UAAAGAUGAAUCAUUUCAAAAUU | 1158 |
| 2839 | siRNA383 | UUUGAAAUGAUUCAUCUUUCA | 775 | UGAAAGAUGAAUCAUUUCAAAUU | 1159 |
| 2858 | siRNA384 | CAGAAAUAAAAGUAUGAAUCA | 776 | UGAUUCAUACUUUUAUUUCUGUU | 1160 |

*‡Each uracil base (U) in any one of the sequences provided in Table 5A may independently and optionally be replaced with a thymine base (T).*

Table 5B: siRNAs for *in vitro* Screening‡

| Position | siRNA#* | Sense (5'-3') | Nucleobase SEQ ID NO: | SEQ ID NO of modified sense strand: | Antisense (5'-3') | Nucleobase SEQ ID NO: | SEQ ID NO of modified antisense strand: |
|---|---|---|---|---|---|---|---|
| 34 | siRNA1-M0 | [mU][mU][mC][mU][mU][mC][mC][mU][C][A][G][A][G][mA][mU][mU][mU][mU][G][mG][mA] | 393 | 1191 | [mU][C][C][mA][A][A][A][U][C][U][mC][mU][G][A][G][mG][A][mA][G][A][mA][mU][mU] | 777 | 1605 |
| 40 | siRNA2-M0 | [mC][mU][mC][mA][mG][mA][mG][mA][U][U][U][U][G][mG][mC][mC][mU][mA][G][mA][mA] | 394 | 1192 | [mU][U][C][mU][A][G][G][C][C][A][mA][mA][A][U][C][mU][C][mU][G][A][mG][mU][mU] | 778 | 1606 |
| 41 | siRNA3-M0 | [mU][mC][mA][mG][mA][mG][mA][mU][U][U][U][G][G][mC][mC][mU][mA][mG][A][mU][mA] | 395 | 1193 | [mU][A][U][mC][U][A][G][G][C][C][mA][mA][A][A][U][mC][U][mC][U][G][mA][mU][mU] | 779 | 1607 |
| 42 | siRNA4-M0 | [mC][mA][mG][mA][mG][mA][mU][mU][U][U][G][G][C][mC][mU][mA][mG][mA][U][mU][mU][mA] | 396 | 1194 | [mU][A][A][mU][C][U][A][G][G][C][mC][mA][A][A][A][mU][C][mU][C][U][mG][mU][mU] | 780 | 1608 |
| 43 | siRNA5-M0 | [mA][mG][mA][mG][mA][mU][mU][mU][U][G][G][C][C][mU][mA][mG][mA][mU][U][mU][mA] | 397 | 1195 | [mU][A][A][mA][U][C][U][A][G][G][mC][mC][A][A][A][mA][U][mC][U][C][mU][mU][mU] | 781 | 1609 |
| 45 | siRNA6-M0 | [mA][mG][mA][mU][mU][mU][mU][mG][G][C][C][U][A][mG][mA][mU][mU][mU][G][mC][mA] | 398 | 1196 | [mU][G][C][mA][A][A][U][C][U][A][mG][mG][C][C][A][mA][A][mA][U][C][mU][mU][mU] | 782 | 1610 |
| 105 | siRNA7-M0 | [mC][mA][mG][mC][mA][mU][mG][mC][U][G][U][U][G][mU][mC][mU][mA][mU][G][mG][mA] | 399 | 1197 | [mU][C][C][mA][U][A][G][A][C][A][mA][mC][A][G][C][mA][U][mG][C][U][mG][mU][mU] | 783 | 1611 |
| 110 | siRNA8-M0 | [mU][mG][mC][mU][mG][mU][mU][mG][U][C][U][A][U][mG][mG][mC][mU][mU][A][mU][mA] | 400 | 1198 | [mU][A][U][mA][A][G][C][C][A][U][mA][mG][A][C][A][mA][C][mA][G][C][mA][mU][mU] | 784 | 1612 |
| 111 | siRNA9-M0 | [mG][mC][mU][mG][mU][mU][mG][mU][C][U][A][U][G][mG][mC][mU][mU][mA][U][mU][mA] | 401 | 1199 | [mU][A][A][mU][A][A][G][C][C][A][mU][mA][G][A][C][mA][A][mC][A][G][mC][mU][mU] | 785 | 1613 |
| 112 | siRNA10-M0 | [mC][mU][mG][mU][mU][mG][mU][mC][U][A][U][G][G][mC][mU][mU][mA][mU][U][mC][mA] | 402 | 1200 | [mU][G][A][mA][U][A][A][G][C][C][mA][mU][A][G][A][mC][A][mA][C][A][mG][mU][mU] | 786 | 1614 |
| 113 | siRNA11-M0 | [mU][mG][mU][mU][mG][mU][mC][mU][A][U][G][G][C] | 403 | 1201 | [mU][A][G][mA][A][U][A][A][G][C][mC][mA][U][ | 787 | 1615 |

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|---|---|---|---|---|---|---|---|
| | | [mU][mU][mA][mU][mU][C][mU][mA] | | | A][G][mA][C][mA][A][C][mA][mU][mU] | | |
| 115 | siRNA12-M0 | [mU][mU][mG][mU][mC][mU][mA][mU][G][G][C][U][U][mA][mU][mU][mC][mU][U][mG][mA] | 404 | 1203 | [mU][C][A][mA][G][A][A][U][A][A][mG][mC][C][A][U][mA][G][mA][C][A][mA][mU][mU] | 788 | 1616 |
| 116 | siRNA13-M0 | [mU][mG][mU][mC][mU][mA][mU][mG][G][C][U][U][A][mU][mU][mC][mU][mU][G][mG][mA] | 405 | 1204 | [mU][C][C][mA][A][G][A][A][U][A][mA][mG][C][C][A][mU][A][mG][A][C][mA][mU][mU] | 789 | 1617 |
| 117 | siRNA14-M0 | [mG][mU][mC][mU][mA][mU][mG][mG][C][U][U][A][U][mU][mC][mU][mU][mG][G][mA][mA] | 406 | 1205 | [mU][U][C][mC][A][A][G][A][A][U][mA][mA][G][C][C][mA][U][mA][G][A][mC][mU][mU] | 790 | 1618 |
| 118 | siRNA15-M0 | [mU][mC][mU][mA][mU][mG][mG][mC][U][U][A][U][U][mC][mU][mU][mG][mG][A][mA][mA] | 407 | 1206 | [mU][U][U][mC][C][A][A][G][A][A][mU][mA][A][G][C][mC][A][mU][A][G][mA][mU][mU] | 791 | 1619 |
| 139 | siRNA16-M0 | [mU][mA][mG][mG][mA][mG][mA][mA][G][G][C][A][A][mA][mC][mG][mG][mG][U][mG][mA] | 408 | 1207 | [mU][C][A][mC][C][C][G][U][U][U][mG][mC][C][U][U][mC][U][mC][C][U][mA][mU][mU] | 792 | 1620 |
| 141 | siRNA17-M0 | [mG][mG][mA][mG][mA][mA][mG][mG][C][A][A][A][C][mG][mG][mG][mU][mG][A][mA][mA] | 409 | 1208 | [mU][U][U][mC][A][C][C][C][G][U][mU][mU][G][C][C][mU][U][mC][U][C][mC][mU][mU] | 793 | 1621 |
| 142 | siRNA18-M0 | [mG][mA][mG][mA][mA][mG][mG][mC][A][A][A][C][G][mG][mG][mG][mU][mG][mA][A][mC][mA] | 410 | 1209 | [mU][G][U][mU][C][A][C][C][C][G][mU][mU][U][G][C][mC][U][mU][C][U][mC][mU][mU] | 794 | 1622 |
| 143 | siRNA19-M0 | [mA][mG][mA][mA][mG][mG][mC][mA][A][A][C][G][G][mG][mU][mG][mA][mA][C][mC][mA] | 411 | 1210 | [mU][G][G][mU][U][C][A][C][C][C][mG][mU][U][U][G][mC][C][mU][U][C][mU][mU][mU] | 795 | 1623 |
| 181 | siRNA20-M0 | [mA][mA][mU][mU][mC][mC][mA][mU][A][C][C][U][G][mG][mG][mC][mU][mG][U][mG][mA] | 412 | 1211 | [mU][C][A][mC][A][G][C][C][C][A][mG][mG][U][A][U][mG][G][mA][A][U][mU][mU][mU] | 796 | 1624 |
| 182 | siRNA21-M0 | [mA][mU][mU][mC][mC][mA][mU][mA][C][C][U][G][G][mG][mC][mU][mG][mU][G][mC][mA] | 413 | 1212 | [mU][G][C][mA][C][A][G][C][C][C][mA][mG][G][U][A][mU][G][mG][A][A][mU][mU][mU] | 797 | 1625 |
| 183 | siRNA22-M0 | [mU][mU][mC][mC][mA][mU][mA][mC][C][U][G][G][G][mC][mU][mG][mU][mG][C][mU][mA] | 414 | 1213 | [mU][A][G][mC][A][C][A][G][C][C][mC][mA][G][G][U][mA][U][mG][G][A][mA][mU][mU] | 798 | 1626 |
| 201 | siRNA23-M0 | [mC][mU][mC][mU][mG][mC][mA][mA][U][U][U][G][G][mU][mG][mC][mC][mA][A][mU][mA] | 415 | 1214 | [mU][A][U][mU][G][G][C][A][C][C][mA][mA][A][U][U][mG][C][mA][G][A][mG][mU][mU] | 799 | 1627 |
| 203 | siRNA24-M0 | [mC][mU][mG][mC][mA][mA][mU][mU][U][G][G][U][G][mC][mC][mA][mA][mU][C][mC][mA] | 416 | 1215 | [mU][G][G][mA][U][U][G][G][C][A][mC][mC][A][A][A][mU][U][mG][C][A][mG][mU][mU] | 800 | 1628 |
| 210 | siRNA25-M0 | [mU][mU][mG][mG][mU][mG][mC][mC][A][A][U][C][C][mU][mC][mU][mU][mG][A][mG][mA] | 417 | 1216 | [mU][C][U][mC][A][A][G][A][G][G][mA][mU][U][G][G][mC][A][mC][C][A][mA][mU][mU] | 801 | 1629 |
| 214 | siRNA26-M0 | [mU][mG][mC][mC][mA][mA][mU][mC][C][U][C][U][U][mG][mA][mG][mU][mU][C][mC][mA] | 418 | 1217 | [mU][G][G][mA][A][C][U][C][A][A][mG][mA][G][G][A][mU][U][mG][G][C][mA][mU][mU] | 802 | 1630 |

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|---|---|---|---|---|---|---|---|
| 223 | siRNA27-M0 | [mU][mC][mU][mU][mG][mA][mG][mU][U][C][C][U][C][mA][mG][mA][mG][mC][A][mA][mA] | 419 | 1218 | [mU][U][U][mG][C][U][C][U][G][A][mG][mG][A][A][C][mU][C][mA][A][G][mA][mU][mU] | 803 | 1631 |
| 225 | siRNA28-M0 | [mU][mU][mG][mA][mG][mU][mU][mC][C][U][C][A][G][mA][mG][mC][mA][mA][A][mU][mA] | 420 | 1220 | [mU][A][U][mU][U][G][C][U][C][U][mG][mA][G][G][A][mA][C][mU][C][A][mA][mU][mU] | 804 | 1632 |
| 229 | siRNA29-M0 | [mG][mU][mU][mC][mC][mU][mC][mA][G][A][G][C][A][mA][mA][mU][mC][mA][A][mA][mA] | 421 | 1221 | [mU][U][U][mU][G][A][U][U][U][G][mC][mU][C][U][G][mA][G][mG][A][A][mC][mU][mU] | 805 | 1633 |
| 230 | siRNA30-M0 | [mU][mU][mC][mC][mU][mC][mA][mG][A][G][C][A][A][mA][mU][mC][mA][mA][A][mG][mA] | 422 | 1222 | [mU][C][U][mU][U][G][A][U][U][U][mG][mC][U][C][U][mG][A][mG][G][A][mA][mU][mU] | 806 | 1634 |
| 236 | siRNA31-M0 | [mA][mG][mA][mG][mC][mA][mA][mA][U][C][A][A][A][mG][mG][mA][mA][mA][C][mA][mA] | 423 | 1223 | [mU][U][G][mU][U][U][C][C][U][U][mU][mG][A][U][U][mU][G][mC][U][C][mU][mU][mU] | 807 | 1635 |
| 238 | siRNA32-M0 | [mA][mG][mC][mA][mA][mA][mU][mC][A][A][A][G][G][mA][mA][mA][mC][mA][U][mG][mA] | 424 | 1224 | [mU][C][A][mU][G][U][U][U][C][C][mU][mU][U][G][A][mU][U][mU][G][C][mU][mU][mU] | 808 | 1636 |
| 242 | siRNA33-M0 | [mA][mA][mU][mC][mA][mA][mA][mG][G][A][A][A][C][mA][mU][mG][mG][mU][C][mA][mA] | 425 | 1225 | [mU][U][G][mA][C][C][A][U][G][U][mU][mU][C][C][U][mU][U][mG][A][U][mU][mU][mU] | 809 | 1637 |
| 243 | siRNA34-M0 | [mA][mU][mC][mA][mA][mA][mG][mG][A][A][A][C][A][mU][mG][mG][mU][mC][A][mU][mA] | 426 | 1226 | [mU][A][U][mG][A][C][C][A][U][G][mU][mU][U][C][C][mU][U][mU][G][A][mU][mU][mU] | 810 | 1638 |
| 244 | siRNA35-M0 | [mU][mC][mA][mA][mA][mG][mG][mA][A][A][C][A][U][mG][mG][mU][mC][mA][U][mG][mA] | 427 | 1227 | [mU][C][A][mU][G][A][C][C][A][U][mG][mU][U][U][C][mC][U][mU][U][G][mA][mU][mU] | 811 | 1639 |
| 245 | siRNA36-M0 | [mC][mA][mA][mA][mG][mG][mA][mA][A][C][A][U][G][mG][mU][mC][mA][mU][G][mU][mA] | 428 | 1228 | [mU][A][C][mA][U][G][A][C][C][A][mU][mG][U][U][U][mC][C][mU][U][U][mG][mU][mU] | 812 | 1640 |
| 246 | siRNA37-M0 | [mA][mA][mA][mG][mG][mA][mA][mA][C][A][U][G][G][mU][mC][mA][mU][mG][U][mU][mA] | 429 | 1229 | [mU][A][A][mC][A][U][G][A][C][C][mA][mU][G][U][U][mU][C][mC][U][U][mU][mU][mU] | 813 | 1641 |
| 251 | siRNA38-M0 | [mA][mA][mA][mC][mA][mU][mG][mG][U][C][A][U][G][mU][mU][mU][mU][mU][A][mC][mA] | 430 | 1230 | [mU][G][U][mA][A][A][A][A][C][A][mU][mG][A][C][C][mA][U][mG][U][U][mU][mU][mU] | 814 | 1642 |
| 253 | siRNA39-M0 | [mA][mC][mA][mU][mG][mG][mU][mC][A][U][G][U][U][mU][mU][mU][mA][mC][C][mU][mA] | 431 | 1232 | [mU][A][G][mG][U][A][A][A][A][A][mC][mA][U][G][A][mC][C][mA][U][G][mU][mU][mU] | 815 | 1643 |
| 254 | siRNA40-M0 | [mC][mA][mU][mG][mG][mU][mC][mA][U][G][U][U][U][mU][mU][mU][mA][mC][mC][U][mG][mA] | 432 | 1233 | [mU][C][A][mG][G][U][A][A][A][A][mA][mC][A][U][G][mA][C][mC][A][U][mG][mU][mU] | 816 | 1644 |
| 262 | siRNA41-M0 | [mU][mG][mU][mU][mU][mU][mU][mA][C][C][U][G][C][mA][mA][mA][mC][mU][A][mA][mA] | 433 | 1234 | [mU][U][U][mA][G][U][U][U][G][C][mA][mG][G][U][A][mA][A][mA][A][C][mA][mU][mU] | 817 | 1645 |
| 264 | siRNA42-M0 | [mU][mU][mU][mU][mU][mA][mC][mC][U][G][C][A][A] | 434 | 1235 | [mU][C][A][mU][U][A][G][U][U][U][mG][mC][A][ | 818 | 1646 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | [mA][mC][mU][mA][mA][U][mG][mA] | | | G][G][mU][A][mA][A][A][mA][mU][mU] | | |
| 266 | siRNA43-M0 | [mU][mU][mU][mA][mC][mC][mU][mG][C][A][A][A][C][mU][mA][mA][mU][mG][G][mG][mA] | 435 | 1236 | [mU][C][C][mC][A][U][U][A][G][U][mU][mU][G][C][A][mG][G][mU][A][A][mA][mU][mU] | 819 | 1647 |
| 267 | siRNA44-M0 | [mU][mU][mA][mC][mC][mU][mG][mC][A][A][A][C][U][mA][mA][mU][mG][mG][G][mA][mA] | 436 | 1237 | [mU][U][C][mC][C][A][U][U][A][G][mU][mU][U][G][C][mA][G][mG][U][A][mA][mU][mU] | 820 | 1648 |
| 268 | siRNA45-M0 | [mU][mA][mC][mC][mU][mG][mC][mA][A][A][C][U][A][mA][mU][mG][mG][mG][A][mA][mA] | 437 | 1238 | [mU][U][U][mC][C][C][A][U][U][A][mG][mU][U][U][G][mC][A][mG][G][U][mA][mU][mU] | 821 | 1649 |
| 291 | siRNA46-M0 | [mA][mU][mG][mU][mC][mC][mA][mU][U][U][C][A][U][mC][mA][mC][mA][mA][A][mU][mA] | 438 | 1239 | [mU][A][U][mU][U][G][U][G][A][U][mG][mA][A][A][U][mG][G][mA][C][A][mU][mU][mU] | 822 | 1650 |
| 292 | siRNA47-M0 | [mU][mG][mU][mC][mC][mA][mU][mU][U][C][A][U][C][mA][mC][mA][mA][mA][U][mC][mA] | 439 | 1240 | [mU][G][A][mU][U][U][G][U][G][A][mU][mG][A][A][A][mU][G][mG][A][C][mA][mU][mU] | 823 | 1651 |
| 294 | siRNA48-M0 | [mU][mC][mC][mA][mU][mU][mU][mC][A][U][C][A][C][mA][mA][mA][mU][mC][C][mC][mA] | 440 | 1242 | [mU][G][G][mG][A][U][U][U][G][U][mG][mA][U][G][A][mA][A][mU][G][G][mA][mU][mU] | 824 | 1652 |
| 300 | siRNA49-M0 | [mU][mC][mA][mU][mC][mA][mC][mA][A][A][U][C][C][mC][mU][mU][mG][mU][C][mA][mA] | 441 | 1243 | [mU][U][G][mA][C][A][A][G][G][G][mA][mU][U][U][G][mU][G][mA][U][G][mA][mU][mU] | 825 | 1653 |
| 302 | siRNA50-M0 | [mA][mU][mC][mA][mC][mA][mA][mA][U][C][C][C][U][mU][mG][mU][mC][mA][U][mA][mA] | 442 | 1244 | [mU][U][A][mU][G][A][C][A][A][G][mG][mG][A][U][U][mU][G][mU][G][A][mU][mU][mU] | 826 | 1654 |
| 303 | siRNA51-M0 | [mU][mC][mA][mC][mA][mA][mA][mU][C][C][C][U][U][mG][mU][mC][mA][mU][A][mC][mA] | 443 | 1245 | [mU][G][U][mA][U][G][A][C][A][A][mG][mG][G][A][U][mU][U][mG][U][G][mA][mU][mU] | 827 | 1655 |
| 304 | siRNA52-M0 | [mC][mA][mC][mA][mA][mA][mU][mC][C][C][U][U][G][mU][mC][mA][mU][mA][C][mC][mA] | 444 | 1247 | [mU][G][G][mU][A][U][G][A][C][A][mA][mG][G][G][A][mU][U][mU][G][U][mG][mU][mU] | 828 | 1656 |
| 305 | siRNA53-M0 | [mA][mC][mA][mA][mA][mU][mC][mC][C][U][U][G][U][mC][mA][mU][mA][mC][C][mA][mA] | 445 | 1248 | [mU][U][G][mG][U][A][U][G][A][C][mA][mA][G][G][G][mA][U][mU][U][G][mU][mU][mU] | 829 | 1657 |
| 309 | siRNA54-M0 | [mA][mU][mC][mC][mC][mU][mU][mG][U][C][A][U][A][mC][mC][mA][mU][mA][A][mG][mA] | 446 | 1249 | [mU][C][U][mU][A][U][G][G][U][A][mU][mG][A][C][A][mA][G][mG][G][A][mU][mU][mU] | 830 | 1658 |
| 310 | siRNA55-M0 | [mU][mC][mC][mC][mU][mU][mG][mU][C][A][U][A][C][mC][mA][mU][mA][mA][G][mG][mA] | 447 | 1250 | [mU][C][C][mU][U][A][U][G][G][U][mA][mU][G][A][C][mA][A][mG][G][G][mA][mU][mU] | 831 | 1659 |
| 311 | siRNA56-M0 | [mC][mC][mC][mU][mU][mG][mU][mC][A][U][A][C][C][mA][mU][mA][mA][mG][G][mU][mA] | 448 | 1251 | [mU][A][C][mC][U][U][A][U][G][G][mU][mA][U][G][A][mC][A][mA][G][G][mG][mU][mU] | 832 | 1660 |
| 312 | siRNA57-M0 | [mC][mC][mU][mU][mG][mU][mC][mA][U][A][C][C][A][mU][mA][mA][mG][mG][U][mG][mA] | 449 | 1252 | [mU][C][A][mC][C][U][U][A][U][G][mG][mU][A][U][G][mA][C][mA][A][G][mG][mU][mU] | 833 | 1661 |

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|---|---|---|---|---|---|---|---|
| 313 | siRNA58-M0 | [mC][mU][mU][mG][mU][mC][mA][mU][A][C][C][A][U][mA][mA][mG][mG][mU][G][mU][mA] | 450 | 1253 | [mU][A][C][mA][C][C][U][U][A][U][mG][mG][U][A][U][mG][A][mC][A][A][mG][mU][mU] | 834 | 1662 |
| 315 | siRNA59-M0 | [mU][mG][mU][mC][mA][mU][mA][mC][C][A][U][A][A][mG][mG][mU][mG][mU][U][mG][mA] | 451 | 1254 | [mU][C][A][mA][C][A][C][C][U][U][mA][mU][G][G][U][mA][U][mG][A][C][mA][mU][mU] | 835 | 1663 |
| 319 | siRNA60-M0 | [mA][mU][mA][mC][mC][mA][mU][mA][A][G][G][U][G][mU][mU][mG][mU][mG][C][mC][mA] | 452 | 1255 | [mU][G][G][mC][A][C][A][A][C][A][mC][mC][U][U][A][mU][G][mG][U][A][mU][mU][mU] | 836 | 1664 |
| 360 | siRNA61-M0 | [mA][mA][mA][mA][mA][mU][mU][mU][C][A][C][U][U][mU][mG][mC][mU][mA][C][mU][mA] | 453 | 1256 | [mU][A][G][mU][A][G][C][A][A][A][mG][mU][G][A][A][mA][U][mU][U][U][mU][mU][mU] | 837 | 1665 |
| 363 | siRNA62-M0 | [mA][mA][mU][mU][mU][mC][mA][mC][U][U][U][G][C][mU][mA][mC][mU][mU][C][mU][mA] | 454 | 1257 | [mU][A][G][mA][A][G][U][A][G][C][mA][mA][A][G][U][mG][A][mA][A][U][mU][mU][mU] | 838 | 1666 |
| 372 | siRNA63-M0 | [mU][mU][mG][mC][mU][mA][mC][mU][U][C][U][G][C][mG][mA][mA][mG][mG][C][mA][mA] | 455 | 1258 | [mU][U][G][mC][C][U][U][C][G][C][mA][mG][A][A][G][mU][A][mG][C][A][mA][mU][mU] | 839 | 1667 |
| 375 | siRNA64-M0 | [mC][mU][mA][mC][mU][mU][mC][mU][G][C][G][A][A][mG][mG][mC][mA][mU][U][mU][mA] | 456 | 1259 | [mU][A][A][mA][U][G][C][C][U][U][mC][mG][C][A][G][mA][A][mG][U][A][mG][mU][mU] | 840 | 1668 |
| 377 | siRNA65-M0 | [mA][mC][mU][mU][mC][mU][mG][mC][G][A][A][G][G][mC][mA][mU][mU][mU][G][mG][mA] | 457 | 1260 | [mU][C][C][mA][A][A][U][G][C][C][mU][mU][C][G][C][mA][G][mA][A][G][mU][mU][mU] | 841 | 1669 |
| 390 | siRNA66-M0 | [mC][mA][mU][mU][mU][mG][mG][mG][C][A][C][A][G][mA][mA][mG][mC][mA][U][mU][mA] | 458 | 1261 | [mU][A][A][mU][G][C][U][U][C][U][mG][mU][G][C][C][mC][A][mA][A][U][mG][mU][mU] | 842 | 1670 |
| 392 | siRNA67-M0 | [mU][mU][mU][mG][mG][mG][mC][mA][C][A][G][A][A][mG][mC][mA][mU][mU][G][mA][mA] | 459 | 1262 | [mU][U][C][mA][A][U][G][C][U][U][mC][mU][G][U][G][mC][C][mC][A][A][mA][mU][mU] | 843 | 1671 |
| 394 | siRNA68-M0 | [mU][mG][mG][mG][mC][mA][mC][mA][G][A][A][G][C][mA][mU][mU][mG][mA][C][mC][mA] | 460 | 1263 | [mU][G][G][mU][C][A][A][U][G][C][mU][mU][C][U][G][mU][G][mC][C][C][mA][mU][mU] | 844 | 1672 |
| 474 | siRNA69-M0 | [mA][mU][mG][mC][mC][mU][mU][mG][A][A][U][U][C][mC][mC][mU][mC][mA][C][mG][mA] | 461 | 1264 | [mU][C][G][mU][G][A][G][G][G][A][mA][mU][U][C][A][mA][G][mG][C][A][mU][mU][mU] | 845 | 1673 |
| 475 | siRNA70-M0 | [mU][mG][mC][mC][mU][mU][mG][mA][A][U][U][C][C][mC][mU][mC][mA][mC][G][mG][mA] | 462 | 1265 | [mU][C][C][mG][U][G][A][G][G][G][mA][mA][U][U][C][mA][A][mG][G][C][mA][mU][mU] | 846 | 1674 |
| 476 | siRNA71-M0 | [mG][mC][mC][mU][mU][mG][mA][mA][U][U][C][C][C][mU][mC][mA][mC][mG][G][mA][mA] | 463 | 1266 | [mU][U][C][mC][G][U][G][A][G][G][mG][mA][A][U][U][mC][A][mA][G][G][mC][mU][mU] | 847 | 1675 |
| 477 | siRNA72-M0 | [mC][mC][mU][mU][mG][mA][mA][mU][U][C][C][C][U][mC][mA][mC][mG][mG][A][mA][mA] | 464 | 1268 | [mU][U][U][mC][C][G][U][G][A][G][mG][mG][A][A][U][mU][C][mA][A][G][mG][mU][mU] | 848 | 1676 |
| 478 | siRNA73-M0 | [mC][mU][mU][mG][mA][mA][mU][mU][C][C][C][U][C] | 465 | 1269 | [mU][U][U][mU][C][C][G][U][G][A][mG][mG][G][ | 849 | 1677 |

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|---|---|---|---|---|---|---|---|
| | | [mA][mC][mG][mG][mA][A][mA][mA] | | | A][A][mU][U][mC][A][A][mG][mU][mU] | | |
| 482 | siRNA74-M0 | [mA][mA][mU][mU][mC][mC][mC][mU][C][A][C][G][G][mA][mA][mA][mG][mC][A][mU][mA] | 466 | 1271 | [mU][A][U][mG][C][U][U][U][C][C][mG][mU][G][A][G][mG][G][mA][A][U][mU][mU][mU] | 850 | 1678 |
| 484 | siRNA75-M0 | [mU][mU][mC][mC][mC][mU][mC][mA][C][G][G][A][A][mA][mG][mC][mA][mU][G][mA][mA] | 467 | 1272 | [mU][U][C][mA][U][G][C][U][U][U][mC][mC][G][U][G][mA][G][mG][G][A][mA][mU][mU] | 851 | 1679 |
| 486 | siRNA76-M0 | [mC][mC][mC][mU][mC][mA][mC][mG][G][A][A][A][G][mC][mA][mU][mG][mA][U][mG][mA] | 468 | 1273 | [mU][C][A][mU][C][A][U][G][C][U][mU][mU][C][C][G][mU][G][mA][G][G][mG][mU][mU] | 852 | 1680 |
| 488 | siRNA77-M0 | [mC][mU][mC][mA][mC][mG][mG][mA][A][A][G][C][A][mU][mG][mA][mU][mG][G][mA][mA] | 469 | 1274 | [mU][U][C][mC][A][U][C][A][U][G][mC][mU][U][U][C][mC][G][mU][G][A][mG][mU][mU] | 853 | 1681 |
| 501 | siRNA78-M0 | [mU][mG][mA][mU][mG][mG][mA][mA][A][A][C][C][U][mC][mC][mA][mA][mC][G][mU][mA] | 470 | 1275 | [mU][A][C][mG][U][U][G][G][A][G][mG][mU][U][U][U][mC][C][mA][U][C][mA][mU][mU] | 854 | 1682 |
| 503 | siRNA79-M0 | [mA][mU][mG][mG][mA][mA][mA][mA][C][C][U][C][C][mA][mA][mC][mG][mU][A][mU][mA] | 471 | 1276 | [mU][A][U][mA][C][G][U][U][G][G][mA][mG][G][U][U][mU][U][mC][C][A][mU][mU][mU] | 855 | 1683 |
| 505 | siRNA80-M0 | [mG][mG][mA][mA][mA][mA][mC][mC][U][C][C][A][A][mC][mG][mU][mA][mU][C][mA][mA] | 472 | 1277 | [mU][U][G][mA][U][A][C][G][U][U][mG][mG][A][G][G][mU][U][mU][U][C][mC][mU][mU] | 856 | 1684 |
| 506 | siRNA81-M0 | [mG][mA][mA][mA][mA][mC][mC][mU][C][C][A][A][C][mG][mU][mA][mU][mC][A][mU][mA] | 473 | 1278 | [mU][A][U][mG][A][U][A][C][G][U][mU][mG][G][A][G][mG][U][mU][U][U][mC][mU][mU] | 857 | 1685 |
| 507 | siRNA82-M0 | [mA][mA][mA][mA][mC][mC][mU][mC][C][A][A][C][G][mU][mA][mU][mC][mA][U][mG][mA] | 474 | 1280 | [mU][C][A][mU][G][A][U][A][C][G][mU][mU][G][G][A][mG][G][mU][U][U][mU][mU][mU] | 858 | 1686 |
| 509 | siRNA83-M0 | [mA][mA][mC][mC][mU][mC][mC][mA][A][C][G][U][A][mU][mC][mA][mU][mG][A][mG][mA] | 475 | 1281 | [mU][C][U][mC][A][U][G][A][U][A][mC][mG][U][U][G][mG][A][mG][G][U][mU][mU][mU] | 859 | 1687 |
| 510 | siRNA84-M0 | [mA][mC][mC][mU][mC][mC][mA][mA][C][G][U][A][U][mC][mA][mU][mG][mA][G][mA][mA] | 476 | 1282 | [mU][U][C][mU][C][A][U][G][A][U][mA][mC][G][U][U][mG][G][mA][G][G][mU][mU][mU] | 860 | 1688 |
| 511 | siRNA85-M0 | [mC][mC][mU][mC][mC][mA][mA][mC][G][U][A][U][C][mA][mU][mG][mA][mG][A][mC][mA] | 477 | 1283 | [mU][G][U][mC][U][C][A][U][G][A][mU][mA][C][G][U][mU][G][mG][A][G][mG][mU][mU] | 861 | 1689 |
| 512 | siRNA86-M0 | [mC][mU][mC][mC][mA][mA][mC][mG][U][A][U][C][A][mU][mG][mA][mG][mA][C][mC][mA] | 478 | 1284 | [mU][G][G][mU][C][U][C][A][U][G][mA][mU][A][C][G][mU][U][mG][G][A][mG][mU][mU] | 862 | 1690 |
| 513 | siRNA87-M0 | [mU][mC][mC][mA][mA][mC][mG][mU][A][U][C][A][U][mG][mA][mG][mA][mC][C][mU][mA] | 479 | 1285 | [mU][A][G][mG][U][C][U][C][A][U][mG][mA][U][A][C][mG][U][mU][G][G][mA][mU][mU] | 863 | 1691 |
| 514 | siRNA88-M0 | [mC][mC][mA][mA][mC][mG][mU][mA][U][C][A][U][G][mA][mG][mA][mC][mC][U][mC][mA] | 480 | 1286 | [mU][G][A][mG][G][U][C][U][C][A][mU][mG][A][U][A][mC][G][mU][U][G][mG][mU][mU] | 864 | 1692 |

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|---|---|---|---|---|---|---|---|
| 539 | siRNA89-M0 | [mU][mC][mC][mU][mC][mU][mA][mA][C][U][C][A][A][mA][mG][mA][mC][mC][G][mC][mA] | 481 | 1287 | [mU][G][C][mG][G][U][C][U][U][U][mG][mA][G][U][U][mA][G][mA][G][G][mA][mU][mU] | 865 | 1693 |
| 552 | siRNA90-M0 | [mA][mG][mA][mC][mC][mG][mC][mU][G][C][C][U][G][mG][mG][mU][mG][mA][C][mA][mA] | 482 | 1288 | [mU][U][G][mU][C][A][C][C][C][A][mG][mG][C][A][G][mC][G][mG][U][C][mU][mU][mU] | 866 | 1694 |
| 562 | siRNA91-M0 | [mC][mU][mG][mG][mG][mU][mG][mA][C][A][G][A][A][mG][mG][mG][mA][mU][G][mU][mA] | 483 | 1289 | [mU][A][C][mA][U][C][C][C][U][U][mC][mU][G][U][C][mA][C][mC][C][A][mG][mU][mU] | 867 | 1695 |
| 563 | siRNA92-M0 | [mU][mG][mG][mG][mU][mG][mA][mC][A][G][A][A][G][mG][mG][mA][mU][mG][U][mA][mA] | 484 | 1290 | [mU][U][A][mC][A][U][C][C][C][U][mU][mC][U][G][U][mC][A][mC][C][C][mA][mU][mU] | 868 | 1696 |
| 564 | siRNA93-M0 | [mG][mG][mG][mU][mG][mA][mC][mA][G][A][A][G][G][mG][mA][mU][mG][mU][A][mU][mA] | 485 | 1291 | [mU][A][U][mA][C][A][U][C][C][C][mU][mU][C][U][G][mU][C][mA][C][C][mC][mU][mU] | 869 | 1697 |
| 585 | siRNA94-M0 | [mC][mU][mU][mU][mC][mU][mG][mC][U][A][C][C][G][mA][mG][mU][mG][mA][U][mG][mA] | 486 | 1292 | [mU][C][A][mU][C][A][C][U][C][G][mG][mU][A][G][C][mA][G][mA][A][A][mG][mU][mU] | 870 | 1698 |
| 587 | siRNA95-M0 | [mU][mU][mC][mU][mG][mC][mU][mA][C][C][G][A][G][mU][mG][mA][mU][mG][U][mU][mA] | 487 | 1293 | [mU][A][A][mC][A][U][C][A][C][U][mC][mG][G][U][A][mG][C][mA][G][A][mA][mU][mU] | 871 | 1699 |
| 588 | siRNA96-M0 | [mU][mC][mU][mG][mC][mU][mA][mC][C][G][A][G][U][mG][mA][mU][mG][mU][U][mU][mA] | 488 | 1294 | [mU][A][A][mA][C][A][U][C][A][C][mU][mC][G][G][U][mA][G][mC][A][G][mA][mU][mU] | 872 | 1700 |
| 589 | siRNA97-M0 | [mC][mU][mG][mC][mU][mA][mC][mC][G][A][G][U][G][mA][mU][mG][mU][mU][U][mG][mA] | 489 | 1295 | [mU][C][A][mA][A][C][A][U][C][A][mC][mU][C][G][G][mU][A][mG][C][A][mG][mU][mU] | 873 | 1701 |
| 593 | siRNA98-M0 | [mU][mA][mC][mC][mG][mA][mG][mU][G][A][U][G][U][mU][mU][mG][mA][mA][G][mC][mA] | 490 | 1296 | [mU][G][C][mU][U][C][A][A][A][C][mA][mU][C][A][C][mU][C][mG][G][U][mA][mU][mU] | 874 | 1702 |
| 594 | siRNA99-M0 | [mA][mC][mC][mG][mA][mG][mU][mG][A][U][G][U][U][mU][mG][mA][mA][mG][C][mU][mA] | 491 | 1297 | [mU][A][G][mC][U][U][C][A][A][A][mC][mA][U][C][A][mC][U][mC][G][G][mU][mU][mU] | 875 | 1703 |
| 595 | siRNA100-M0 | [mC][mC][mG][mA][mG][mU][mG][mA][U][G][U][U][U][mG][mA][mA][mG][mC][U][mG][mA] | 492 | 1298 | [mU][C][A][mG][C][U][U][C][A][A][mA][mC][A][U][C][mA][C][mU][C][G][mG][mU][mU] | 876 | 1704 |
| 602 | siRNA101-M0 | [mA][mU][mG][mU][mU][mU][mG][mA][A][G][C][U][G][mG][mG][mU][mA][mU][U][mU][mA] | 493 | 1300 | [mU][A][A][mA][U][A][C][C][C][A][mG][mC][U][U][C][mA][A][mA][C][A][mU][mU][mU] | 877 | 1705 |
| 605 | siRNA102-M0 | [mU][mU][mU][mG][mA][mA][mG][mC][U][G][G][G][U][mA][mU][mU][mU][mA][A][mC][mA] | 494 | 1302 | [mU][G][U][mU][A][A][A][U][A][C][mC][mC][A][G][C][mU][U][mC][A][A][mA][mU][mU] | 878 | 1706 |
| 606 | siRNA103-M0 | [mU][mU][mG][mA][mA][mG][mC][mU][G][G][G][U][A][mU][mU][mU][mA][mA][C][mU][mA] | 495 | 1303 | [mU][A][G][mU][U][A][A][A][U][A][mC][mC][C][A][G][mC][U][mU][C][A][mA][mU][mU] | 879 | 1707 |
| 607 | siRNA104-M0 | [mU][mG][mA][mA][mG][mC][mU][mG][G][G][U][A][U] | 496 | 1304 | [mU][U][A][mG][U][U][A][A][A][U][mA][mC][C][ | 880 | 1708 |

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|---|---|---|---|---|---|---|---|
| | | [mU][mU][mA][mA][mC][U][mA][mA] | | | C][A][mG][C][mU][U][C][mA][mU][mU] | | |
| 608 | siRNA105-M0 | [mG][mA][mA][mG][mC][mU][mG][mG][G][U][A][U][U][mU][mA][mA][mC][mU][A][mU][mA] | 497 | 1305 | [mU][A][U][mA][G][U][U][A][A][A][mU][mA][C][C][C][mA][G][mC][U][U][mC][mU][mU] | 881 | 1709 |
| 609 | siRNA106-M0 | [mA][mA][mG][mC][mU][mG][mG][mG][U][A][U][U][U][mA][mA][mC][mU][mA][U][mC][mA] | 498 | 1306 | [mU][G][A][mU][A][G][U][U][A][A][mA][mU][A][C][C][mC][A][mG][C][U][mU][mU][mU] | 882 | 1710 |
| 610 | siRNA107-M0 | [mA][mG][mC][mU][mG][mG][mG][mU][A][U][U][U][A][mA][mC][mU][mA][mU][C][mU][mA] | 499 | 1307 | [mU][A][G][mA][U][A][G][U][U][A][mA][mA][U][A][C][mC][C][mA][G][C][mU][mU][mU] | 883 | 1711 |
| 612 | siRNA108-M0 | [mC][mU][mG][mG][mG][mU][mA][mU][U][U][A][A][C][mU][mA][mU][mC][mU][U][mU][mA] | 500 | 1308 | [mU][A][A][mA][G][A][U][A][G][U][mU][mA][A][A][U][mA][C][mC][C][A][mG][mU][mU] | 884 | 1712 |
| 613 | siRNA109-M0 | [mU][mG][mG][mG][mU][mA][mU][mU][U][A][A][C][U][mA][mU][mC][mU][mU][U][mG][mA] | 501 | 1309 | [mU][C][A][mA][A][G][A][U][A][G][mU][mU][A][A][A][mU][A][mC][C][C][mA][mU][mU] | 885 | 1713 |
| 653 | siRNA110-M0 | [mG][mA][mC][mA][mC][mA][mC][mA][G][A][A][A][G][mC][mA][mC][mA][mU][A][mU][mA] | 502 | 1310 | [mU][A][U][mA][U][G][U][G][C][U][mU][mU][C][U][G][mU][G][mU][G][U][mC][mU][mU] | 886 | 1714 |
| 663 | siRNA111-M0 | [mA][mA][mG][mC][mA][mC][mA][mU][A][U][U][C][U][mA][mA][mA][mC][mA][A][mU][mA] | 503 | 1311 | [mU][A][U][mU][G][U][U][U][A][G][mA][mA][U][A][U][mG][U][mG][C][U][mU][mU][mU] | 887 | 1715 |
| 665 | siRNA112-M0 | [mG][mC][mA][mC][mA][mU][mA][mU][U][C][U][A][A][mA][mC][mA][mA][mU][C][mU][mA] | 504 | 1312 | [mU][A][G][mA][U][U][G][U][U][U][mA][mG][A][A][U][mA][U][mG][U][G][mC][mU][mU] | 888 | 1716 |
| 666 | siRNA113-M0 | [mC][mA][mC][mA][mU][mA][mU][mU][C][U][A][A][A][mC][mA][mA][mU][mC][U][mU][mA] | 505 | 1313 | [mU][A][A][mG][A][U][U][G][U][U][mU][mA][G][A][A][mU][A][mU][G][U][mG][mU][mU] | 889 | 1717 |
| 667 | siRNA114-M0 | [mA][mC][mA][mU][mA][mU][mU][mC][U][A][A][A][C][mA][mA][mU][mC][mU][U][mG][mA] | 506 | 1314 | [mU][C][A][mA][G][A][U][U][G][U][mU][mU][A][G][A][mA][U][mA][U][G][mU][mU][mU] | 890 | 1718 |
| 669 | siRNA115-M0 | [mA][mU][mA][mU][mU][mC][mU][mA][A][A][C][A][A][mU][mC][mU][mU][mG][A][mC][mA] | 507 | 1315 | [mU][G][U][mC][A][A][G][A][U][U][mG][mU][U][U][A][mG][A][mA][U][A][mU][mU][mU] | 891 | 1719 |
| 670 | siRNA116-M0 | [mU][mA][mU][mU][mC][mU][mA][mA][A][C][A][A][U][mC][mU][mU][mG][mA][C][mA][mA] | 508 | 1316 | [mU][U][G][mU][C][A][A][G][A][U][mU][mG][U][U][U][mA][G][mA][A][U][mA][mU][mU] | 892 | 1720 |
| 671 | siRNA117-M0 | [mA][mU][mU][mC][mU][mA][mA][mA][C][A][A][U][C][mU][mU][mG][mA][mC][A][mA][mA] | 509 | 1317 | [mU][U][U][mG][U][C][A][A][G][A][mU][mU][G][U][U][mU][A][mG][A][A][mU][mU][mU] | 893 | 1721 |
| 673 | siRNA118-M0 | [mU][mC][mU][mA][mA][mA][mC][mA][A][U][C][U][U][mG][mA][mC][mA][mA][C][mU][mA] | 510 | 1318 | [mU][A][G][mU][U][G][U][C][A][A][mG][mA][U][U][G][mU][U][mU][A][G][mA][mU][mU] | 894 | 1722 |
| 679 | siRNA119-M0 | [mC][mA][mA][mU][mC][mU][mU][mG][A][C][A][A][C][mU][mU][mC][mA][mA][G][mC][mA] | 511 | 1320 | [mU][G][C][mU][U][G][A][A][G][U][mU][mG][U][C][A][mA][G][mA][U][U][mG][mU][mU] | 895 | 1723 |

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|---|---|---|---|---|---|---|---|
| 712 | siRNA120-M0 | [mC][mU][mU][mU][mC][mC][mA][mG][C][C][C][U][G][mG][mU][mA][mG][mC][A][mG][mA] | 512 | 1321 | [mU][C][U][mG][C][U][A][C][C][A][mG][mG][G][C][U][mG][G][mA][A][A][mG][mU][mU] | 896 | 1724 |
| 771 | siRNA121-M0 | [mG][mG][mG][mA][mG][mA][mA][mA][C][U][G][G][C][mA][mG][mA][mG][mA][G][mC][mA] | 513 | 1322 | [mU][G][C][mU][C][U][C][U][G][C][mC][mA][G][U][U][mU][C][mU][C][C][mC][mU][mU] | 897 | 1725 |
| 781 | siRNA122-M0 | [mG][mG][mC][mA][mG][mA][mG][mA][G][C][U][U][G][mA][mG][mG][mC][mA][C][mG][mA] | 514 | 1323 | [mU][C][G][mU][G][C][C][U][C][A][mA][mG][C][U][C][mU][C][mU][G][C][mC][mU][mU] | 898 | 1726 |
| 788 | siRNA123-M0 | [mA][mG][mC][mU][mU][mG][mA][mG][G][C][A][C][G][mA][mG][mA][mA][mC][C][mU][mA] | 515 | 1325 | [mU][A][G][mG][U][U][C][U][C][G][mU][mG][C][C][U][mC][A][mA][G][C][mU][mU][mU] | 899 | 1727 |
| 841 | siRNA124-M0 | [mC][mC][mU][mG][mC][mG][mC][mA][U][G][U][U][U][mC][mU][mC][mA][mA][U][mG][mA] | 516 | 1326 | [mU][C][A][mU][U][G][A][G][A][A][mA][mC][A][U][G][mC][G][mC][A][G][mG][mU][mU] | 900 | 1728 |
| 844 | siRNA125-M0 | [mG][mC][mG][mC][mA][mU][mG][mU][U][U][C][U][C][mA][mA][mU][mG][mA][C][mA][mA] | 517 | 1328 | [mU][U][G][mU][C][A][U][U][G][A][mG][mA][A][A][C][mA][U][mG][C][G][mC][mU][mU] | 901 | 1729 |
| 846 | siRNA126-M0 | [mG][mC][mA][mU][mG][mU][mU][mU][C][U][C][A][A][mU][mG][mA][mC][mA][C][mU][mA] | 518 | 1330 | [mU][A][G][mU][G][U][C][A][U][U][mG][mA][G][A][A][mA][C][mA][U][G][mC][mU][mU] | 902 | 1730 |
| 850 | siRNA127-M0 | [mG][mU][mU][mU][mC][mU][mC][mA][A][U][G][A][C][mA][mC][mU][mU][mU][G][mU][mA] | 519 | 1331 | [mU][A][C][mA][A][A][G][U][G][U][mC][mA][U][U][G][mA][G][mA][A][A][mC][mU][mU] | 903 | 1731 |
| 851 | siRNA128-M0 | [mU][mU][mU][mC][mU][mC][mA][mA][U][G][A][C][A][mC][mU][mU][mU][mG][U][mC][mA] | 520 | 1332 | [mU][G][A][mC][A][A][A][G][U][G][mU][mC][A][U][U][mG][A][mG][A][A][mA][mU][mU] | 904 | 1732 |
| 852 | siRNA129-M0 | [mU][mU][mC][mU][mC][mA][mA][mU][G][A][C][A][C][mU][mU][mU][mG][mU][C][mC][mA] | 521 | 1333 | [mU][G][G][mA][C][A][A][A][G][U][mG][mU][C][A][U][mU][G][mA][G][A][mA][mU][mU] | 905 | 1733 |
| 861 | siRNA130-M0 | [mA][mC][mA][mC][mU][mU][mU][mG][U][C][C][A][C][mC][mU][mU][mU][mG][A][mU][mA] | 522 | 1334 | [mU][A][U][mC][A][A][A][G][G][U][mG][mG][A][C][A][mA][A][mG][U][G][mU][mU][mU] | 906 | 1734 |
| 862 | siRNA131-M0 | [mC][mA][mC][mU][mU][mU][mG][mU][C][C][A][C][C][mU][mU][mU][mG][mA][U][mG][mA] | 523 | 1335 | [mU][C][A][mU][C][A][A][A][G][G][mU][mG][G][A][C][mA][A][mA][G][U][mG][mU][mU] | 907 | 1735 |
| 901 | siRNA132-M0 | [mA][mC][mA][mC][mC][mU][mC][mG][U][G][G][U][C][mC][mU][mC][mU][mG][G][mG][mA] | 524 | 1336 | [mU][C][C][mC][A][G][A][G][G][A][mC][mC][A][C][G][mA][G][mG][U][G][mU][mU][mU] | 908 | 1736 |
| 903 | siRNA133-M0 | [mA][mC][mC][mU][mC][mG][mU][mG][G][U][C][C][U][mC][mU][mG][mG][mG][C][mA][mA] | 525 | 1337 | [mU][U][G][mC][C][C][A][G][A][G][mG][mA][C][C][A][mC][G][mA][G][G][mU][mU][mU] | 909 | 1737 |
| 904 | siRNA134-M0 | [mC][mC][mU][mC][mG][mU][mG][mG][U][C][C][U][C][mU][mG][mG][mG][mC][A][mU][mA] | 526 | 1338 | [mU][A][U][mG][C][C][C][A][G][A][mG][mG][A][C][C][mA][C][mG][A][G][mG][mU][mU] | 910 | 1738 |
| 905 | siRNA135-M0 | [mC][mU][mC][mG][mU][mG][mG][mU][C][C][U][C][U] | 527 | 1339 | [mU][G][A][mU][G][C][C][C][A][G][mA][mG][G][ | 911 | 1739 |

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|---|---|---|---|---|---|---|---|
| | | [mG][mG][mG][mC][mA][U][mC][mA] | | | A][C][mC][A][mC][G][A][mG][mU][mU] | | |
| 906 | siRNA136-M0 | [mU][mC][mG][mU][mG][mG][mU][mC][C][U][C][U][G][mG][mG][mC][mA][mU][C][mG][mA] | 528 | 1340 | [mU][C][G][mA][U][G][C][C][C][A][mG][mA][G][G][A][mC][C][mA][C][G][mA][mU][mU] | 912 | 1740 |
| 912 | siRNA137-M0 | [mU][mC][mC][mU][mC][mU][mG][mG][G][C][A][U][C][mG][mC][mA][mA][mG][C][mA][mA] | 529 | 1341 | [mU][U][G][mC][U][U][G][C][G][A][mU][mG][C][C][C][mA][G][mA][G][G][mA][mU][mU] | 913 | 1741 |
| 915 | siRNA138-M0 | [mU][mC][mU][mG][mG][mG][mC][mA][U][C][G][C][A][mA][mG][mC][mA][mA][A][mC][mA] | 530 | 1342 | [mU][G][U][mU][U][G][C][U][U][G][mC][mG][A][U][G][mC][C][mC][A][G][mA][mU][mU] | 914 | 1742 |
| 916 | siRNA139-M0 | [mC][mU][mG][mG][mG][mC][mA][mU][C][G][C][A][A][mG][mC][mA][mA][mA][C][mA][mA] | 531 | 1343 | [mU][U][G][mU][U][U][G][C][U][U][mG][mC][G][A][U][mG][C][mC][C][A][mG][mU][mU] | 915 | 1743 |
| 917 | siRNA140-M0 | [mU][mG][mG][mG][mC][mA][mU][mC][G][C][A][A][G][mC][mA][mA][mA][mC][A][mC][mA] | 532 | 1344 | [mU][G][U][mG][U][U][U][G][C][U][mU][mG][C][G][A][mU][G][mC][C][C][mA][mU][mU] | 916 | 1744 |
| 920 | siRNA141-M0 | [mG][mC][mA][mU][mC][mG][mC][mA][A][G][C][A][A][mA][mC][mA][mC][mC][C][A][mU][mA] | 533 | 1345 | [mU][A][U][mG][G][U][G][U][U][U][mG][mC][U][U][G][mC][G][mA][U][G][mC][mU][mU] | 917 | 1745 |
| 921 | siRNA142-M0 | [mC][mA][mU][mC][mG][mC][mA][mA][G][C][A][A][A][mC][mA][mC][mC][mA][U][mU][mA] | 534 | 1346 | [mU][A][A][mU][G][G][U][G][U][U][mU][mG][C][U][U][mG][C][mG][A][U][mG][mU][mU] | 918 | 1746 |
| 950 | siRNA143-M0 | [mU][mU][mC][mU][mG][mG][mA][mG][U][U][U][A][U][mU][mU][mC][mA][mA][A][mU][mA] | 535 | 1347 | [mU][A][U][mU][U][G][A][A][A][U][mA][mA][A][C][U][mC][C][mA][G][A][mA][mU][mU] | 919 | 1747 |
| 954 | siRNA144-M0 | [mG][mG][mA][mG][mU][mU][mU][mU][mA][U][U][U][C][A][mA][mA][mU][mG][mA][U][mU][mA] | 536 | 1348 | [mU][A][A][mU][C][A][U][U][U][G][mA][mA][A][U][A][mA][A][mC][U][C][mC][mU][mU] | 920 | 1748 |
| 955 | siRNA145-M0 | [mG][mA][mG][mU][mU][mU][mA][mU][U][U][C][A][A][mA][mU][mG][mA][mU][U][mA][mA] | 537 | 1349 | [mU][U][A][mA][U][C][A][U][U][U][mG][mA][A][A][U][mA][A][mA][C][U][mC][mU][mU] | 921 | 1749 |
| 956 | siRNA146-M0 | [mA][mG][mU][mU][mU][mA][mU][mU][U][C][A][A][A][mU][mG][mA][mU][mU][A][mG][mA] | 538 | 1350 | [mU][C][U][mA][A][U][C][A][U][U][mU][mG][A][A][A][mU][A][mA][A][C][mU][mU][mU] | 922 | 1750 |
| 957 | siRNA147-M0 | [mG][mU][mU][mU][mA][mU][mU][mU][C][A][A][A][U][mG][mA][mU][mU][mA][G][mG][mA] | 539 | 1351 | [mU][C][C][mU][A][A][U][C][A][U][mU][mU][G][A][A][mA][U][mA][A][A][mC][mU][mU] | 923 | 1751 |
| 980 | siRNA148-M0 | [mC][mC][mA][mG][mA][mA][mG][mC][A][A][U][G][A][mA][mA][mG][mC][mA][G][mC][mA] | 540 | 1352 | [mU][G][C][mU][G][C][U][U][U][C][mA][mU][U][G][C][mU][U][mC][U][G][mG][mU][mU] | 924 | 1752 |
| 982 | siRNA149-M0 | [mA][mG][mA][mA][mG][mC][mA][mA][U][G][A][A][A][mG][mC][mA][mG][mC][U][mA][mA] | 541 | 1353 | [mU][U][A][mG][C][U][G][C][U][U][mU][mC][A][U][U][mG][C][mU][U][C][mU][mU][mU] | 925 | 1753 |
| 983 | siRNA150-M0 | [mG][mA][mA][mG][mC][mA][mA][mU][G][A][A][A][G][mC][mA][mG][mC][mU][A][mC][mA] | 542 | 1354 | [mU][G][U][mA][G][C][U][G][C][U][mU][mU][C][A][U][mU][G][mC][U][U][mC][mU][mU] | 926 | 1754 |

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|---|---|---|---|---|---|---|---|
| 988 | siRNA151-M0 | [mA][mA][mU][mG][mA][mA][mA][mG][C][A][G][C][U][mA][mC][mU][mG][mA][A][mG][mA] | 543 | 1355 | [mU][C][U][mU][C][A][G][U][A][G][mC][mU][G][C][U][mU][U][mC][A][U][mU][mU][mU] | 927 | 1755 |
| 989 | siRNA152-M0 | [mA][mU][mG][mA][mA][mA][mG][mC][A][G][C][U][A][mC][mU][mG][mA][mA][G][mA][mA] | 544 | 1356 | [mU][U][C][mU][U][C][A][G][U][A][mG][mC][U][G][C][mU][U][mU][C][A][mU][mU][mU] | 928 | 1756 |
| 992 | siRNA153-M0 | [mA][mA][mA][mG][mC][mA][mG][mC][U][A][C][U][G][mA][mA][mG][mA][mA][G][mU][mA] | 545 | 1357 | [mU][A][C][mU][U][C][U][U][C][A][mG][mU][A][G][C][mU][G][mC][U][U][mU][mU][mU] | 929 | 1757 |
| 993 | siRNA154-M0 | [mA][mA][mG][mC][mA][mG][mC][mU][A][C][U][G][A][mA][mG][mA][mA][mG][U][mG][mA] | 546 | 1358 | [mU][C][A][mC][U][U][C][U][U][C][mA][mG][U][A][G][mC][U][mG][C][U][mU][mU][mU] | 930 | 1758 |
| 994 | siRNA155-M0 | [mA][mG][mC][mA][mG][mC][mU][mA][C][U][G][A][A][mG][mA][mA][mG][mU][G][mA][mA] | 547 | 1359 | [mU][U][C][mA][C][U][U][C][U][U][mC][mA][G][U][A][mG][C][mU][G][C][mU][mU][mU] | 931 | 1759 |
| 995 | siRNA156-M0 | [mG][mC][mA][mG][mC][mU][mA][mC][U][G][A][A][G][mA][mA][mG][mU][mG][A][mA][mA] | 548 | 1360 | [mU][U][U][mC][A][C][U][U][C][U][mU][mC][A][G][U][mA][G][mC][U][G][mC][mU][mU] | 932 | 1760 |
| 996 | siRNA157-M0 | [mC][mA][mG][mC][mU][mA][mC][mU][G][A][A][G][A][mA][mG][mU][mG][mA][A][mA][mA] | 549 | 1361 | [mU][U][U][mU][C][A][C][U][U][C][mU][mU][C][A][G][mU][A][mG][C][U][mG][mU][mU] | 933 | 1761 |
| 1005 | siRNA158-M0 | [mA][mA][mG][mA][mA][mG][mU][mG][A][A][A][A][G][mA][mA][mC][mA][mU][U][mA][mA] | 550 | 1362 | [mU][U][A][mA][U][G][U][U][C][U][mU][mU][U][C][A][mC][U][mU][C][U][mU][mU][mU] | 934 | 1762 |
| 1006 | siRNA159-M0 | [mA][mG][mA][mA][mG][mU][mG][mA][A][A][A][G][A][mA][mC][mA][mU][mU][A][mG][mA] | 551 | 1364 | [mU][C][U][mA][A][U][G][U][U][C][mU][mU][U][U][C][mA][C][mU][U][C][mU][mU][mU] | 935 | 1763 |
| 1007 | siRNA160-M0 | [mG][mA][mA][mG][mU][mG][mA][mA][A][A][G][A][A][mC][mA][mU][mU][mA][G][mA][mA] | 552 | 1365 | [mU][U][C][mU][A][A][U][G][U][U][mC][mU][U][U][U][mC][A][mC][U][U][mC][mU][mU] | 936 | 1764 |
| 1011 | siRNA161-M0 | [mU][mG][mA][mA][mA][mA][mG][mA][A][C][A][U][U][mA][mG][mA][mG][mA][A][mU][mA] | 553 | 1366 | [mU][A][U][mU][C][U][C][U][A][A][mU][mG][U][U][C][mU][U][mU][U][C][mA][mU][mU] | 937 | 1765 |
| 1012 | siRNA162-M0 | [mG][mA][mA][mA][mA][mG][mA][mA][C][A][U][U][A][mG][mA][mG][mA][mA][U][mG][mA] | 554 | 1368 | [mU][C][A][mU][U][C][U][C][U][A][mA][mU][G][U][U][mC][U][mU][U][U][mC][mU][mU] | 938 | 1766 |
| 1013 | siRNA163-M0 | [mA][mA][mA][mA][mG][mA][mA][mC][A][U][U][A][G][mA][mG][mA][mA][mU][G][mC][mA] | 555 | 1369 | [mU][G][C][mA][U][U][C][U][C][U][mA][mA][U][G][U][mU][C][mU][U][U][mU][mU][mU] | 939 | 1767 |
| 1018 | siRNA164-M0 | [mA][mA][mC][mA][mU][mU][mA][mG][A][G][A][A][U][mG][mC][mU][mG][mG][U][mC][mA] | 556 | 1370 | [mU][G][A][mC][C][A][G][C][A][U][mU][mC][U][C][U][mA][A][mU][G][U][mU][mU][mU] | 940 | 1768 |
| 1019 | siRNA165-M0 | [mA][mC][mA][mU][mU][mA][mG][mA][G][A][A][U][G][mC][mU][mG][mG][mU][C][mA][mA] | 557 | 1371 | [mU][U][G][mA][C][C][A][G][C][A][mU][mU][C][U][C][mU][A][mA][U][G][mU][mU][mU] | 941 | 1769 |
| 1020 | siRNA166-M0 | [mC][mA][mU][mU][mA][mG][mA][mG][A][A][U][G][C] | 558 | 1372 | [mU][U][U][mG][A][C][C][A][G][C][mA][mU][U][ | 942 | 1770 |

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|---|---|---|---|---|---|---|---|
| | | [mU][mG][mG][mU][mC][A][mA][mA] | | | C][U][mC][U][mA][A][U][mG][mU][mU] | | |
| 1021 | siRNA167-M0 | [mA][mU][mU][mA][mG][mA][mG][mA][A][U][G][C][U][mG][mG][mU][mC][mA][A][mA][mA] | 559 | 1373 | [mU][U][U][mU][G][A][C][C][A][G][mC][mA][U][U][C][mU][C][mU][A][A][mU][mU][mU] | 943 | 1771 |
| 1026 | siRNA168-M0 | [mA][mG][mA][mA][mU][mG][mC][mU][G][G][U][C][A][mA][mA][mA][mA][mG][U][mC][mA] | 560 | 1374 | [mU][G][A][mC][U][U][U][U][U][G][mA][mC][C][A][G][mC][A][mU][U][C][mU][mU][mU] | 944 | 1772 |
| 1027 | siRNA169-M0 | [mG][mA][mA][mU][mG][mC][mU][mG][G][U][C][A][A][mA][mA][mA][mG][mU][C][mA][mA] | 561 | 1375 | [mU][U][G][mA][C][U][U][U][U][U][mG][mA][C][C][A][mG][C][mA][U][U][mC][mU][mU] | 945 | 1773 |
| 1029 | siRNA170-M0 | [mA][mU][mG][mC][mU][mG][mG][mU][C][A][A][A][A][mA][mG][mU][mC][mA][G][mC][mA] | 562 | 1376 | [mU][G][C][mU][G][A][C][U][U][U][mU][mU][G][A][C][mC][A][mG][C][A][mU][mU][mU] | 946 | 1774 |
| 1036 | siRNA171-M0 | [mU][mC][mA][mA][mA][mA][mA][mG][U][C][A][G][C][mU][mU][mG][mG][mA][A][mG][mA] | 563 | 1377 | [mU][C][U][mU][C][C][A][A][G][C][mU][mG][A][C][U][mU][U][mU][U][G][mA][mU][mU] | 947 | 1775 |
| 1039 | siRNA172-M0 | [mA][mA][mA][mA][mG][mU][mC][mA][G][C][U][U][G][mG][mA][mA][mG][mG][C][mA][mA] | 564 | 1378 | [mU][U][G][mC][C][U][U][C][C][A][mA][mG][C][U][G][mA][C][mU][U][U][mU][mU][mU] | 948 | 1776 |
| 1042 | siRNA173-M0 | [mA][mG][mU][mC][mA][mG][mC][mU][U][G][G][A][A][mG][mG][mC][mA][mA][U][mC][mA] | 565 | 1380 | [mU][G][A][mU][U][G][C][C][U][U][mC][mC][A][A][G][mC][U][mG][A][C][mU][mU][mU] | 949 | 1777 |
| 1084 | siRNA174-M0 | [mA][mC][mU][mG][mA][mA][mU][mG][A][C][C][U][G][mC][mC][mA][mG][mU][A][mU][mA] | 566 | 1381 | [mU][A][U][mA][C][U][G][G][C][A][mG][mG][U][C][A][mU][U][mC][A][G][mU][mU][mU] | 950 | 1778 |
| 1087 | siRNA175-M0 | [mG][mA][mA][mU][mG][mA][mC][mC][U][G][C][C][A][mG][mU][mA][mU][mU][A][mG][mA] | 567 | 1383 | [mU][C][U][mA][A][U][A][C][U][G][mG][mC][A][G][G][mU][C][mA][U][U][mC][mU][mU] | 951 | 1779 |
| 1134 | siRNA176-M0 | [mU][mU][mU][mC][mC][mA][mG][mU][G][C][C][U][C][mC][mC][mU][mC][mA][A][mC][mA] | 568 | 1384 | [mU][G][U][mU][G][A][G][G][G][A][mG][mG][C][A][C][mU][G][mG][A][A][mA][mU][mU] | 952 | 1780 |
| 1151 | siRNA177-M0 | [mA][mA][mC][mA][mU][mC][mC][mG][G][A][C][A][G][mC][mU][mA][mA][mG][G][mA][mA] | 569 | 1385 | [mU][U][C][mC][U][U][A][G][C][U][mG][mU][C][C][G][mG][A][mU][G][U][mU][mU][mU] | 953 | 1781 |
| 1152 | siRNA178-M0 | [mA][mC][mA][mU][mC][mC][mG][mG][A][C][A][G][C][mU][mA][mA][mG][mG][A][mG][mA] | 570 | 1386 | [mU][C][U][mC][C][U][U][A][G][C][mU][mG][U][C][C][mG][G][mA][U][G][mU][mU][mU] | 954 | 1782 |
| 1155 | siRNA179-M0 | [mU][mC][mC][mG][mG][mA][mC][mA][G][C][U][A][A][mG][mG][mA][mG][mG][A][mU][mA] | 571 | 1387 | [mU][A][U][mC][C][U][C][C][U][U][mA][mG][C][U][G][mU][C][mC][G][G][mA][mU][mU] | 955 | 1783 |
| 1158 | siRNA180-M0 | [mG][mG][mA][mC][mA][mG][mC][mU][A][A][G][G][A][mG][mG][mA][mU][mU][U][mC][mA] | 572 | 1388 | [mU][G][A][mA][A][U][C][C][U][C][mC][mU][U][A][G][mC][U][mG][U][C][mC][mU][mU] | 956 | 1784 |
| 1160 | siRNA181-M0 | [mA][mC][mA][mG][mC][mU][mA][mA][G][G][A][G][G][mA][mU][mU][mU][mC][A][mC][mA] | 573 | 1389 | [mU][G][U][mG][A][A][A][U][C][C][mU][mC][C][U][U][mA][G][mC][U][G][mU][mU][mU] | 957 | 1785 |

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| 1162 | siRNA182-M0 | [mA][mG][mC][mU][mA][mA][mG][mG][A][G][G][A][U][mU][mU][mC][mA][mC][U][mU][mA] | 574 | 1390 | [mU][A][A][mG][U][G][A][A][A][U][mC][mC][U][C][C][mU][U][mA][G][C][mU][mU][mU] | 958 | 1786 |
| 1165 | siRNA183-M0 | [mU][mA][mA][mG][mG][mA][mG][mG][A][U][U][U][C][mA][mC][mU][mU][mU][G][mC][mA] | 575 | 1391 | [mU][G][C][mA][A][A][G][U][G][A][mA][mA][U][C][C][mU][C][mC][U][U][mA][mU][mU] | 959 | 1787 |
| 1168 | siRNA184-M0 | [mG][mG][mA][mG][mG][mA][mU][mU][U][C][A][C][U][mU][mU][mG][mC][mA][C][mC][mA] | 576 | 1392 | [mU][G][G][mU][G][C][A][A][A][G][mU][mG][A][A][A][mU][C][mC][U][C][mC][mU][mU] | 960 | 1788 |
| 1169 | siRNA185-M0 | [mG][mA][mG][mG][mA][mU][mU][mU][C][A][C][U][U][mU][mG][mC][mA][mC][C][mU][mA] | 577 | 1393 | [mU][A][G][mG][U][G][C][A][A][A][mG][mU][G][A][A][mA][U][mC][C][U][mC][mU][mU] | 961 | 1789 |
| 1174 | siRNA186-M0 | [mU][mU][mU][mC][mA][mC][mU][mU][U][G][C][A][C][mC][mU][mU][mG][mA][G][mG][mA] | 578 | 1394 | [mU][C][C][mU][C][A][A][G][G][U][mG][mC][A][A][A][mG][U][mG][A][A][mA][mU][mU] | 962 | 1790 |
| 1177 | siRNA187-M0 | [mC][mA][mC][mU][mU][mU][mG][mC][A][C][C][U][U][mG][mA][mG][mG][mA][C][mG][mA] | 579 | 1395 | [mU][C][G][mU][C][C][U][C][A][A][mG][mG][U][G][C][mA][A][mA][G][U][mG][mU][mU] | 963 | 1791 |
| 1178 | siRNA188-M0 | [mA][mC][mU][mU][mU][mG][mC][mA][C][C][U][U][G][mA][mG][mG][mA][mC][G][mG][mA] | 580 | 1396 | [mU][C][C][mG][U][C][C][U][C][A][mA][mG][G][U][G][mC][A][mA][A][G][mU][mU][mU] | 964 | 1792 |
| 1189 | siRNA189-M0 | [mU][mG][mA][mG][mG][mA][mC][mG][G][U][U][C][C][mU][mA][mC][mA][mA][C][mA][mA] | 581 | 1397 | [mU][U][G][mU][U][G][U][A][G][G][mA][mA][C][C][G][mU][C][mC][U][C][mA][mU][mU] | 965 | 1793 |
| 1191 | siRNA190-M0 | [mA][mG][mG][mA][mC][mG][mG][mU][U][C][C][U][A][mC][mA][mA][mC][mA][U][mC][mA] | 582 | 1398 | [mU][G][A][mU][G][U][U][G][U][A][mG][mG][A][A][C][mC][G][mU][C][C][mU][mU][mU] | 966 | 1794 |
| 1194 | siRNA191-M0 | [mA][mC][mG][mG][mU][mU][mC][mC][U][A][C][A][A][mC][mA][mU][mC][mC][G][mA][mA] | 583 | 1400 | [mU][U][C][mG][G][A][U][G][U][U][mG][mU][A][G][G][mA][A][mC][C][G][mU][mU][mU] | 967 | 1795 |
| 1196 | siRNA192-M0 | [mG][mG][mU][mU][mC][mC][mU][mA][C][A][A][C][A][mU][mC][mC][mG][mA][A][mA][mA] | 584 | 1401 | [mU][U][U][mU][C][G][G][A][U][G][mU][mU][G][U][A][mG][G][mA][A][C][mC][mU][mU] | 968 | 1796 |
| 1200 | siRNA193-M0 | [mC][mC][mU][mA][mC][mA][mA][mC][A][U][C][C][G][mA][mA][mA][mA][mG][A][mU][mA] | 585 | 1402 | [mU][A][U][mC][U][U][U][U][C][G][mG][mA][U][G][U][mU][G][mU][A][G][mG][mU][mU] | 969 | 1797 |
| 1201 | siRNA194-M0 | [mC][mU][mA][mC][mA][mA][mC][mA][U][C][C][G][A][mA][mA][mA][mG][mA][U][mG][mA] | 586 | 1403 | [mU][C][A][mU][C][U][U][U][U][C][mG][mG][A][U][G][mU][U][mG][U][A][mG][mU][mU] | 970 | 1798 |
| 1203 | siRNA195-M0 | [mA][mC][mA][mA][mC][mA][mU][mC][C][G][A][A][A][mA][mG][mA][mU][mG][A][mC][mA] | 587 | 1404 | [mU][G][U][mC][A][U][C][U][U][U][mU][mC][G][G][A][mU][G][mU][U][G][mU][mU][mU] | 971 | 1799 |
| 1204 | siRNA196-M0 | [mC][mA][mA][mC][mA][mU][mC][mC][G][A][A][A][A][mG][mA][mU][mG][mA][C][mA][mA] | 588 | 1405 | [mU][U][G][mU][C][A][U][C][U][U][mU][mU][C][G][G][mA][U][mG][U][U][mG][mU][mU] | 972 | 1800 |
| 1205 | siRNA197-M0 | [mA][mA][mC][mA][mU][mC][mC][mG][A][A][A][A][G] | 589 | 1406 | [mU][A][U][mG][U][C][A][U][C][U][mU][mU][U][ | 973 | 1801 |

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|---|---|---|---|---|---|---|---|
| | | [mA][mU][mG][mA][mC][A][mU][mA] | | | C][G][mG][A][mU][G][U][mU][mU][mU] | | |
| 1207 | siRNA198-M0 | [mC][mA][mU][mC][mC][mG][mA][mA][A][A][G][A][U][mG][mA][mC][mA][mU][C][mA][mA] | 590 | 1407 | [mU][U][G][mA][U][G][U][C][A][U][mC][mU][U][U][U][mC][G][mG][A][U][mG][mU][mU] | 974 | 1802 |
| 1209 | siRNA199-M0 | [mU][mC][mC][mG][mA][mA][mA][mA][G][A][U][G][A][mC][mA][mU][mC][mA][U][mA][mA] | 591 | 1408 | [mU][U][A][mU][G][A][U][G][U][C][mA][mU][C][U][U][mU][U][mC][G][G][mA][mU][mU] | 975 | 1803 |
| 1212 | siRNA200-M0 | [mG][mA][mA][mA][mA][mG][mA][mU][G][A][C][A][U][mC][mA][mU][mA][mG][C][mU][mA] | 592 | 1410 | [mU][A][G][mC][U][A][U][G][A][U][mG][mU][C][A][U][mC][U][mU][U][U][mC][mU][mU] | 976 | 1804 |
| 1214 | siRNA201-M0 | [mA][mA][mA][mG][mA][mU][mG][mA][C][A][U][C][A][mU][mA][mG][mC][mU][C][mU][mA] | 593 | 1411 | [mU][A][G][mA][G][C][U][A][U][G][mA][mU][G][U][C][mA][U][mC][U][U][mU][mU][mU] | 977 | 1805 |
| 1215 | siRNA202-M0 | [mA][mA][mG][mA][mU][mG][mA][mC][A][U][C][A][U][mA][mG][mC][mU][mC][U][mU][mA] | 594 | 1412 | [mU][A][A][mG][A][G][C][U][A][U][mG][mA][U][G][U][mC][A][mU][C][U][mU][mU][mU] | 978 | 1806 |
| 1217 | siRNA203-M0 | [mG][mA][mU][mG][mA][mC][mA][mU][C][A][U][A][G][mC][mU][mC][mU][mU][U][mA][mA] | 595 | 1413 | [mU][U][A][mA][A][G][A][G][C][U][mA][mU][G][A][U][mG][U][mC][A][U][mC][mU][mU] | 979 | 1807 |
| 1226 | siRNA204-M0 | [mA][mU][mA][mG][mC][mU][mC][mU][U][U][A][C][C][mC][mA][mC][mA][mG][U][mU][mA] | 596 | 1415 | [mU][A][A][mC][U][G][U][G][G][G][mU][mA][A][A][G][mA][G][mC][U][A][mU][mU][mU] | 980 | 1808 |
| 1227 | siRNA205-M0 | [mU][mA][mG][mC][mU][mC][mU][mU][U][A][C][C][C][mA][mC][mA][mG][mU][U][mA][mA] | 597 | 1416 | [mU][U][A][mA][C][U][G][U][G][G][mG][mU][A][A][A][mG][A][mG][C][U][mA][mU][mU] | 981 | 1809 |
| 1228 | siRNA206-M0 | [mA][mG][mC][mU][mC][mU][mU][mU][A][C][C][C][A][mC][mA][mG][mU][mU][A][mA][mA] | 598 | 1419 | [mU][U][U][mA][A][C][U][G][U][G][mG][mG][U][A][A][mA][G][mA][G][C][mU][mU][mU] | 982 | 1810 |
| 1229 | siRNA207-M0 | [mG][mC][mU][mC][mU][mU][mU][mA][C][C][C][A][C][mA][mG][mU][mU][mA][A][mU][mA] | 599 | 1420 | [mU][A][U][mU][A][A][C][U][G][U][mG][mG][G][U][A][mA][A][mG][A][G][mC][mU][mU] | 983 | 1811 |
| 1230 | siRNA208-M0 | [mC][mU][mC][mU][mU][mU][mA][mC][C][C][A][C][A][mG][mU][mU][mA][mA][U][mG][mA] | 600 | 1421 | [mU][C][A][mU][U][A][A][C][U][G][mU][mG][G][G][U][mA][A][mA][G][A][mG][mU][mU] | 984 | 1812 |
| 1231 | siRNA209-M0 | [mU][mC][mU][mU][mU][mA][mC][mC][C][A][C][A][G][mU][mU][mA][mA][mU][G][mC][mA] | 601 | 1422 | [mU][G][C][mA][U][U][A][A][C][U][mG][mU][G][G][G][mU][A][mA][A][G][mA][mU][mU] | 985 | 1813 |
| 1233 | siRNA210-M0 | [mU][mU][mU][mA][mC][mC][mC][mA][C][A][G][U][U][mA][mA][mU][mG][mC][A][mC][mA] | 602 | 1423 | [mU][G][U][mG][C][A][U][U][A][A][mC][mU][G][U][G][mG][G][mU][A][A][mA][mU][mU] | 986 | 1814 |
| 1236 | siRNA211-M0 | [mA][mC][mC][mC][mA][mC][mA][mG][U][U][A][A][U][mG][mC][mA][mC][mU][U][mA][mA] | 603 | 1424 | [mU][U][A][mA][G][U][G][C][A][U][mU][mA][A][C][U][mG][U][mG][G][G][mU][mU][mU] | 987 | 1815 |
| 1237 | siRNA212-M0 | [mC][mC][mC][mA][mC][mA][mG][mU][U][A][A][U][G][mC][mA][mC][mU][mU][A][mG][mA] | 604 | 1426 | [mU][C][U][mA][A][G][U][G][C][A][mU][mU][A][A][C][mU][G][mU][G][G][mG][mU][mU] | 988 | 1816 |

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| 1238 | siRNA213-M0 | [mC][mC][mA][mC][mA][mG][mU][mU][A][A][U][G][C][mA][mC][mU][mU][mA][G][mA][mA] | 605 | 1427 | [mU][U][C][mU][A][A][G][U][G][C][mA][mU][U][A][A][mC][U][mG][U][G][mG][mU][mU] | 989 | 1817 |
| 1239 | siRNA214-M0 | [mC][mA][mC][mA][mG][mU][mU][mA][A][U][G][C][A][mC][mU][mU][mA][mG][A][mU][mA] | 606 | 1428 | [mU][A][U][mC][U][A][A][G][U][G][mC][mA][U][U][A][mA][C][mU][G][U][mG][mU][mU] | 990 | 1818 |
| 1240 | siRNA215-M0 | [mA][mC][mA][mG][mU][mU][mA][mA][U][G][C][A][C][mU][mU][mA][mG][mA][U][mC][mA] | 607 | 1429 | [mU][G][A][mU][C][U][A][A][G][U][mG][mC][A][U][U][mA][A][mC][U][G][mU][mU][mU] | 991 | 1819 |
| 1241 | siRNA216-M0 | [mC][mA][mG][mU][mU][mA][mA][mU][G][C][A][C][U][mU][mA][mG][mA][mU][C][mC][mA] | 608 | 1430 | [mU][G][G][mA][U][C][U][A][A][G][mU][mG][C][A][U][mU][A][mA][C][U][mG][mU][mU] | 992 | 1820 |
| 1242 | siRNA217-M0 | [mA][mG][mU][mU][mA][mA][mU][mG][C][A][C][U][U][mA][mG][mA][mU][mC][C][mA][mA] | 609 | 1431 | [mU][U][G][mG][A][U][C][U][A][A][mG][mU][G][C][A][mU][U][mA][A][C][mU][mU][mU] | 993 | 1821 |
| 1248 | siRNA218-M0 | [mU][mG][mC][mA][mC][mU][mU][mA][G][A][U][C][C][mA][mG][mA][mA][mA][U][mC][mA] | 610 | 1432 | [mU][G][A][mU][U][U][C][U][G][G][mA][mU][C][U][A][mA][G][mU][G][C][mA][mU][mU] | 994 | 1822 |
| 1249 | siRNA219-M0 | [mG][mC][mA][mC][mU][mU][mA][mG][A][U][C][C][A][mG][mA][mA][mA][mU][C][mU][mA] | 611 | 1433 | [mU][A][G][mA][U][U][U][C][U][G][mG][mA][U][C][U][mA][A][mG][U][G][mC][mU][mU] | 995 | 1823 |
| 1261 | siRNA220-M0 | [mA][mG][mA][mA][mA][mU][mC][mU][A][C][C][C][A][mG][mA][mC][mC][mC][U][mU][mA] | 612 | 1434 | [mU][A][A][mG][G][G][U][C][U][G][mG][mG][U][A][G][mA][U][mU][U][C][mU][mU][mU] | 996 | 1824 |
| 1283 | siRNA221-M0 | [mA][mC][mU][mU][mU][mU][mA][mA][A][U][A][U][G][mA][mU][mA][mG][mG][U][mA][mA] | 613 | 1435 | [mU][U][A][mC][C][U][A][U][C][A][mU][mA][U][U][U][mA][A][mA][A][G][mU][mU][mU] | 997 | 1825 |
| 1285 | siRNA222-M0 | [mU][mU][mU][mU][mA][mA][mA][mU][A][U][G][A][U][mA][mG][mG][mU][mA][U][mC][mA] | 614 | 1436 | [mU][G][A][mU][A][C][C][U][A][U][mC][mA][U][A][U][mU][U][mA][A][A][mA][mU][mU] | 998 | 1826 |
| 1286 | siRNA223-M0 | [mU][mU][mU][mA][mA][mA][mU][mA][U][G][A][U][A][mG][mG][mU][mA][mU][C][mU][mA] | 615 | 1437 | [mU][A][G][mA][U][A][C][C][U][A][mU][mC][A][U][A][mU][U][mU][A][A][mA][mU][mU] | 999 | 1827 |
| 1288 | siRNA224-M0 | [mU][mA][mA][mA][mU][mA][mU][mG][A][U][A][G][G][mU][mA][mU][mC][mU][U][mG][mA] | 616 | 1438 | [mU][C][A][mA][G][A][U][A][C][C][mU][mA][U][C][A][mU][A][mU][U][U][mA][mU][mU] | 1000 | 1828 |
| 1290 | siRNA225-M0 | [mA][mA][mU][mA][mU][mG][mA][mU][A][G][G][U][A][mU][mC][mU][mU][mG][A][mU][mA] | 617 | 1439 | [mU][A][U][mC][A][A][G][A][U][A][mC][mC][U][A][U][mC][A][mU][A][U][mU][mU][mU] | 1001 | 1829 |
| 1291 | siRNA226-M0 | [mA][mU][mA][mU][mG][mA][mU][mA][G][G][U][A][U][mC][mU][mU][mG][mA][U][mG][mA] | 618 | 1440 | [mU][C][A][mU][C][A][A][G][A][U][mA][mC][C][U][A][mU][C][mA][U][A][mU][mU][mU] | 1002 | 1830 |
| 1292 | siRNA227-M0 | [mU][mA][mU][mG][mA][mU][mA][mG][G][U][A][U][C][mU][mU][mG][mA][mU][G][mA][mA] | 619 | 1442 | [mU][U][C][mA][U][C][A][A][G][A][mU][mA][C][C][U][mA][U][mC][A][U][mA][mU][mU] | 1003 | 1831 |
| 1293 | siRNA228-M0 | [mA][mU][mG][mA][mU][mA][mG][mG][U][A][U][C][U] | 620 | 1443 | [mU][U][U][mC][A][U][C][A][A][G][mA][mU][A][ | 1004 | 1832 |

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|---|---|---|---|---|---|---|---|
| | | [mU][mG][mA][mU][mG][A][mA][mA] | | | C][C][mU][A][mU][C][A][mU][mU][mU] | | |
| 1296 | siRNA229-M0 | [mA][mU][mA][mG][mG][mU][mA][mU][C][U][U][G][A][mU][mG][mA][mA][mA][A][mC][mA] | 621 | 1444 | [mU][G][U][mU][U][U][C][A][U][C][mA][mA][G][A][U][mA][C][mC][U][A][mU][mU][mU] | 1005 | 1833 |
| 1297 | siRNA230-M0 | [mU][mA][mG][mG][mU][mA][mU][mC][U][U][G][A][U][mG][mA][mA][mA][mA][C][mG][mA] | 622 | 1445 | [mU][C][G][mU][U][U][U][C][A][U][mC][mA][A][G][A][mU][A][mC][C][U][mA][mU][mU] | 1006 | 1834 |
| 1298 | siRNA231-M0 | [mA][mG][mG][mU][mA][mU][mC][mU][U][G][A][U][G][mA][mA][mA][mA][mC][G][mG][mA] | 623 | 1446 | [mU][C][C][mG][U][U][U][U][C][A][mU][mC][A][A][G][mA][U][mA][C][C][mU][mU][mU] | 1007 | 1835 |
| 1299 | siRNA232-M0 | [mG][mG][mU][mA][mU][mC][mU][mU][G][A][U][G][A][mA][mA][mA][mC][mG][G][mG][mA] | 624 | 1448 | [mU][C][C][mC][G][U][U][U][U][C][mA][mU][C][A][A][mG][A][mU][A][C][mC][mU][mU] | 1008 | 1836 |
| 1300 | siRNA233-M0 | [mG][mU][mA][mU][mC][mU][mU][mG][A][U][G][A][A][mA][mA][mC][mG][mG][G][mA][mA] | 625 | 1449 | [mU][U][C][mC][C][G][U][U][U][U][mC][mA][U][C][A][mA][G][mA][U][A][mC][mU][mU] | 1009 | 1837 |
| 1301 | siRNA234-M0 | [mU][mA][mU][mC][mU][mU][mG][mA][U][G][A][A][A][mA][mC][mG][mG][mG][A][mA][mA] | 626 | 1450 | [mU][U][U][mC][C][C][G][U][U][U][mU][mC][A][U][C][mA][A][mG][A][U][mA][mU][mU] | 1010 | 1838 |
| 1302 | siRNA235-M0 | [mA][mU][mC][mU][mU][mG][mA][mU][G][A][A][A][A][mC][mG][mG][mG][mA][A][mG][mA] | 627 | 1451 | [mU][C][U][mU][C][C][C][G][U][U][mU][mU][C][A][U][mC][A][mA][G][A][mU][mU][mU] | 1011 | 1839 |
| 1303 | siRNA236-M0 | [mU][mC][mU][mU][mG][mA][mU][mG][A][A][A][A][C][mG][mG][mG][mA][mA][G][mA][mA] | 628 | 1452 | [mU][U][C][mU][U][C][C][C][G][U][mU][mU][U][C][A][mU][C][mA][A][G][mA][mU][mU] | 1012 | 1840 |
| 1306 | siRNA237-M0 | [mU][mG][mA][mU][mG][mA][mA][mA][A][C][G][G][G][mA][mA][mA][mG][mA][mC][A][mA][mA] | 629 | 1453 | [mU][U][U][mG][U][C][U][U][C][C][mC][mG][U][U][U][mU][C][mA][U][C][mA][mU][mU] | 1013 | 1841 |
| 1307 | siRNA238-M0 | [mG][mA][mU][mG][mA][mA][mA][mA][C][G][G][G][A][mA][mG][mA][mC][mA][A][mA][mA] | 630 | 1454 | [mU][U][U][mU][G][U][C][U][U][C][mC][mC][G][U][U][mU][U][mC][A][U][mC][mU][mU] | 1014 | 1842 |
| 1311 | siRNA239-M0 | [mA][mA][mA][mA][mC][mG][mG][mG][A][A][G][A][C][mA][mA][mA][mG][mA][C][mU][mA] | 631 | 1455 | [mU][A][G][mU][C][U][U][U][U][G][U][mC][mU][U][C][C][mC][G][mU][U][U][mU][mU][mU] | 1015 | 1843 |
| 1313 | siRNA240-M0 | [mA][mA][mC][mG][mG][mG][mA][mA][G][A][C][A][A][mA][mG][mA][mC][mU][A][mC][mA] | 632 | 1456 | [mU][G][U][mA][G][U][C][U][U][U][mG][mU][C][U][U][mC][C][mC][G][U][mU][mU][mU] | 1016 | 1844 |
| 1314 | siRNA241-M0 | [mA][mC][mG][mG][mG][mA][mA][mG][A][C][A][A][A][mG][mA][mC][mU][mA][C][mC][mA] | 633 | 1457 | [mU][G][G][mU][A][G][U][C][U][U][mU][mG][U][C][U][mU][C][mC][C][G][mU][mU][mU] | 1017 | 1845 |
| 1353 | siRNA242-M0 | [mA][mG][mU][mU][mA][mA][mA][mG][U][A][U][U][A][mC][mU][mA][mC][mA][U][mG][mA] | 634 | 1458 | [mU][C][A][mU][G][U][A][G][U][A][mA][mU][A][C][U][mU][U][mA][A][C][mU][mU][mU] | 1018 | 1846 |
| 1354 | siRNA243-M0 | [mG][mU][mU][mA][mA][mA][mG][mU][A][U][U][A][C][mU][mA][mC][mA][mU][G][mC][mA] | 635 | 1459 | [mU][G][C][mA][U][G][U][A][G][U][mA][mA][U][A][C][mU][U][mU][A][A][mC][mU][mU] | 1019 | 1847 |

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|---|---|---|---|---|---|---|---|
| 1358 | siRNA244-M0 | [mA][mA][mG][mU][mA][mU][mU][mA][C][U][A][C][A][mU][mG][mC][mC][mC][U][mU][mA] | 636 | 1460 | [mU][A][A][mG][G][G][C][A][U][G][mU][mA][G][U][A][mA][U][mA][C][U][mU][mU][mU] | 1020 | 1848 |
| 1360 | siRNA245-M0 | [mG][mU][mA][mU][mU][mA][mC][mU][A][C][A][U][G][mC][mC][mC][mU][mU][U][mG][mA] | 637 | 1461 | [mU][C][A][mA][A][G][G][G][C][A][mU][mG][U][A][G][mU][A][mA][U][A][mC][mU][mU] | 1021 | 1849 |
| 1361 | siRNA246-M0 | [mU][mA][mU][mU][mA][mC][mU][mA][C][A][U][G][C][mC][mC][mU][mU][mU][G][mG][mA] | 638 | 1462 | [mU][C][C][mA][A][A][G][G][G][C][mA][mU][G][U][A][mG][U][mA][A][U][mA][mU][mU] | 1022 | 1850 |
| 1363 | siRNA247-M0 | [mU][mU][mA][mC][mU][mA][mC][mA][U][G][C][C][C][mU][mU][mU][mG][mG][A][mU][mA] | 639 | 1463 | [mU][A][U][mC][C][A][A][A][G][G][mG][mC][A][U][G][mU][A][mG][U][A][mA][mU][mU] | 1023 | 1851 |
| 1364 | siRNA248-M0 | [mU][mA][mC][mU][mA][mC][mA][mU][G][C][C][C][U][mU][mU][mG][mG][mA][U][mC][mA] | 640 | 1464 | [mU][G][A][mU][C][C][A][A][A][G][mG][mG][C][A][U][mG][U][mA][G][U][mA][mU][mU] | 1024 | 1852 |
| 1382 | siRNA249-M0 | [mU][mC][mG][mG][mG][mA][mG][mC][U][A][C][A][A][mU][mA][mU][mG][mU][C][mC][mA] | 641 | 1465 | [mU][G][G][mA][C][A][U][A][U][U][mG][mU][A][G][C][mU][C][mC][C][G][mA][mU][mU] | 1025 | 1853 |
| 1386 | siRNA250-M0 | [mG][mA][mG][mC][mU][mA][mC][mA][A][U][A][U][G][mU][mC][mC][mU][mG][G][mA][mA] | 642 | 1466 | [mU][U][C][mC][A][G][G][A][C][A][mU][mA][U][U][G][mU][A][mG][C][U][mC][mU][mU] | 1026 | 1854 |
| 1388 | siRNA251-M0 | [mG][mC][mU][mA][mC][mA][mA][mU][A][U][G][U][C][mC][mC][mU][mG][mG][mA][A][mG][mA] | 643 | 1468 | [mU][C][U][mU][C][C][A][G][G][A][mC][mA][U][A][U][mU][G][mU][A][G][mC][mU][mU] | 1027 | 1855 |
| 1389 | siRNA252-M0 | [mC][mU][mA][mC][mA][mA][mU][mA][U][G][U][C][C][mU][mG][mG][mA][mA][G][mA][mA] | 644 | 1469 | [mU][U][C][mU][U][C][C][A][G][G][mA][mC][A][U][A][mU][U][mG][U][A][mG][mU][mU] | 1028 | 1856 |
| 1413 | siRNA253-M0 | [mU][mC][mG][mC][mU][mA][mU][mC][C][A][C][G][A][mA][mA][mU][mC][mA][A][mG][mA] | 645 | 1470 | [mU][C][U][mU][G][A][U][U][U][C][mG][mU][G][G][A][mU][A][mG][C][G][mA][mU][mU] | 1029 | 1857 |
| 1415 | siRNA254-M0 | [mG][mC][mU][mA][mU][mC][mC][mA][C][G][A][A][A][mU][mC][mA][mA][mG][C][mA][mA] | 646 | 1471 | [mU][U][G][mC][U][U][G][A][U][U][mU][mC][G][U][G][mG][A][mU][A][G][mC][mU][mU] | 1030 | 1858 |
| 1416 | siRNA255-M0 | [mC][mU][mA][mU][mC][mC][mA][mC][G][A][A][A][U][mC][mA][mA][mG][mC][A][mA][mA] | 647 | 1472 | [mU][U][U][mG][C][U][U][G][A][U][mU][mU][C][G][U][mG][G][mA][U][A][mG][mU][mU] | 1031 | 1859 |
| 1417 | siRNA256-M0 | [mU][mA][mU][mC][mC][mA][mC][mG][A][A][A][U][C][mA][mA][mG][mC][mA][A][mU][mA] | 648 | 1473 | [mU][A][U][mU][G][C][U][U][G][A][mU][mU][U][C][G][mU][G][mG][A][U][mA][mU][mU] | 1032 | 1860 |
| 1424 | siRNA257-M0 | [mG][mA][mA][mA][mU][mC][mA][mA][G][C][A][A][U][mU][mU][mU][mU][mG][A][mU][mA] | 649 | 1475 | [mU][A][U][mC][A][A][A][A][A][U][mU][mG][C][U][U][mG][A][mU][U][U][mC][mU][mU] | 1033 | 1861 |
| 1425 | siRNA258-M0 | [mA][mA][mA][mU][mC][mA][mA][mG][C][A][A][U][U][mU][mU][mU][mG][mA][U][mU][mA] | 650 | 1476 | [mU][A][A][mU][C][A][A][A][A][A][mU][mU][G][C][U][mU][G][mA][U][U][mU][mU][mU] | 1034 | 1862 |
| 1431 | siRNA259-M0 | [mA][mG][mC][mA][mA][mU][mU][mU][U][U][G][A][U | 651 | 1477 | [mU][C][A][mU][C][A][G][A][A][U][mC][mA][A][ | 1035 | 1863 |

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|---|---|---|---|---|---|---|---|
| | | ][mU][mC][mU][mG][mA][U][mG][mA] | | | A][A][mA][U][mU][G][C][mU][mU][mU] | | |
| 1433 | siRNA260-M0 | [mC][mA][mA][mU][mU][mU][mU][mU][G][A][U][U][C][mU][mG][mA][mU][mG][C][mU][mA] | 652 | 1478 | [mU][A][G][mC][A][U][C][A][G][A][mA][mU][C][A][A][mA][A][mA][U][U][mG][mU][mU] | 1036 | 1864 |
| 1506 | siRNA261-M0 | [mA][mC][mC][mA][mG][mU][mC][mC][C][G][G][G][C][mA][mG][mG][mC][mU][U][mG][mA] | 653 | 1480 | [mU][C][A][mA][G][C][C][U][G][C][mC][mC][G][G][G][mA][C][mU][G][G][mU][mU][mU] | 1037 | 1865 |
| 1507 | siRNA262-M0 | [mC][mC][mA][mG][mU][mC][mC][mC][G][G][G][C][A][mG][mG][mC][mU][mU][G][mG][mA] | 654 | 1481 | [mU][C][C][mA][A][G][C][C][U][G][mC][mC][C][G][G][mG][A][mC][U][G][mG][mU][mU] | 1038 | 1866 |
| 1510 | siRNA263-M0 | [mG][mU][mC][mC][mC][mG][mG][mG][C][A][G][G][C][mU][mU][mG][mG][mG][C][mA][mA] | 655 | 1482 | [mU][U][G][mC][C][C][A][A][G][C][mC][mU][G][C][C][mC][G][mG][G][A][mC][mU][mU] | 1039 | 1867 |
| 1511 | siRNA264-M0 | [mU][mC][mC][mC][mG][mG][mG][mC][A][G][G][C][U][mU][mG][mG][mG][mC][A][mU][mA] | 656 | 1483 | [mU][A][U][mG][C][C][C][A][A][G][mC][mC][U][G][C][mC][C][mG][G][G][mA][mU][mU] | 1040 | 1868 |
| 1513 | siRNA265-M0 | [mC][mC][mG][mG][mG][mC][mA][mG][G][C][U][U][G][mG][mG][mC][mA][mU][U][mU][mA] | 657 | 1484 | [mU][A][A][mA][U][G][C][C][C][A][mA][mG][C][C][U][mG][C][mC][C][G][mG][mU][mU] | 1041 | 1869 |
| 1518 | siRNA266-M0 | [mC][mA][mG][mG][mC][mU][mU][mG][G][G][C][A][U][mU][mU][mU][mG][mC][C][mG][mA] | 658 | 1485 | [mU][C][G][mG][C][A][A][A][A][U][mG][mC][C][C][A][mA][G][mC][C][U][mG][mU][mU] | 1042 | 1870 |
| 1555 | siRNA267-M0 | [mA][mU][mU][mU][mA][mA][mA][mU][A][U][A][A][A][mU][mU][mC][mA][mA][G][mC][mA] | 659 | 1486 | [mU][G][C][mU][U][G][A][A][U][U][mU][mA][U][A][U][mU][U][mA][A][A][mU][mU][mU] | 1043 | 1871 |
| 1556 | siRNA268-M0 | [mU][mU][mU][mA][mA][mA][mU][mA][U][A][A][A][U][mU][mC][mA][mA][mG][C][mA][mA] | 660 | 1487 | [mU][U][G][mC][U][U][G][A][A][U][mU][mU][A][U][A][mU][U][mU][A][A][mA][mU][mU] | 1044 | 1872 |
| 1557 | siRNA269-M0 | [mU][mU][mA][mA][mA][mU][mA][mU][A][A][A][U][U][mC][mA][mA][mG][mC][A][mU][mA] | 661 | 1488 | [mU][A][U][mG][C][U][U][G][A][A][mU][mU][U][A][U][mA][U][mU][U][A][mA][mU][mU] | 1045 | 1873 |
| 1558 | siRNA270-M0 | [mU][mA][mA][mA][mU][mA][mU][mA][A][A][U][U][C][mA][mA][mG][mC][mA][U][mU][mA] | 662 | 1489 | [mU][A][A][mU][G][C][U][U][G][A][mA][mU][U][U][A][mU][A][mU][U][U][mA][mU][mU] | 1046 | 1874 |
| 1559 | siRNA271-M0 | [mA][mA][mA][mU][mA][mU][mA][mA][A][U][U][C][A][mA][mG][mC][mA][mU][U][mU][mA] | 663 | 1490 | [mU][A][A][mA][U][G][C][U][U][G][mA][mA][U][U][U][mA][U][mA][U][U][mU][mU][mU] | 1047 | 1875 |
| 1561 | siRNA272-M0 | [mA][mU][mA][mU][mA][mA][mA][mU][U][C][A][A][G][mC][mA][mU][mU][mU][G][mU][mA] | 664 | 1491 | [mU][A][C][mA][A][A][U][G][C][U][mU][mG][A][A][U][mU][U][mA][U][A][mU][mU][mU] | 1048 | 1876 |
| 1593 | siRNA273-M0 | [mG][mG][mA][mA][mU][mA][mA][mG][A][G][G][A][C][mA][mC][mU][mA][mG][A][mU][mA] | 665 | 1493 | [mU][A][U][mC][U][A][G][U][G][U][mC][mC][U][C][U][mU][A][mU][U][C][mC][mU][mU] | 1049 | 1877 |
| 1594 | siRNA274-M0 | [mG][mA][mA][mU][mA][mA][mG][mA][G][G][A][C][A][mC][mU][mA][mG][mA][U][mG][mA] | 666 | 1494 | [mU][C][A][mU][C][U][A][G][U][G][mU][mC][C][U][C][mU][U][mA][U][U][mC][mU][mU] | 1050 | 1878 |

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|---|---|---|---|---|---|---|---|
| 1597 | siRNA275-M0 | [mU][mA][mA][mG][mA][mG][mG][mA][C][A][C][U][A][mG][mA][mU][mG][mA][U][mA][mA] | 667 | 1495 | [mU][U][A][mU][C][A][U][C][U][A][mG][mU][G][U][C][mC][U][mC][U][U][mA][mU][mU] | 1051 | 1879 |
| 1599 | siRNA276-M0 | [mA][mG][mA][mG][mG][mA][mC][mA][C][U][A][G][A][mU][mG][mA][mU][mA][U][mU][mA] | 668 | 1496 | [mU][A][A][mU][A][U][C][A][U][C][mU][mA][G][U][G][mU][C][mC][U][C][mU][mU][mU] | 1052 | 1880 |
| 1601 | siRNA277-M0 | [mA][mG][mG][mA][mC][mA][mC][mU][A][G][A][U][G][mA][mU][mA][mU][mU][A][mC][mA] | 669 | 1497 | [mU][G][U][mA][A][U][A][U][C][A][mU][mC][U][A][G][mU][G][mU][C][C][mU][mU][mU] | 1053 | 1881 |
| 1605 | siRNA278-M0 | [mC][mA][mC][mU][mA][mG][mA][mU][G][A][U][A][U][mU][mA][mC][mA][mG][G][mA][mA] | 670 | 1498 | [mU][U][C][mC][U][G][U][A][A][U][mA][mU][C][A][U][mC][U][mA][G][U][mG][mU][mU] | 1054 | 1882 |
| 1606 | siRNA279-M0 | [mA][mC][mU][mA][mG][mA][mU][mG][A][U][A][U][U][mA][mC][mA][mG][mG][A][mC][mA] | 671 | 1499 | [mU][G][U][mC][C][U][G][U][A][A][mU][mA][U][C][A][mU][C][mU][A][G][mU][mU][mU] | 1055 | 1883 |
| 1609 | siRNA280-M0 | [mA][mG][mA][mU][mG][mA][mU][mA][U][U][A][C][A][mG][mG][mA][mC][mU][G][mC][mA] | 672 | 1500 | [mU][G][C][mA][G][U][C][C][U][G][mU][mA][A][U][A][mU][C][mA][U][C][mU][mU][mU] | 1056 | 1884 |
| 1610 | siRNA281-M0 | [mG][mA][mU][mG][mA][mU][mA][mU][U][A][C][A][G][mG][mA][mC][mU][mG][C][mA][mA] | 673 | 1501 | [mU][U][G][mC][A][G][U][C][C][U][mG][mU][A][A][U][mA][U][mC][A][U][mC][mU][mU] | 1057 | 1885 |
| 1614 | siRNA282-M0 | [mA][mU][mA][mU][mU][mA][mC][mA][G][G][A][C][U][mG][mC][mA][mG][mA][A][mC][mA] | 674 | 1502 | [mU][G][U][mU][C][U][G][C][A][G][mU][mC][C][U][G][mU][A][mA][U][A][mU][mU][mU] | 1058 | 1886 |
| 1638 | siRNA283-M0 | [mU][mC][mA][mC][mC][mA][mC][mA][C][A][G][U][C][mC][mC][mU][mU][mU][G][mG][mA] | 675 | 1503 | [mU][C][C][mA][A][A][G][G][G][A][mC][mU][G][U][G][mU][G][mG][U][G][mA][mU][mU] | 1059 | 1887 |
| 1660 | siRNA284-M0 | [mA][mA][mA][mU][mG][mC][mA][mU][U][U][A][G][U][mG][mG][mU][mG][mG][U][mA][mA] | 676 | 1504 | [mU][U][A][mC][C][A][C][C][A][C][mU][mA][A][A][U][mG][C][mA][U][U][mU][mU][mU] | 1060 | 1888 |
| 1661 | siRNA285-M0 | [mA][mA][mU][mG][mC][mA][mU][mU][U][A][G][U][G][mG][mU][mG][mG][mU][A][mG][mA] | 677 | 1505 | [mU][C][U][mA][C][C][A][C][C][A][mC][mU][A][A][A][mU][G][mC][A][U][mU][mU][mU] | 1061 | 1889 |
| 1662 | siRNA286-M0 | [mA][mU][mG][mC][mA][mU][mU][mU][A][G][U][G][G][mU][mG][mG][mU][mA][G][mA][mA] | 678 | 1506 | [mU][U][C][mU][A][C][C][A][C][C][mA][mC][U][A][A][mA][U][mG][C][A][mU][mU][mU] | 1062 | 1890 |
| 1663 | siRNA287-M0 | [mU][mG][mC][mA][mU][mU][mU][mA][G][U][G][G][U][mG][mG][mU][mA][mG][A][mA][mA] | 679 | 1507 | [mU][U][U][mC][U][A][C][C][A][C][mC][mA][C][U][A][mA][A][mU][G][C][mA][mU][mU] | 1063 | 1891 |
| 1669 | siRNA288-M0 | [mU][mA][mG][mU][mG][mG][mU][mG][G][U][A][G][A][mA][mA][mU][mG][mA][U][mU][mA] | 680 | 1508 | [mU][A][A][mU][C][A][U][U][U][C][mU][mA][C][C][A][mC][C][mA][C][U][mA][mU][mU] | 1064 | 1892 |
| 1671 | siRNA289-M0 | [mG][mU][mG][mG][mU][mG][mG][mU][A][G][A][A][A][mU][mG][mA][mU][mU][C][mA][mA] | 681 | 1509 | [mU][U][G][mA][A][U][C][A][U][U][mU][mC][U][A][C][mC][A][mC][C][A][mC][mU][mU] | 1065 | 1893 |
| 1673 | siRNA290-M0 | [mG][mG][mU][mG][mG][mU][mA][mG][A][A][A][U][G | 682 | 1510 | [mU][G][G][mU][G][A][A][U][C][A][mU][mU][U][ | 1066 | 1894 |

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|---|---|---|---|---|---|---|---|
| | | ][mA][mU][mU][mC][mA][C][mC][mA] | | | C][U][mA][C][mC][A][C][mC][mU][mU] | | |
| 1676 | siRNA291-M0 | [mG][mG][mU][mA][mG][mA][mA][mA][U][G][A][U][U][mC][mA][mC][mC][mA][G][mG][mA] | 683 | 1511 | [mU][C][C][mU][G][G][U][G][A][A][mU][mC][A][U][U][mU][C][mU][A][C][mC][mU][mU] | 1067 | 1895 |
| 1683 | siRNA292-M0 | [mA][mU][mG][mA][mU][mU][mC][mA][C][C][A][G][G][mU][mC][mC][mA][mA][U][mG][mA] | 684 | 1512 | [mU][C][A][mU][U][G][G][A][C][C][mU][mG][G][U][G][mA][A][mU][C][A][mU][mU][mU] | 1068 | 1896 |
| 1686 | siRNA293-M0 | [mA][mU][mU][mC][mA][mC][mC][mA][G][G][U][C][C][mA][mA][mU][mG][mU][U][mG][mA] | 685 | 1513 | [mU][C][A][mA][C][A][U][U][G][G][mA][mC][C][U][G][mG][U][mG][A][A][mU][mU][mU] | 1069 | 1897 |
| 1689 | siRNA294-M0 | [mC][mA][mC][mC][mA][mG][mG][mU][C][C][A][A][U][mG][mU][mU][mG][mU][U][mC][mA] | 686 | 1514 | [mU][G][A][mA][C][A][A][C][A][U][mU][mG][G][A][C][mC][U][mG][G][U][mG][mU][mU] | 1070 | 1898 |
| 1690 | siRNA295-M0 | [mA][mC][mC][mA][mG][mG][mU][mC][C][A][A][U][G][mU][mU][mG][mU][mU][C][mA][mA] | 687 | 1515 | [mU][U][G][mA][A][C][A][A][C][A][mU][mU][G][G][A][mC][C][mU][G][G][mU][mU][mU] | 1071 | 1899 |
| 1692 | siRNA296-M0 | [mC][mA][mG][mG][mU][mC][mC][mA][A][U][G][U][U][mG][mU][mU][mC][mA][C][mC][mA] | 688 | 1516 | [mU][G][G][mU][G][A][A][C][A][A][mC][mA][U][U][G][mG][A][mC][C][U][mG][mU][mU] | 1072 | 1900 |
| 1712 | siRNA297-M0 | [mA][mG][mU][mG][mC][mU][mU][mG][C][U][U][G][U][mG][mA][mA][mU][mC][U][mU][mA] | 689 | 1517 | [mU][A][A][mG][A][U][U][C][A][C][mA][mA][G][C][A][mA][G][mC][A][C][mU][mU][mU] | 1073 | 1901 |
| 1713 | siRNA298-M0 | [mG][mU][mG][mC][mU][mU][mG][mC][U][U][G][U][G][mA][mA][mU][mC][mU][U][mA][mA] | 690 | 1518 | [mU][U][A][mA][G][A][U][U][C][A][mC][mA][A][G][C][mA][A][mG][C][A][mC][mU][mU] | 1074 | 1902 |
| 1769 | siRNA299-M0 | [mU][mC][mU][mG][mC][mU][mA][mG][U][G][A][A][A][mA][mG][mA][mA][mC][U][mA][mA] | 691 | 1519 | [mU][U][A][mG][U][U][C][U][U][U][mU][mC][A][C][U][mA][G][mC][A][G][mA][mU][mU] | 1075 | 1903 |
| 1770 | siRNA300-M0 | [mC][mU][mG][mC][mU][mA][mG][mU][G][A][A][A][A][mG][mA][mA][mC][mU][A][mG][mA] | 692 | 1520 | [mU][C][U][mA][G][U][U][C][U][U][mU][mU][C][A][C][mU][A][mG][C][A][mG][mU][mU] | 1076 | 1904 |
| 1824 | siRNA301-M0 | [mU][mA][mA][mG][mU][mC][mC][mA][U][G][A][A][U][mG][mU][mU][mC][mA][U][mA][mA] | 693 | 1521 | [mU][U][A][mU][G][A][A][C][A][U][mU][mC][A][U][G][mG][A][mC][U][U][mA][mU][mU] | 1077 | 1905 |
| 1825 | siRNA302-M0 | [mA][mA][mG][mU][mC][mC][mA][mU][G][A][A][U][G][mU][mU][mC][mA][mU][A][mU][mA] | 694 | 1522 | [mU][A][U][mA][U][G][A][A][C][A][mU][mU][C][A][U][mG][G][mA][C][U][mU][mU][mU] | 1078 | 1906 |
| 1826 | siRNA303-M0 | [mA][mG][mU][mC][mC][mA][mU][mG][A][A][U][G][U][mU][mC][mA][mU][mA][U][mA][mA] | 695 | 1523 | [mU][U][A][mU][A][U][G][A][A][C][mA][mU][U][C][A][mU][G][mG][A][C][mU][mU][mU] | 1079 | 1907 |
| 1827 | siRNA304-M0 | [mG][mU][mC][mC][mA][mU][mG][mA][A][U][G][U][U][mC][mA][mU][mA][mU][A][mG][mA] | 696 | 1524 | [mU][C][U][mA][U][A][U][G][A][A][mC][mA][U][U][C][mA][U][mG][G][A][mC][mU][mU] | 1080 | 1908 |
| 1828 | siRNA305-M0 | [mU][mC][mC][mA][mU][mG][mA][mA][U][G][U][U][C][mA][mU][mA][mU][mA][G][mC][mA] | 697 | 1525 | [mU][G][C][mU][A][U][A][U][G][A][mA][mC][A][U][U][mC][A][mU][G][G][mA][mU][mU] | 1081 | 1909 |

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|---|---|---|---|---|---|---|---|
| 1829 | siRNA306-M0 | [mC][mC][mA][mU][mG][mA][mA][mU][G][U][U][C][A][mU][mA][mU][mA][mG][C][mC][mA] | 698 | 1526 | [mU][G][G][mC][U][A][U][A][U][G][mA][mA][C][A][U][mU][C][mA][U][G][mG][mU][mU] | 1082 | 1910 |
| 1830 | siRNA307-M0 | [mC][mA][mU][mG][mA][mA][mU][mG][U][U][C][A][U][mA][mU][mA][mG][mC][C][mA][mA] | 699 | 1527 | [mU][U][G][mG][C][U][A][U][A][U][mG][mA][A][C][A][mU][U][mC][A][U][mG][mU][mU] | 1083 | 1911 |
| 1895 | siRNA308-M0 | [mU][mU][mU][mU][mU][mU][mU][mC][A][A][A][A][U][mG][mA][mA][mG][mA][U][mA][mA] | 700 | 1528 | [mU][U][A][mU][C][U][U][C][A][U][mU][mU][U][G][A][mA][A][mA][A][A][mA][mU][mU] | 1084 | 1912 |
| 2052 | siRNA309-M0 | [mU][mG][mU][mA][mU][mU][mC][mU][A][A][U][U][G][mG][mC][mA][mG][mA][U][mU][mA] | 701 | 1529 | [mU][A][A][mU][C][U][G][C][C][A][mA][mU][U][A][G][mA][A][mU][A][C][mA][mU][mU] | 1085 | 1913 |
| 2053 | siRNA310-M0 | [mG][mU][mA][mU][mU][mC][mU][mA][A][U][U][G][G][mC][mA][mG][mA][mU][U][mG][mA] | 702 | 1530 | [mU][C][A][mA][U][C][U][G][C][C][mA][mA][U][U][A][mG][A][mA][U][A][mC][mU][mU] | 1086 | 1914 |
| 2075 | siRNA311-M0 | [mU][mU][mU][mU][mC][mC][mU][mA][A][G][G][A][A][mA][mC][mU][mG][mC][U][mU][mA] | 703 | 1531 | [mU][A][A][mG][C][A][G][U][U][U][mC][mC][U][U][A][mG][G][mA][A][A][mA][mU][mU] | 1087 | 1915 |
| 2127 | siRNA312-M0 | [mA][mA][mA][mU][mG][mU][mU][mC][A][A][A][U][U][mC][mA][mC][mG][mU][U][mC][mA] | 704 | 1532 | [mU][G][A][mA][C][G][U][G][A][A][mU][mU][U][G][A][mA][C][mA][U][U][mU][mU][mU] | 1088 | 1916 |
| 2128 | siRNA313-M0 | [mA][mA][mU][mG][mU][mU][mC][mA][A][A][U][U][C][mA][mC][mG][mU][mU][C][mU][mA] | 705 | 1533 | [mU][A][G][mA][A][C][G][U][G][A][mA][mU][U][U][G][mA][A][mC][A][U][mU][mU][mU] | 1089 | 1917 |
| 2132 | siRNA314-M0 | [mU][mU][mC][mA][mA][mA][mU][mU][C][A][C][G][U][mU][mC][mU][mA][mG][U][mG][mA] | 706 | 1534 | [mU][C][A][mC][U][A][G][A][A][C][mG][mU][G][A][A][mU][U][mU][G][A][mA][mU][mU] | 1090 | 1918 |
| 2133 | siRNA315-M0 | [mU][mC][mA][mA][mA][mU][mU][mC][A][C][G][U][U][mC][mU][mA][mG][mU][G][mA][mA] | 707 | 1535 | [mU][U][C][mA][C][U][A][G][A][A][mC][mG][U][G][A][mA][U][mU][U][G][mA][mU][mU] | 1091 | 1919 |
| 2134 | siRNA316-M0 | [mC][mA][mA][mA][mU][mU][mC][mA][C][G][U][U][C][mU][mA][mG][mU][mG][A][mA][mA] | 708 | 1536 | [mU][U][U][mC][A][C][U][A][G][A][mA][mC][G][U][G][mA][A][mU][U][U][mG][mU][mU] | 1092 | 1920 |
| 2135 | siRNA317-M0 | [mA][mA][mA][mU][mU][mC][mA][mC][G][U][U][C][U][mA][mG][mU][mG][mA][A][mA][mA] | 709 | 1537 | [mU][U][U][mU][C][A][C][U][A][G][mA][mA][C][G][U][mG][A][mA][U][U][mU][mU][mU] | 1093 | 1921 |
| 2136 | siRNA318-M0 | [mA][mA][mU][mU][mC][mA][mC][mG][U][U][C][U][A][mG][mU][mG][mA][mA][A][mC][mA] | 710 | 1538 | [mU][G][U][mU][U][C][A][C][U][A][mG][mA][A][C][G][mU][G][mA][A][U][mU][mU][mU] | 1094 | 1922 |
| 2137 | siRNA319-M0 | [mA][mU][mU][mC][mA][mC][mG][mU][U][C][U][A][G][mU][mG][mA][mA][mA][C][mU][mA] | 711 | 1539 | [mU][A][G][mU][U][U][C][A][C][U][mA][mG][A][A][C][mG][U][mG][A][A][mU][mU][mU] | 1095 | 1923 |
| 2139 | siRNA320-M0 | [mU][mC][mA][mC][mG][mU][mU][mC][U][A][G][U][G][mA][mA][mA][mC][mU][G][mC][mA] | 712 | 1540 | [mU][G][C][mA][G][U][U][U][C][A][mC][mU][A][G][A][mA][C][mG][U][G][mA][mU][mU] | 1096 | 1924 |
| 2140 | siRNA321-M0 | [mC][mA][mC][mG][mU][mU][mC][mU][A][G][U][G][A] | 713 | 1541 | [mU][U][G][mC][A][G][U][U][U][C][mA][mC][U][ | 1097 | 1925 |

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|---|---|---|---|---|---|---|---|
| | | [mA][mA][mC][mU][mG][C][mA][mA] | | | A][G][mA][A][mC][G][U][mG][mU][mU] | | |
| 2144 | siRNA322-M0 | [mU][mU][mC][mU][mA][mG][mU][mG][A][A][A][C][U][mG][mC][mA][mU][mU][A][mU][mA] | 714 | 1542 | [mU][A][U][mA][A][U][G][C][A][G][mU][mU][U][C][A][mC][U][mA][G][A][mA][mU][mU] | 1098 | 1926 |
| 2189 | siRNA323-M0 | [mC][mG][mG][mG][mU][mG][mU][mG][A][U][C][A][U][mA][mU][mA][mU][mC][A][mU][mA] | 715 | 1543 | [mU][A][U][mG][A][U][A][U][A][U][mG][mA][U][C][A][mC][A][mC][C][C][mG][mU][mU] | 1099 | 1927 |
| 2190 | siRNA324-M0 | [mG][mG][mG][mU][mG][mU][mG][mA][U][C][A][U][A][mU][mA][mU][mC][mA][U][mA][mA] | 716 | 1544 | [mU][U][A][mU][G][A][U][A][U][A][mU][mG][A][U][C][mA][C][mA][C][C][mC][mU][mU] | 1100 | 1928 |
| 2191 | siRNA325-M0 | [mG][mG][mU][mG][mU][mG][mA][mU][C][A][U][A][U][mA][mU][mC][mA][mU][A][mA][mA] | 717 | 1545 | [mU][U][U][mA][U][G][A][U][A][U][mA][mU][G][A][U][mC][A][mC][A][C][mC][mU][mU] | 1101 | 1929 |
| 2192 | siRNA326-M0 | [mG][mU][mG][mU][mG][mA][mU][mC][A][U][A][U][A][mU][mC][mA][mU][mA][A][mA][mA] | 718 | 1546 | [mU][U][U][mU][A][U][G][A][U][A][mU][mA][U][G][A][mU][C][mA][C][A][mC][mU][mU] | 1102 | 1930 |
| 2193 | siRNA327-M0 | [mU][mG][mU][mG][mA][mU][mC][mA][U][A][U][A][U][mC][mA][mU][mA][mA][A][mG][mA] | 719 | 1547 | [mU][C][U][mU][U][A][U][G][A][U][mA][mU][A][U][G][mA][U][mC][A][C][mA][mU][mU] | 1103 | 1931 |
| 2194 | siRNA328-M0 | [mG][mU][mG][mA][mU][mC][mA][mU][A][U][A][U][C][mA][mU][mA][mA][mA][G][mG][mA] | 720 | 1548 | [mU][C][C][mU][U][U][A][U][G][A][mU][mA][U][A][U][mG][A][mU][C][A][mC][mU][mU] | 1104 | 1932 |
| 2195 | siRNA329-M0 | [mU][mG][mA][mU][mC][mA][mU][mA][U][A][U][C][A][mU][mA][mA][mA][mG][G][mA][mA] | 721 | 1549 | [mU][U][C][mC][U][U][U][A][U][G][mA][mU][A][U][A][mU][G][mA][U][C][mA][mU][mU] | 1105 | 1933 |
| 2199 | siRNA330-M0 | [mC][mA][mU][mA][mU][mA][mU][mC][A][U][A][A][A][mG][mG][mA][mU][mA][U][mU][mA] | 722 | 1550 | [mU][A][A][mU][A][U][C][C][U][U][mU][mA][U][G][A][mU][A][mU][A][U][mG][mU][mU] | 1106 | 1934 |
| 2209 | siRNA331-M0 | [mA][mA][mA][mG][mG][mA][mU][mA][U][U][U][C][A][mA][mA][mU][mG][mA][U][mU][mA] | 723 | 1551 | [mU][A][A][mU][C][A][U][U][U][G][mA][mA][A][U][A][mU][C][mC][U][U][mU][mU][mU] | 1107 | 1935 |
| 2210 | siRNA332-M0 | [mA][mA][mG][mG][mA][mU][mA][mU][U][U][C][A][A][mA][mU][mG][mA][mU][U][mA][mA] | 724 | 1552 | [mU][U][A][mA][U][C][A][U][U][U][mG][mA][A][A][U][mA][U][mC][C][U][mU][mU][mU] | 1108 | 1936 |
| 2211 | siRNA333-M0 | [mA][mG][mG][mA][mU][mA][mU][mU][U][C][A][A][A][mU][mG][mA][mU][mU][A][mU][mA] | 725 | 1553 | [mU][A][U][mA][A][U][C][A][U][U][mU][mG][A][A][A][mU][A][mU][C][C][mU][mU][mU] | 1109 | 1937 |
| 2215 | siRNA334-M0 | [mU][mA][mU][mU][mU][mC][mA][mA][A][U][G][A][U][mU][mA][mU][mG][mA][U][mU][mA] | 726 | 1554 | [mU][A][A][mU][C][A][U][A][A][U][mC][mA][U][U][U][mG][A][mA][A][U][mA][mU][mU] | 1110 | 1938 |
| 2216 | siRNA335-M0 | [mA][mU][mU][mU][mC][mA][mA][mA][U][G][A][U][U][mA][mU][mG][mA][mU][U][mA][mA] | 727 | 1555 | [mU][U][A][mA][U][C][A][U][A][A][mU][mC][A][U][U][mU][G][mA][A][A][mU][mU][mU] | 1111 | 1939 |
| 2217 | siRNA336-M0 | [mU][mU][mU][mC][mA][mA][mA][mU][G][A][U][U][A][mU][mG][mA][mU][mU][A][mG][mA] | 728 | 1556 | [mU][C][U][mA][A][U][C][A][U][A][mA][mU][C][A][U][mU][U][mG][A][A][mA][mU][mU] | 1112 | 1940 |

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|---|---|---|---|---|---|---|---|
| 2218 | siRNA337-M0 | [mU][mU][mC][mA][mA][mA][mU][mG][A][U][U][A][U][mG][mA][mU][mU][mA][G][mU][mA] | 729 | 1557 | [mU][A][C][mU][A][A][U][C][A][U][mA][mA][U][C][A][mU][U][mU][G][A][mA][mU][mU] | 1113 | 1941 |
| 2220 | siRNA338-M0 | [mC][mA][mA][mA][mU][mG][mA][mU][U][A][U][G][A][mU][mU][mA][mG][mU][U][mA][mA] | 730 | 1558 | [mU][U][A][mA][C][U][A][A][U][C][mA][mU][A][A][U][mC][A][mU][U][U][mG][mU][mU] | 1114 | 1942 |
| 2221 | siRNA339-M0 | [mA][mA][mA][mU][mG][mA][mU][mU][A][U][G][A][U][mU][mA][mG][mU][mU][A][mU][mA] | 731 | 1559 | [mU][A][U][mA][A][C][U][A][A][U][mC][mA][U][A][A][mU][C][mA][U][U][mU][mU][mU] | 1115 | 1943 |
| 2223 | siRNA340-M0 | [mA][mU][mG][mA][mU][mU][mA][mU][G][A][U][U][A][mG][mU][mU][mA][mU][G][mU][mA] | 732 | 1560 | [mU][A][C][mA][U][A][A][C][U][A][mA][mU][C][A][U][mA][A][mU][C][A][mU][mU][mU] | 1116 | 1944 |
| 2224 | siRNA341-M0 | [mU][mG][mA][mU][mU][mA][mU][mG][A][U][U][A][G][mU][mU][mA][mU][mG][U][mC][mA] | 733 | 1561 | [mU][G][A][mC][A][U][A][A][C][U][mA][mA][U][C][A][mU][A][mA][U][C][mA][mU][mU] | 1117 | 1945 |
| 2225 | siRNA342-M0 | [mG][mA][mU][mU][mA][mU][mG][mA][U][U][A][G][U][mU][mA][mU][mG][mU][C][mU][mA] | 734 | 1562 | [mU][A][G][mA][C][A][U][A][A][C][mU][mA][A][U][C][mA][U][mA][A][U][mC][mU][mU] | 1118 | 1946 |
| 2313 | siRNA343-M0 | [mU][mU][mG][mA][mU][mU][mU][mC][C][C][A][A][A][mA][mA][mC][mA][mC][U][mA][mA] | 735 | 1563 | [mU][U][A][mG][U][G][U][U][U][U][mU][mG][G][G][A][mA][A][mU][C][A][mA][mU][mU] | 1119 | 1947 |
| 2316 | siRNA344-M0 | [mA][mU][mU][mU][mC][mC][mC][mA][A][A][A][A][C][mA][mC][mU][mA][mA][A][mG][mA] | 736 | 1564 | [mU][C][U][mU][U][A][G][U][G][U][mU][mU][U][U][G][mG][G][mA][A][A][mU][mU][mU] | 1120 | 1948 |
| 2317 | siRNA345-M0 | [mU][mU][mU][mC][mC][mC][mA][mA][A][A][A][C][A][mC][mU][mA][mA][mA][G][mG][mA] | 737 | 1565 | [mU][C][C][mU][U][U][A][G][U][G][mU][mU][U][U][U][mG][G][mG][A][A][mA][mU][mU] | 1121 | 1949 |
| 2319 | siRNA346-M0 | [mU][mC][mC][mC][mA][mA][mA][mA][A][C][A][C][U][mA][mA][mA][mG][mG][U][mG][mA] | 738 | 1566 | [mU][C][A][mC][C][U][U][U][A][G][mU][mG][U][U][U][mU][U][mG][G][G][mA][mU][mU] | 1122 | 1950 |
| 2320 | siRNA347-M0 | [mC][mC][mC][mA][mA][mA][mA][C][A][C][U][A][mA][mA][mG][mG][mU][G][mG][mA] | 739 | 1567 | [mU][C][C][mA][C][C][U][U][U][A][mG][mU][G][U][U][mU][U][mU][G][G][mG][mU][mU] | 1123 | 1951 |
| 2321 | siRNA348-M0 | [mC][mC][mA][mA][mA][mA][mA][mC][A][C][U][A][A][mA][mG][mG][mU][mG][G][mU][mA] | 740 | 1568 | [mU][A][C][mC][A][C][C][U][U][U][mA][mG][U][G][U][mU][U][mU][U][G][mG][mU][mU] | 1124 | 1952 |
| 2353 | siRNA349-M0 | [mU][mC][mA][mU][mG][mU][mU][mU][U][A][A][C][U][mU][mA][mU][mU][mG][U][mU][mA] | 741 | 1569 | [mU][A][A][mC][A][A][U][A][A][G][mU][mU][A][A][A][mA][C][mA][U][G][mA][mU][mU] | 1125 | 1953 |
| 2355 | siRNA350-M0 | [mA][mU][mG][mU][mU][mU][mU][mA][A][C][U][U][A][mU][mU][mG][mU][mU][G][mC][mA] | 742 | 1570 | [mU][G][C][mA][A][C][A][A][U][A][mA][mG][U][U][A][mA][A][mA][C][A][mU][mU][mU] | 1126 | 1954 |
| 2356 | siRNA351-M0 | [mU][mG][mU][mU][mU][mU][mA][mA][C][U][U][A][U][mU][mG][mU][mU][mG][C][mU][mA] | 743 | 1571 | [mU][A][G][mC][A][A][C][A][A][U][mA][mA][G][U][U][mA][A][mA][A][C][mA][mU][mU] | 1127 | 1955 |
| 2362 | siRNA352-M0 | [mA][mA][mC][mU][mU][mA][mU][mU][G][U][U][G][C] | 744 | 1572 | [mU][G][U][mU][U][U][C][A][G][C][mA][mA][C][ | 1128 | 1956 |

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|---|---|---|---|---|---|---|---|
| | | [mU][mG][mA][mA][mA][A][mC][mA] | | | A][A][mU][A][mA][G][U][mU][mU][mU] | | |
| 2364 | siRNA353-M0 | [mC][mU][mU][mA][mU][mU][mG][mU][U][G][C][U][G][mA][mA][mA][mA][mC][U][mC][mA] | 745 | 1573 | [mU][G][A][mG][U][U][U][U][C][A][mG][mC][A][A][C][mA][A][mU][A][A][mG][mU][mU] | 1129 | 1957 |
| 2365 | siRNA354-M0 | [mU][mU][mA][mU][mU][mG][mU][mU][G][C][U][G][A][mA][mA][mA][mC][mU][C][mU][mA] | 746 | 1574 | [mU][A][G][mA][G][U][U][U][U][C][mA][mG][C][A][A][mC][A][mA][U][A][mA][mU][mU] | 1130 | 1958 |
| 2366 | siRNA355-M0 | [mU][mA][mU][mU][mG][mU][mU][mG][C][U][G][A][A][mA][mA][mC][mU][mC][U][mA][mA] | 747 | 1575 | [mU][U][A][mG][A][G][U][U][U][U][mC][mA][G][C][A][mA][C][mA][A][U][mA][mU][mU] | 1131 | 1959 |
| 2370 | siRNA356-M0 | [mG][mU][mU][mG][mC][mU][mG][mA][A][A][A][C][U][mC][mU][mA][mU][mG][U][mC][mA] | 748 | 1576 | [mU][G][A][mC][A][U][A][G][A][G][mU][mU][U][U][C][mA][G][mC][A][A][mC][mU][mU] | 1132 | 1960 |
| 2503 | siRNA357-M0 | [mA][mA][mA][mA][mA][mU][mG][mU][A][G][C][U][U][mU][mU][mA][mU][mG][U][mG][mA] | 749 | 1577 | [mU][C][A][mC][A][U][A][A][A][A][mG][mC][U][A][C][mA][U][mU][U][U][mU][mU][mU] | 1133 | 1961 |
| 2562 | siRNA358-M0 | [mG][mG][mA][mA][mG][mC][mU][mU][U][G][G][U][U][mA][mU][mG][mA][mA][A][mC][mA] | 750 | 1578 | [mU][G][U][mU][U][C][A][U][A][A][mC][mC][A][A][A][mG][C][mU][U][C][mC][mU][mU] | 1134 | 1962 |
| 2563 | siRNA359-M0 | [mG][mA][mA][mG][mC][mU][mU][mU][G][G][U][U][A][mU][mG][mA][mA][mA][C][mA][mA] | 751 | 1579 | [mU][U][G][mU][U][U][C][A][U][A][mA][mC][C][A][A][mA][G][mC][U][U][mC][mU][mU] | 1135 | 1963 |
| 2620 | siRNA360-M0 | [mA][mU][mU][mU][mA][mA][mA][mU][G][C][U][U][U][mU][mU][mU][mA][mU][mC][G][mC][mA] | 752 | 1580 | [mU][G][C][mG][A][U][A][A][A][A][mA][mG][C][A][U][mU][U][mA][A][A][mU][mU][mU] | 1136 | 1964 |
| 2621 | siRNA361-M0 | [mU][mU][mU][mA][mA][mA][mU][mG][C][U][U][U][U][mU][mA][mU][mC][mG][C][mU][mA] | 753 | 1581 | [mU][A][G][mC][G][A][U][A][A][A][mA][mA][G][C][A][mU][U][mU][A][A][mA][mU][mU] | 1137 | 1965 |
| 2622 | siRNA362-M0 | [mU][mU][mA][mA][mA][mU][mG][mC][U][U][U][U][U][mA][mU][mC][mG][mC][U][mA][mA] | 754 | 1582 | [mU][U][A][mG][C][G][A][U][A][A][mA][mA][A][G][C][mA][U][mU][U][A][mA][mU][mU] | 1138 | 1966 |
| 2623 | siRNA363-M0 | [mU][mA][mA][mA][mU][mG][mC][mU][U][U][U][U][A][mU][mC][mG][mC][mU][A][mA][mA] | 755 | 1583 | [mU][U][U][mA][G][C][G][A][U][A][mA][mA][A][A][G][mC][A][mU][U][U][mA][mU][mU] | 1139 | 1967 |
| 2624 | siRNA364-M0 | [mA][mA][mA][mU][mG][mC][mU][mU][U][U][U][A][U][mC][mG][mC][mU][mA][A][mA][mA] | 756 | 1584 | [mU][U][U][mU][A][G][C][G][A][U][mA][mA][A][A][A][mG][C][mA][U][U][mU][mU][mU] | 1140 | 1968 |
| 2625 | siRNA365-M0 | [mA][mA][mU][mG][mC][mU][mU][mU][U][U][A][U][C][mG][mC][mU][mA][mA][A][mU][mA] | 757 | 1585 | [mU][A][U][mU][U][A][G][C][G][A][mU][mA][A][A][A][mA][G][mC][A][U][mU][mU][mU] | 1141 | 1969 |
| 2629 | siRNA366-M0 | [mC][mU][mU][mU][mU][mU][mA][mU][C][G][C][U][A][mA][mA][mU][mG][mA][C][mU][mA] | 758 | 1586 | [mU][A][G][mU][C][A][U][U][U][A][mG][mC][G][A][U][mA][A][mA][A][A][mG][mU][mU] | 1142 | 1970 |
| 2630 | siRNA367-M0 | [mU][mU][mU][mU][mU][mA][mU][mC][G][C][U][A][A][mA][mU][mG][mA][mC][U][mU][mA] | 759 | 1587 | [mU][A][A][mG][U][C][A][U][U][U][mA][mG][C][G][A][mU][A][mA][A][A][mA][mU][mU] | 1143 | 1971 |

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|---|---|---|---|---|---|---|---|
| 2632 | siRNA368-M0 | [mU][mU][mU][mA][mU][mC][mG][mC][U][A][A][A][U][mG][mA][mC][mU][mU][G][mC][mA] | 760 | 1588 | [mU][G][C][mA][A][G][U][C][A][U][mU][mU][A][G][C][mG][A][mU][A][A][mA][mU][mU] | 1144 | 1972 |
| 2633 | siRNA369-M0 | [mU][mU][mA][mU][mC][mG][mC][mU][A][A][A][U][G][mA][mC][mU][mU][mG][C][mA][mA] | 761 | 1589 | [mU][U][G][mC][A][A][G][U][C][A][mU][mU][U][A][G][mC][G][mA][U][A][mA][mU][mU] | 1145 | 1973 |
| 2635 | siRNA370-M0 | [mA][mU][mC][mG][mC][mU][mA][mA][A][U][G][A][C][mU][mU][mG][mC][mA][G][mA][mA] | 762 | 1590 | [mU][U][C][mU][G][C][A][A][G][U][mC][mA][U][U][U][mA][G][mC][G][A][mU][mU][mU] | 1146 | 1974 |
| 2639 | siRNA371-M0 | [mC][mU][mA][mA][mA][mU][mG][mA][C][U][U][G][C][mA][mG][mA][mU][mG][A][mA][mA] | 763 | 1591 | [mU][U][U][mC][A][U][C][U][G][C][mA][mA][G][U][C][mA][U][mU][U][A][mG][mU][mU] | 1147 | 1975 |
| 2679 | siRNA372-M0 | [mU][mG][mU][mU][mU][mA][mA][mA][U][G][C][U][G][mU][mG][mU][mA][mC][A][mA][mA] | 764 | 1592 | [mU][U][U][mG][U][A][C][A][C][A][mG][mC][A][U][U][mU][A][mA][A][C][mA][mU][mU] | 1148 | 1976 |
| 2682 | siRNA373-M0 | [mU][mU][mA][mA][mA][mU][mG][mC][U][G][U][G][U][mA][mC][mA][mA][mC][A][mA][mA] | 765 | 1593 | [mU][U][U][mG][U][U][G][U][A][C][mA][mC][A][G][C][mA][U][mU][U][A][mA][mU][mU] | 1149 | 1977 |
| 2683 | siRNA374-M0 | [mU][mA][mA][mA][mU][mG][mC][mU][G][U][G][U][A][mC][mA][mA][mC][mA][A][mU][mA] | 766 | 1594 | [mU][A][U][mU][G][U][U][G][U][A][mC][mA][C][A][G][mC][A][mU][U][U][mA][mU][mU] | 1150 | 1978 |
| 2687 | siRNA375-M0 | [mU][mG][mC][mU][mG][mU][mG][mU][A][C][A][A][C][mA][mA][mU][mG][mC][U][mU][mA] | 767 | 1595 | [mU][A][A][mG][C][A][U][U][G][U][mU][mG][U][A][C][mA][C][mA][G][C][mA][mU][mU] | 1151 | 1979 |
| 2690 | siRNA376-M0 | [mU][mG][mU][mG][mU][mA][mC][mA][A][C][A][A][U][mG][mC][mU][mU][mU][G][mA][mA] | 768 | 1596 | [mU][U][C][mA][A][A][G][C][A][U][mU][mG][U][U][G][mU][A][mC][A][C][mA][mU][mU] | 1152 | 1980 |
| 2691 | siRNA377-M0 | [mG][mU][mG][mU][mA][mC][mA][mA][C][A][A][U][G][mC][mU][mU][mU][mG][A][mU][mA] | 769 | 1597 | [mU][A][U][mC][A][A][A][G][C][A][mU][mU][G][U][U][mG][U][mA][C][A][mC][mU][mU] | 1153 | 1981 |
| 2692 | siRNA378-M0 | [mU][mG][mU][mA][mC][mA][mA][mC][A][A][U][G][C][mU][mU][mU][mG][mA][U][mA][mA] | 770 | 1598 | [mU][U][A][mU][C][A][A][A][G][C][mA][mU][U][G][U][mU][G][mU][A][C][mA][mU][mU] | 1154 | 1982 |
| 2833 | siRNA379-M0 | [mG][mA][mU][mA][mA][mU][mU][mU][U][G][A][A][A][mU][mG][mA][mU][mU][C][mA][mA] | 771 | 1599 | [mU][U][G][mA][A][U][C][A][U][U][mU][mC][A][A][A][mA][U][mU][A][U][mC][mU][mU] | 1155 | 1983 |
| 2834 | siRNA380-M0 | [mA][mU][mA][mA][mU][mU][mU][mU][G][A][A][A][U][mG][mA][mU][mU][mC][A][mU][mA] | 772 | 1600 | [mU][A][U][mG][A][A][U][C][A][U][mU][mU][C][A][A][mA][A][mU][U][A][mU][mU][mU] | 1156 | 1984 |
| 2835 | siRNA381-M0 | [mU][mA][mA][mU][mU][mU][mU][mG][A][A][A][U][G][mA][mU][mU][mC][mA][U][mC][mA] | 773 | 1601 | [mU][G][A][mU][G][A][A][U][C][A][mU][mU][U][C][A][mA][A][mA][U][U][mA][mU][mU] | 1157 | 1985 |
| 2838 | siRNA382-M0 | [mU][mU][mU][mU][mG][mA][mA][mA][U][G][A][U][U][mC][mA][mU][mC][mU][U][mU][mA] | 774 | 1602 | [mU][A][A][mA][G][A][U][G][A][A][mU][mC][A][U][U][mU][C][mA][A][A][mA][mU][mU] | 1158 | 1986 |
| 2839 | siRNA383-M0 | [mU][mU][mU][mG][mA][mA][mA][mU][G][A][U][U][C] | 775 | 1603 | [mU][G][A][mA][A][G][A][U][G][A][mA][mU][C][ | 1159 | 1987 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | [mA][mU][mC][mU][mU][U][mC][mA] | | | A][U][mU][U][mC][A][A][mA][mU][mU] | | |
| 2858 | siRNA384-M0 | [mC][mA][mG][mA][mA][mA][mU][mA][A][A][A][G][U][mA][mU][mG][mA][mA][U][mC][mA] | 776 | 1604 | [mU][G][A][mU][U][C][A][U][A][C][mU][mU][U][U][A][mU][U][mU][C][U][mG][mU][mU] | 1160 | 1988 |

**siRNA# in Table 5B correspond to the siRNA# in Table 5A, but with "M0" added to indicate modification pattern. M0 refers to the modification pattern of the siRNAs in Table 5B. In the sense strands, the nucleosides at positions 9-13 and 19 are ribonucleosides and the nucleosides at the rest of the positions are 2'-O-Me modified nucleosides. In the antisense strands, the nucleosides at positions 2, 3, 5-10, 13-15, 17, 19, and 20 are 2'-hydroxy nucleosides and the nucleosides at the rest of the positions are 2'-O-Me modified nucleosides.*

*‡Each uracil base (U) in any one of the sequences provided in Table 5B may independently and optionally be replaced with a thymine base (T).*

**Example 3: *In vitro* screening of siRNA agents**

Initially, a library of 384 siRNAs as listed in Table 5B with the generic 21/23-mer partially modified design were prioritized for synthesis for a primary in-vitro screen. For this initial set of constructs, a combination of 2'-OH (RNA) and 2'O-Me RNA nucleosides were used. The chosen modification pattern is minimally modified and is expected to maintain the intrinsic potency of a given siRNA sequence.

To determine knockdown efficiency of the synthesized siRNAs, a stable cell line overexpressing human CYP7A1 was generated. The whole library was then tested for their mRNA knockdown efficiency *in vitro* under transfection conditions. Two different concentrations (0.1 nM and 1nM) were tested and siRNA that achieved 50%, or greater knockdown at the lowest concentration were selected.

**Generation of CYP7A1 over-expressing cells**

U2OS cells were obtained from ATCC (Manassas, VA). Cells were thawed and sub-cultured according to manufacturer's recommendations. A stable over-expressing CYP7A11 U2OS cell line was generated by cloning hCyp7a1 DNA into a lentivirus expression vector and then co-transfecting along with lentivirus packaging plasmids into U2OS cells. After several weeks of growth under antibiotic selection, a stable pool was identified and expanded after evaluating mRNA expression of CYP7A1 by RT-PCR.

***In Vitro* RNAi activity procedure**

U2OS cells overexpressing CYP7A1 mRNA were plated in a 96-well plate at 10,000 cells/well containing antibiotic-free medium and placed at 37°C in an atmosphere with 5% CO2 in a humidified incubator. Within 24 hours after seeding, cells were transfected with biological duplicates for each treatment. Two concentrations (1nM and 0.1nM) of cyp7a1 siRNA as well as control siRNAs (ON-TARGETplus Human CYP7A1 control pool, Horizon Discovery) were

applied to the cells following the manufacturer's recommendation (Thermofisher) for Lipofectamine RNAiMax. Cells were returned to the incubator for 24 hours. Cell lysates were prepared 24 hour post-transfection by removing medium and washing with cold PBS and the addition of lysis buffer (following the manufacturer's recommendation for Cells-to-CT 1-step TaqMan Kit, Thermofisher). Reverse transcription was performed on cell lysates which were then used for qPCR. qPCR was performed in duplicate for each sample in a 10ul reaction mix, multiplexing the probe for CYP7A1 with the housekeeping gene probe(GAPDH): Nuclease-free water 5.5ul + qRT-PCR Master Mix 2.5ul + TaqMan Primer-1, 0.5ul + TaqMan Primer-2, 0.5ul.

For each well, the target mRNA level was normalized to the respective GAPDH mRNA level. The activity of a given siRNA was expressed as percent mRNA concentration of the respective target (normalized to GAPDH mRNA) in treated cells, relative to the target mRNA concentration (normalized to GAPDH mRNA) averaged across mock control wells. Table 6 summarizes the *in vitro* screening results.

Table 6: *In vitro* Screening of siRNAs

| Start_19mer | **siRNA#*** | **% mRNA remaining_1nM** | **KD_1nM** | **% mRNA remaining_0.1nM** | **KD_0.1nM** |
|---|---|---|---|---|---|
| 34 | siRNA1-M0 | 97.0 | 3.0 | 91.3 | 8.7 |
| 40 | siRNA2-M0 | 95.3 | 4.7 | 79.0 | 21.0 |
| 41 | siRNA3-M0 | 84.4 | 15.6 | 81.5 | 18.5 |
| 42 | siRNA4-M0 | 87.3 | 12.7 | 78.3 | 21.7 |
| 43 | siRNA5-M0 | 86.1 | 13.9 | 83.0 | 17.0 |
| 45 | siRNA6-M0 | 90.0 | 10.0 | 75.8 | 24.2 |
| 105 | siRNA7-M0 | 48.9 | 51.1 | 87.9 | 12.1 |
| 110 | siRNA8-M0 | 46.6 | 53.4 | 81.8 | 18.2 |
| 111 | siRNA9-M0 | 40.5 | 59.5 | 80.8 | 19.2 |
| 112 | siRNA10-M0 | 21.6 | 78.4 | 73.6 | 26.4 |
| 113 | siRNA11-M0 | 86.5 | 13.5 | 89.0 | 11.0 |
| 115 | siRNA12-M0 | 14.3 | 85.7 | 48.6 | 51.4 |
| 116 | siRNA13-M0 | 33.1 | 66.9 | 70.6 | 29.4 |
| 117 | siRNA14-M0 | 80.2 | 19.8 | 91.2 | 8.8 |
| 118 | siRNA15-M0 | 31.4 | 68.6 | 55.5 | 44.5 |
| 139 | siRNA16-M0 | 53.3 | 46.7 | 100.7 | -0.7 |
| 141 | siRNA17-M0 | 55.4 | 44.6 | 88.7 | 11.3 |
| 142 | siRNA18-M0 | 57.4 | 42.6 | 81.1 | 18.9 |
| 143 | siRNA19-M0 | 34.1 | 65.9 | 69.3 | 30.7 |
| 181 | siRNA20-M0 | 54.3 | 45.7 | 96.6 | 3.4 |
| 182 | siRNA21-M0 | 63.2 | 36.8 | 85.8 | 14.2 |
| 183 | siRNA22-M0 | 68.6 | 31.4 | 94.7 | 5.3 |
| 201 | siRNA23-M0 | 29.0 | 71.0 | 73.8 | 26.2 |
| 203 | siRNA24-M0 | 28.3 | 71.7 | 66.3 | 33.7 |
| 210 | siRNA25-M0 | 99.2 | 0.8 | 96.1 | 3.9 |
| 214 | siRNA26-M0 | 41.0 | 59.0 | 55.5 | 44.5 |
| 223 | siRNA27-M0 | 18.3 | 81.7 | 18.0 | 82.0 |
| 225 | siRNA28-M0 | 81.2 | 18.8 | 84.3 | 15.7 |

| | | | | | |
|---|---|---|---|---|---|
| 229 | siRNA29-M0 | 62.3 | 37.7 | 90.3 | 9.7 |
| 230 | siRNA30-M0 | 32.4 | 67.6 | 74.2 | 25.8 |
| 236 | siRNA31-M0 | 28.5 | 71.5 | 78.7 | 21.3 |
| 238 | siRNA32-M0 | 64.3 | 35.7 | 84.8 | 15.2 |
| 242 | siRNA33-M0 | 45.9 | 54.1 | 76.3 | 23.7 |
| 243 | siRNA34-M0 | 41.5 | 58.5 | 86.1 | 13.9 |
| 244 | siRNA35-M0 | 80.3 | 19.7 | 70.6 | 29.4 |
| 245 | siRNA36-M0 | 38.8 | 61.2 | 53.7 | 46.3 |
| 246 | siRNA37-M0 | 81.0 | 19.0 | 75.5 | 24.5 |
| 251 | siRNA38-M0 | 29.6 | 70.4 | 50.1 | 49.9 |
| 253 | siRNA39-M0 | 68.2 | 31.8 | 199.0 | -99.0 |
| 254 | siRNA40-M0 | 18.7 | 81.3 | 70.7 | 29.3 |
| 262 | siRNA41-M0 | 37.7 | 62.3 | 62.7 | 37.3 |
| 264 | siRNA42-M0 | 48.7 | 51.3 | 87.2 | 12.8 |
| 266 | siRNA43-M0 | 66.8 | 33.2 | 72.8 | 27.2 |
| 267 | siRNA44-M0 | 75.4 | 24.6 | 90.9 | 9.1 |
| 268 | siRNA45-M0 | 17.6 | 82.4 | 56.3 | 43.7 |
| 291 | siRNA46-M0 | 53.0 | 47.0 | 74.6 | 25.4 |
| 292 | siRNA47-M0 | 17.4 | 82.6 | 36.7 | 63.3 |
| 294 | siRNA48-M0 | 73.0 | 27.0 | 90.1 | 9.9 |
| 300 | siRNA49-M0 | 37.2 | 62.8 | 58.8 | 41.2 |
| 302 | siRNA50-M0 | 32.2 | 67.8 | 69.4 | 30.6 |
| 303 | siRNA51-M0 | 23.6 | 76.4 | 50.3 | 49.7 |
| 304 | siRNA52-M0 | 22.7 | 77.3 | 67.5 | 32.5 |
| 305 | siRNA53-M0 | 58.9 | 41.1 | 83.0 | 17.0 |
| 309 | siRNA54-M0 | 33.2 | 66.8 | 76.9 | 23.1 |
| 310 | siRNA55-M0 | 79.3 | 20.7 | 74.7 | 25.3 |
| 311 | siRNA56-M0 | 87.0 | 13.0 | 80.3 | 19.7 |
| 312 | siRNA57-M0 | 65.3 | 34.7 | 94.3 | 5.7 |
| 313 | siRNA58-M0 | 63.3 | 36.7 | 88.1 | 11.9 |
| 315 | siRNA59-M0 | 78.7 | 21.3 | 82.2 | 17.8 |
| 319 | siRNA60-M0 | 68.6 | 31.4 | 76.6 | 23.4 |
| 360 | siRNA61-M0 | 50.5 | 49.5 | 75.9 | 24.1 |
| 363 | siRNA62-M0 | 52.4 | 47.6 | 86.7 | 13.3 |
| 372 | siRNA63-M0 | 26.8 | 73.2 | 79.6 | 20.4 |
| 375 | siRNA64-M0 | 45.3 | 54.7 | 109.1 | -9.1 |
| 377 | siRNA65-M0 | 62.0 | 38.0 | 90.3 | 9.7 |
| 390 | siRNA66-M0 | 59.6 | 40.4 | 62.0 | 38.0 |
| 392 | siRNA67-M0 | 78.8 | 21.2 | 99.8 | 0.2 |
| 394 | siRNA68-M0 | 43.1 | 56.9 | 90.5 | 9.5 |
| 474 | siRNA69-M0 | 53.5 | 46.5 | 89.6 | 10.4 |
| 475 | siRNA70-M0 | 70.0 | 30.0 | 80.9 | 19.1 |
| 476 | siRNA71-M0 | 64.7 | 35.3 | 80.0 | 20.0 |
| 477 | siRNA72-M0 | 23.2 | 76.8 | 48.9 | 51.1 |
| 478 | siRNA73-M0 | 25.9 | 74.1 | 46.0 | 54.0 |
| 482 | siRNA74-M0 | 61.0 | 39.0 | 82.6 | 17.4 |
| 484 | siRNA75-M0 | 66.9 | 33.1 | 76.9 | 23.1 |
| 486 | siRNA76-M0 | 61.2 | 38.8 | 87.4 | 12.6 |
| 488 | siRNA77-M0 | 62.0 | 38.0 | 86.3 | 13.7 |
| 501 | siRNA78-M0 | 51.1 | 48.9 | 73.7 | 26.3 |
| 503 | siRNA79-M0 | 28.5 | 71.5 | 76.7 | 23.3 |
| 505 | siRNA80-M0 | 37.2 | 62.8 | 74.7 | 25.3 |
| 506 | siRNA81-M0 | 15.8 | 84.2 | 52.8 | 47.2 |
| 507 | siRNA82-M0 | 83.3 | 16.7 | 83.8 | 16.2 |
| 509 | siRNA83-M0 | 75.5 | 24.5 | 103.0 | -3.0 |
| 510 | siRNA84-M0 | 70.6 | 29.4 | 108.8 | -8.8 |
| 511 | siRNA85-M0 | 166.5 | -66.5 | 86.8 | 13.2 |

| | | | | | |
|---|---|---|---|---|---|
| 512 | siRNA86-M0 | 60.3 | 39.7 | 96.0 | 4.0 |
| 513 | siRNA87-M0 | 71.0 | 29.0 | 82.7 | 17.3 |
| 514 | siRNA88-M0 | 65.6 | 34.4 | 58.5 | 41.5 |
| 539 | siRNA89-M0 | 85.3 | 14.7 | 77.3 | 22.7 |
| 552 | siRNA90-M0 | 46.8 | 53.2 | 68.3 | 31.7 |
| 562 | siRNA91-M0 | 82.4 | 17.6 | 87.1 | 12.9 |
| 563 | siRNA92-M0 | 60.7 | 39.3 | 93.3 | 6.7 |
| 564 | siRNA93-M0 | 36.6 | 63.4 | 71.0 | 29.0 |
| 585 | siRNA94-M0 | 68.5 | 31.5 | 99.6 | 0.4 |
| 587 | siRNA95-M0 | 63.2 | 36.8 | 93.8 | 6.2 |
| 588 | siRNA96-M0 | 59.7 | 40.3 | 96.2 | 3.8 |
| 589 | siRNA97-M0 | 29.5 | 70.5 | 73.1 | 26.9 |
| 593 | siRNA98-M0 | 58.5 | 41.5 | 99.3 | 0.7 |
| 594 | siRNA99-M0 | 70.2 | 29.8 | 70.8 | 29.2 |
| 595 | siRNA100-M0 | 15.0 | 85.0 | 46.2 | 53.8 |
| 602 | siRNA101-M0 | 17.7 | 82.3 | 52.6 | 47.4 |
| 605 | siRNA102-M0 | 61.8 | 38.2 | 88.4 | 11.6 |
| 606 | siRNA103-M0 | 19.1 | 80.9 | 62.5 | 37.5 |
| 607 | siRNA104-M0 | 70.8 | 29.2 | 65.4 | 34.6 |
| 608 | siRNA105-M0 | 50.4 | 49.6 | 82.4 | 17.6 |
| 609 | siRNA106-M0 | 36.2 | 63.8 | 63.7 | 36.3 |
| 610 | siRNA107-M0 | 61.5 | 38.5 | 69.4 | 30.6 |
| 612 | siRNA108-M0 | 30.1 | 69.9 | 68.4 | 31.6 |
| 613 | siRNA109-M0 | 22.6 | 77.4 | 65.7 | 34.3 |
| 653 | siRNA110-M0 | 20.4 | 79.6 | 70.4 | 29.6 |
| 663 | siRNA111-M0 | 19.1 | 80.9 | 65.1 | 34.9 |
| 665 | siRNA112-M0 | 32.2 | 67.8 | 55.1 | 44.9 |
| 666 | siRNA113-M0 | 34.6 | 65.4 | 58.6 | 41.4 |
| 667 | siRNA114-M0 | 34.2 | 65.8 | 83.3 | 16.7 |
| 669 | siRNA115-M0 | 75.7 | 24.3 | 96.6 | 3.4 |
| 670 | siRNA116-M0 | 26.2 | 73.8 | 65.4 | 34.6 |
| 671 | siRNA117-M0 | 60.5 | 39.5 | 71.7 | 28.3 |
| 673 | siRNA118-M0 | 9.7 | 90.3 | 38.7 | 61.3 |
| 679 | siRNA119-M0 | 55.5 | 44.5 | 84.2 | 15.8 |
| 712 | siRNA120-M0 | 65.2 | 34.8 | 72.5 | 27.5 |
| 771 | siRNA121-M0 | 82.5 | 17.5 | 77.6 | 22.4 |
| 781 | siRNA122-M0 | 20.7 | 79.3 | 52.3 | 47.7 |
| 788 | siRNA123-M0 | 77.9 | 22.1 | 94.1 | 5.9 |
| 841 | siRNA124-M0 | 13.0 | 87.0 | 51.1 | 48.9 |
| 844 | siRNA125-M0 | 7.6 | 92.4 | 28.0 | 72.0 |
| 846 | siRNA126-M0 | 61.9 | 38.1 | 84.7 | 15.3 |
| 850 | siRNA127-M0 | 88.0 | 12.0 | 85.0 | 15.0 |
| 851 | siRNA128-M0 | 72.2 | 27.8 | 85.3 | 14.7 |
| 852 | siRNA129-M0 | 48.8 | 51.2 | 73.3 | 26.7 |
| 861 | siRNA130-M0 | 84.9 | 15.1 | 72.1 | 27.9 |
| 862 | siRNA131-M0 | 50.4 | 49.6 | 79.9 | 20.1 |
| 901 | siRNA132-M0 | 98.1 | 1.9 | 79.6 | 20.4 |
| 903 | siRNA133-M0 | 99.1 | 0.9 | 93.7 | 6.3 |
| 904 | siRNA134-M0 | 79.7 | 20.3 | 75.1 | 24.9 |
| 905 | siRNA135-M0 | 84.8 | 15.2 | 100.5 | -0.5 |
| 906 | siRNA136-M0 | 70.7 | 29.3 | 86.1 | 13.9 |
| 912 | siRNA137-M0 | 35.4 | 64.6 | 90.7 | 9.3 |
| 915 | siRNA138-M0 | 43.9 | 56.1 | 86.4 | 13.6 |
| 916 | siRNA139-M0 | 63.9 | 36.1 | 91.5 | 8.5 |
| 917 | siRNA140-M0 | 41.6 | 58.4 | 80.4 | 19.6 |
| 920 | siRNA141-M0 | 20.2 | 79.8 | 74.2 | 25.8 |
| 921 | siRNA142-M0 | 104.4 | -4.4 | 74.8 | 25.2 |

| | | | | | |
|---|---|---|---|---|---|
| 950 | siRNA143-M0 | 67.8 | 32.2 | 55.3 | 44.7 |
| 954 | siRNA144-M0 | 28.9 | 71.1 | 79.0 | 21.0 |
| 955 | siRNA145-M0 | 61.3 | 38.7 | 71.7 | 28.3 |
| 956 | siRNA146-M0 | 70.6 | 29.4 | 74.0 | 26.0 |
| 957 | siRNA147-M0 | 93.4 | 6.6 | 82.9 | 17.1 |
| 980 | siRNA148-M0 | 63.1 | 36.9 | 96.1 | 3.9 |
| 982 | siRNA149-M0 | 40.3 | 59.7 | 72.4 | 27.6 |
| 983 | siRNA150-M0 | 72.8 | 27.2 | 80.2 | 19.8 |
| 988 | siRNA151-M0 | 46.7 | 53.3 | 84.6 | 15.4 |
| 989 | siRNA152-M0 | 18.4 | 81.6 | 60.0 | 40.0 |
| 992 | siRNA153-M0 | 43.2 | 56.8 | 63.3 | 36.7 |
| 993 | siRNA154-M0 | 47.7 | 52.3 | 81.5 | 18.5 |
| 994 | siRNA155-M0 | 53.3 | 46.7 | 64.3 | 35.7 |
| 995 | siRNA156-M0 | 24.7 | 75.3 | 77.8 | 22.2 |
| 996 | siRNA157-M0 | 17.3 | 82.7 | 73.5 | 26.5 |
| 1005 | siRNA158-M0 | 10.9 | 89.1 | 49.9 | 50.1 |
| 1006 | siRNA159-M0 | 49.9 | 50.1 | 80.6 | 19.4 |
| 1007 | siRNA160-M0 | 28.5 | 71.5 | 71.7 | 28.3 |
| 1011 | siRNA161-M0 | 20.7 | 79.3 | 33.4 | 66.6 |
| 1012 | siRNA162-M0 | 42.4 | 57.6 | 84.6 | 15.4 |
| 1013 | siRNA163-M0 | 81.6 | 18.4 | 83.4 | 16.6 |
| 1018 | siRNA164-M0 | 84.5 | 15.5 | 89.5 | 10.5 |
| 1019 | siRNA165-M0 | 24.9 | 75.1 | 55.2 | 44.8 |
| 1020 | siRNA166-M0 | 25.7 | 74.3 | 76.2 | 23.8 |
| 1021 | siRNA167-M0 | 67.7 | 32.3 | 83.9 | 16.1 |
| 1026 | siRNA168-M0 | 55.9 | 44.1 | 76.2 | 23.8 |
| 1027 | siRNA169-M0 | 75.7 | 24.3 | 71.9 | 28.1 |
| 1029 | siRNA170-M0 | 85.4 | 14.6 | 100.6 | -0.6 |
| 1036 | siRNA171-M0 | 39.8 | 60.2 | 68.1 | 31.9 |
| 1039 | siRNA172-M0 | 9.0 | 91.0 | 31.4 | 68.6 |
| 1042 | siRNA173-M0 | 49.2 | 50.8 | 86.8 | 13.2 |
| 1084 | siRNA174-M0 | 10.5 | 89.5 | 33.0 | 67.0 |
| 1087 | siRNA175-M0 | 39.2 | 60.8 | 73.1 | 26.9 |
| 1134 | siRNA176-M0 | 81.8 | 18.2 | 87.0 | 13.0 |
| 1151 | siRNA177-M0 | 67.9 | 32.1 | 88.7 | 11.3 |
| 1152 | siRNA178-M0 | 41.0 | 59.0 | 90.1 | 9.9 |
| 1155 | siRNA179-M0 | 46.8 | 53.2 | 71.1 | 28.9 |
| 1158 | siRNA180-M0 | 62.8 | 37.2 | 81.5 | 18.5 |
| 1160 | siRNA181-M0 | 87.0 | 13.0 | 90.4 | 9.6 |
| 1162 | siRNA182-M0 | 28.9 | 71.1 | 94.0 | 6.0 |
| 1165 | siRNA183-M0 | 73.3 | 26.7 | 71.3 | 28.7 |
| 1168 | siRNA184-M0 | 29.1 | 70.9 | 76.2 | 23.8 |
| 1169 | siRNA185-M0 | 19.9 | 80.1 | 76.9 | 23.1 |
| 1174 | siRNA186-M0 | 42.3 | 57.7 | 69.9 | 30.1 |
| 1177 | siRNA187-M0 | 32.2 | 67.8 | 83.9 | 16.1 |
| 1178 | siRNA188-M0 | 49.5 | 50.5 | 101.8 | -1.8 |
| 1189 | siRNA189-M0 | 76.9 | 23.1 | 76.3 | 23.7 |
| 1191 | siRNA190-M0 | 19.6 | 80.4 | 33.9 | 66.1 |
| 1194 | siRNA191-M0 | 63.8 | 36.2 | 94.1 | 5.9 |
| 1196 | siRNA192-M0 | 85.1 | 14.9 | 83.6 | 16.4 |
| 1200 | siRNA193-M0 | 48.4 | 51.6 | 78.3 | 21.7 |
| 1201 | siRNA194-M0 | 25.3 | 74.7 | 60.4 | 39.6 |
| 1203 | siRNA195-M0 | 63.4 | 36.6 | 92.5 | 7.5 |
| 1204 | siRNA196-M0 | 192.8 | -92.8 | 147.7 | -47.7 |
| 1205 | siRNA197-M0 | 17.4 | 82.6 | 58.1 | 41.9 |
| 1207 | siRNA198-M0 | 40.2 | 59.8 | 82.9 | 17.1 |
| 1209 | siRNA199-M0 | 14.3 | 85.7 | 50.9 | 49.1 |

| | | | | | |
|---|---|---|---|---|---|
| 1212 | siRNA200-M0 | 19.7 | 80.3 | 67.2 | 32.8 |
| 1214 | siRNA201-M0 | 26.4 | 73.6 | 81.1 | 18.9 |
| 1215 | siRNA202-M0 | 23.8 | 76.2 | 60.0 | 40.0 |
| 1217 | siRNA203-M0 | 14.6 | 85.4 | 44.6 | 55.4 |
| 1226 | siRNA204-M0 | 69.0 | 31.0 | 92.1 | 7.9 |
| 1227 | siRNA205-M0 | 10.9 | 89.1 | 32.1 | 67.9 |
| 1228 | siRNA206-M0 | 9.2 | 90.8 | 44.2 | 55.8 |
| 1229 | siRNA207-M0 | 23.4 | 76.6 | 78.7 | 21.3 |
| 1230 | siRNA208-M0 | 16.8 | 83.2 | 70.6 | 29.4 |
| 1231 | siRNA209-M0 | 18.5 | 81.5 | 72.0 | 28.0 |
| 1233 | siRNA210-M0 | 15.8 | 84.2 | 71.0 | 29.0 |
| 1236 | siRNA211-M0 | 73.4 | 26.6 | 80.9 | 19.1 |
| 1237 | siRNA212-M0 | 24.5 | 75.5 | 44.1 | 55.9 |
| 1238 | siRNA213-M0 | 36.5 | 63.5 | 98.3 | 1.7 |
| 1239 | siRNA214-M0 | 41.1 | 58.9 | 79.0 | 21.0 |
| 1240 | siRNA215-M0 | 16.5 | 83.5 | 65.4 | 34.6 |
| 1241 | siRNA216-M0 | 18.8 | 81.2 | 55.0 | 45.0 |
| 1242 | siRNA217-M0 | 13.0 | 87.0 | 53.6 | 46.4 |
| 1248 | siRNA218-M0 | 42.3 | 57.7 | 57.4 | 42.6 |
| 1249 | siRNA219-M0 | 37.1 | 62.9 | 67.3 | 32.7 |
| 1261 | siRNA220-M0 | 30.8 | 69.2 | 77.4 | 22.6 |
| 1283 | siRNA221-M0 | 52.0 | 48.0 | 88.6 | 11.4 |
| 1285 | siRNA222-M0 | 91.2 | 8.8 | 88.3 | 11.7 |
| 1286 | siRNA223-M0 | 54.9 | 45.1 | 88.2 | 11.8 |
| 1288 | siRNA224-M0 | 49.7 | 50.3 | 79.4 | 20.6 |
| 1290 | siRNA225-M0 | 26.9 | 73.1 | 77.2 | 22.8 |
| 1291 | siRNA226-M0 | 42.0 | 58.0 | 47.1 | 52.9 |
| 1292 | siRNA227-M0 | 38.2 | 61.8 | 72.9 | 27.1 |
| 1293 | siRNA228-M0 | 31.7 | 68.3 | 71.0 | 29.0 |
| 1296 | siRNA229-M0 | 33.3 | 66.7 | 72.3 | 27.7 |
| 1297 | siRNA230-M0 | 34.0 | 66.0 | 78.5 | 21.5 |
| 1298 | siRNA231-M0 | 18.8 | 81.2 | 39.3 | 60.7 |
| 1299 | siRNA232-M0 | 40.9 | 59.1 | 76.8 | 23.2 |
| 1300 | siRNA233-M0 | 131.7 | -31.7 | 83.1 | 16.9 |
| 1301 | siRNA234-M0 | 31.8 | 68.2 | 74.9 | 25.1 |
| 1302 | siRNA235-M0 | 61.4 | 38.6 | 80.7 | 19.3 |
| 1303 | siRNA236-M0 | 31.1 | 68.9 | 69.7 | 30.3 |
| 1306 | siRNA237-M0 | 24.4 | 75.6 | 67.8 | 32.2 |
| 1307 | siRNA238-M0 | 39.1 | 60.9 | 84.7 | 15.3 |
| 1311 | siRNA239-M0 | 42.0 | 58.0 | 65.8 | 34.2 |
| 1313 | siRNA240-M0 | 55.4 | 44.6 | 87.5 | 12.5 |
| 1314 | siRNA241-M0 | 24.0 | 76.0 | 80.3 | 19.7 |
| 1353 | siRNA242-M0 | 19.9 | 80.1 | 68.9 | 31.1 |
| 1354 | siRNA243-M0 | 54.1 | 45.9 | 74.2 | 25.8 |
| 1358 | siRNA244-M0 | 77.0 | 23.0 | 84.7 | 15.3 |
| 1360 | siRNA245-M0 | 23.7 | 76.3 | 61.7 | 38.3 |
| 1361 | siRNA246-M0 | 48.1 | 51.9 | 82.5 | 17.5 |
| 1363 | siRNA247-M0 | 26.4 | 73.6 | 83.7 | 16.3 |
| 1364 | siRNA248-M0 | 24.1 | 75.9 | 67.2 | 32.8 |
| 1382 | siRNA249-M0 | 56.8 | 43.2 | 96.7 | 3.3 |
| 1386 | siRNA250-M0 | 16.1 | 83.9 | 44.4 | 55.6 |
| 1388 | siRNA251-M0 | 21.5 | 78.5 | 64.7 | 35.3 |
| 1389 | siRNA252-M0 | 28.5 | 71.5 | 83.3 | 16.7 |
| 1413 | siRNA253-M0 | 35.5 | 64.5 | 77.6 | 22.4 |
| 1415 | siRNA254-M0 | 34.9 | 65.1 | 76.4 | 23.6 |
| 1416 | siRNA255-M0 | 61.0 | 39.0 | 75.9 | 24.1 |
| 1417 | siRNA256-M0 | 14.6 | 85.4 | 29.7 | 70.3 |

| | | | | | |
|---|---|---|---|---|---|
| 1424 | siRNA257-M0 | 25.8 | 74.2 | 76.1 | 23.9 |
| 1425 | siRNA258-M0 | 61.4 | 38.6 | 95.1 | 4.9 |
| 1431 | siRNA259-M0 | 18.1 | 81.9 | 61.2 | 38.8 |
| 1433 | siRNA260-M0 | 13.9 | 86.1 | 52.7 | 47.3 |
| 1506 | siRNA261-M0 | 75.2 | 24.8 | 97.4 | 2.6 |
| 1507 | siRNA262-M0 | 68.1 | 31.9 | 87.1 | 12.9 |
| 1510 | siRNA263-M0 | 94.6 | 5.4 | 88.2 | 11.8 |
| 1511 | siRNA264-M0 | 220.8 | -120.8 | 89.1 | 10.9 |
| 1513 | siRNA265-M0 | 69.5 | 30.5 | 80.5 | 19.5 |
| 1518 | siRNA266-M0 | 73.7 | 26.3 | 87.5 | 12.5 |
| 1555 | siRNA267-M0 | 77.4 | 22.6 | 83.5 | 16.5 |
| 1556 | siRNA268-M0 | 61.1 | 38.9 | 72.1 | 27.9 |
| 1557 | siRNA269-M0 | 66.4 | 33.6 | 66.5 | 33.5 |
| 1558 | siRNA270-M0 | 42.5 | 57.5 | 79.8 | 20.2 |
| 1559 | siRNA271-M0 | 75.3 | 24.7 | 81.0 | 19.0 |
| 1561 | siRNA272-M0 | 20.7 | 79.3 | 39.8 | 60.2 |
| 1593 | siRNA273-M0 | 99.5 | 0.5 | 95.6 | 4.4 |
| 1594 | siRNA274-M0 | 81.6 | 18.4 | 77.0 | 23.0 |
| 1597 | siRNA275-M0 | 70.3 | 29.7 | 99.0 | 1.0 |
| 1599 | siRNA276-M0 | 89.3 | 10.7 | 74.0 | 26.0 |
| 1601 | siRNA277-M0 | 86.2 | 13.8 | 69.5 | 30.5 |
| 1605 | siRNA278-M0 | 83.1 | 16.9 | 84.7 | 15.3 |
| 1606 | siRNA279-M0 | 87.5 | 12.5 | 74.1 | 25.9 |
| 1609 | siRNA280-M0 | 92.3 | 7.7 | 70.9 | 29.1 |
| 1610 | siRNA281-M0 | 77.7 | 22.3 | 81.0 | 19.0 |
| 1614 | siRNA282-M0 | 76.2 | 23.8 | 76.5 | 23.5 |
| 1638 | siRNA283-M0 | 91.0 | 9.0 | 87.1 | 12.9 |
| 1660 | siRNA284-M0 | 83.7 | 16.3 | 87.9 | 12.1 |
| 1661 | siRNA285-M0 | 88.0 | 12.0 | 74.9 | 25.1 |
| 1662 | siRNA286-M0 | 95.5 | 4.5 | 84.6 | 15.4 |
| 1663 | siRNA287-M0 | 100.1 | -0.1 | 88.9 | 11.1 |
| 1669 | siRNA288-M0 | 84.0 | 16.0 | 85.6 | 14.4 |
| 1671 | siRNA289-M0 | 81.0 | 19.0 | 76.3 | 23.7 |
| 1673 | siRNA290-M0 | 66.1 | 33.9 | 84.0 | 16.0 |
| 1676 | siRNA291-M0 | 70.8 | 29.2 | 60.1 | 39.9 |
| 1683 | siRNA292-M0 | 93.8 | 6.2 | 87.5 | 12.5 |
| 1686 | siRNA293-M0 | 89.6 | 10.4 | 79.9 | 20.1 |
| 1689 | siRNA294-M0 | 72.5 | 27.5 | 86.6 | 13.4 |
| 1690 | siRNA295-M0 | 78.9 | 21.1 | 84.9 | 15.1 |
| 1692 | siRNA296-M0 | 82.3 | 17.7 | 83.8 | 16.2 |
| 1712 | siRNA297-M0 | 85.0 | 15.0 | 67.3 | 32.7 |
| 1713 | siRNA298-M0 | 81.0 | 19.0 | 82.0 | 18.0 |
| 1769 | siRNA299-M0 | 89.8 | 10.2 | 72.7 | 27.3 |
| 1770 | siRNA300-M0 | 84.2 | 15.8 | 91.3 | 8.7 |
| 1824 | siRNA301-M0 | 95.1 | 4.9 | 88.3 | 11.7 |
| 1825 | siRNA302-M0 | 82.2 | 17.8 | 82.2 | 17.8 |
| 1826 | siRNA303-M0 | 94.2 | 5.8 | 97.6 | 2.4 |
| 1827 | siRNA304-M0 | 90.7 | 9.3 | 70.4 | 29.6 |
| 1828 | siRNA305-M0 | 73.2 | 26.8 | 97.4 | 2.6 |
| 1829 | siRNA306-M0 | 84.5 | 15.5 | 82.5 | 17.5 |
| 1830 | siRNA307-M0 | 87.6 | 12.4 | 72.7 | 27.3 |
| 1895 | siRNA308-M0 | 73.2 | 26.8 | 93.4 | 6.6 |
| 2052 | siRNA309-M0 | 89.0 | 11.0 | 71.4 | 28.6 |
| 2053 | siRNA310-M0 | 82.2 | 17.8 | 82.6 | 17.4 |
| 2075 | siRNA311-M0 | 87.6 | 12.4 | 94.8 | 5.2 |
| 2127 | siRNA312-M0 | 70.3 | 29.7 | 83.7 | 16.3 |
| 2128 | siRNA313-M0 | 88.9 | 11.1 | 75.2 | 24.8 |

| | | | | | |
|---|---|---|---|---|---|
| 2132 | siRNA314-M0 | 81.8 | 18.2 | 96.2 | 3.8 |
| 2133 | siRNA315-M0 | 76.5 | 23.5 | 83.6 | 16.4 |
| 2134 | siRNA316-M0 | 77.5 | 22.5 | 102.2 | -2.2 |
| 2135 | siRNA317-M0 | 215.9 | -115.9 | 84.5 | 15.5 |
| 2136 | siRNA318-M0 | 74.7 | 25.3 | 91.1 | 8.9 |
| 2137 | siRNA319-M0 | 77.0 | 23.0 | 77.9 | 22.1 |
| 2139 | siRNA320-M0 | 79.1 | 20.9 | 89.0 | 11.0 |
| 2140 | siRNA321-M0 | 91.0 | 9.0 | 87.2 | 12.8 |
| 2144 | siRNA322-M0 | 92.0 | 8.0 | 109.0 | -9.0 |
| 2189 | siRNA323-M0 | 91.3 | 8.7 | 75.1 | 24.9 |
| 2190 | siRNA324-M0 | 66.9 | 33.1 | 95.5 | 4.5 |
| 2191 | siRNA325-M0 | 90.3 | 9.7 | 76.3 | 23.7 |
| 2192 | siRNA326-M0 | 80.4 | 19.6 | 89.2 | 10.8 |
| 2193 | siRNA327-M0 | 68.6 | 31.4 | 90.3 | 9.7 |
| 2194 | siRNA328-M0 | 87.0 | 13.0 | 67.6 | 32.4 |
| 2195 | siRNA329-M0 | 70.1 | 29.9 | 98.0 | 2.0 |
| 2199 | siRNA330-M0 | 80.0 | 20.0 | 74.5 | 25.5 |
| 2209 | siRNA331-M0 | 67.3 | 32.7 | 94.6 | 5.4 |
| 2210 | siRNA332-M0 | 91.9 | 8.1 | 72.9 | 27.1 |
| 2211 | siRNA333-M0 | 78.6 | 21.4 | 75.7 | 24.3 |
| 2215 | siRNA334-M0 | 86.8 | 13.2 | 73.1 | 26.9 |
| 2216 | siRNA335-M0 | 70.6 | 29.4 | 98.7 | 1.3 |
| 2217 | siRNA336-M0 | 89.4 | 10.6 | 77.7 | 22.3 |
| 2218 | siRNA337-M0 | 78.0 | 22.0 | 103.6 | -3.6 |
| 2220 | siRNA338-M0 | 77.2 | 22.8 | 107.3 | -7.3 |
| 2221 | siRNA339-M0 | 76.6 | 23.4 | 87.1 | 12.9 |
| 2223 | siRNA340-M0 | 78.4 | 21.6 | 84.5 | 15.5 |
| 2224 | siRNA341-M0 | 88.3 | 11.7 | 76.5 | 23.5 |
| 2225 | siRNA342-M0 | 73.1 | 26.9 | 64.5 | 35.5 |
| 2313 | siRNA343-M0 | 100.1 | -0.1 | 78.6 | 21.4 |
| 2316 | siRNA344-M0 | 78.0 | 22.0 | 80.5 | 19.5 |
| 2317 | siRNA345-M0 | 91.2 | 8.8 | 94.7 | 5.3 |
| 2319 | siRNA346-M0 | 87.0 | 13.0 | 76.9 | 23.1 |
| 2320 | siRNA347-M0 | 67.4 | 32.6 | 90.1 | 9.9 |
| 2321 | siRNA348-M0 | 82.4 | 17.6 | 93.8 | 6.2 |
| 2353 | siRNA349-M0 | 87.9 | 12.1 | 71.9 | 28.1 |
| 2355 | siRNA350-M0 | 92.7 | 7.3 | 73.7 | 26.3 |
| 2356 | siRNA351-M0 | 89.1 | 10.9 | 70.4 | 29.6 |
| 2362 | siRNA352-M0 | 78.1 | 21.9 | 78.9 | 21.1 |
| 2364 | siRNA353-M0 | 71.1 | 28.9 | 108.2 | -8.2 |
| 2365 | siRNA354-M0 | 78.5 | 21.5 | 86.1 | 13.9 |
| 2366 | siRNA355-M0 | 80.7 | 19.3 | 96.5 | 3.5 |
| 2370 | siRNA356-M0 | 80.5 | 19.5 | 85.4 | 14.6 |
| 2503 | siRNA357-M0 | 71.6 | 28.4 | 72.1 | 27.9 |
| 2562 | siRNA358-M0 | 87.5 | 12.5 | 79.4 | 20.6 |
| 2563 | siRNA359-M0 | 96.3 | 3.7 | 78.3 | 21.7 |
| 2620 | siRNA360-M0 | 87.4 | 12.6 | 73.9 | 26.1 |
| 2621 | siRNA361-M0 | 66.9 | 33.1 | 90.9 | 9.1 |
| 2622 | siRNA362-M0 | 83.8 | 16.2 | 79.2 | 20.8 |
| 2623 | siRNA363-M0 | 84.1 | 15.9 | 76.1 | 23.9 |
| 2624 | siRNA364-M0 | 83.8 | 16.2 | 88.9 | 11.1 |
| 2625 | siRNA365-M0 | 78.8 | 21.2 | 81.5 | 18.5 |
| 2629 | siRNA366-M0 | 71.5 | 28.5 | 87.7 | 12.3 |
| 2630 | siRNA367-M0 | 96.6 | 3.4 | 87.7 | 12.3 |
| 2632 | siRNA368-M0 | 96.0 | 4.0 | 85.8 | 14.2 |
| 2633 | siRNA369-M0 | 80.4 | 19.6 | 92.6 | 7.4 |
| 2635 | siRNA370-M0 | 185.9 | -85.9 | 80.3 | 19.7 |

| | | | | | |
|---|---|---|---|---|---|
| 2639 | siRNA371-M0 | 97.2 | 2.8 | 75.4 | 24.6 |
| 2679 | siRNA372-M0 | 69.8 | 30.2 | 97.7 | 2.3 |
| 2682 | siRNA373-M0 | 92.1 | 7.9 | 84.9 | 15.1 |
| 2683 | siRNA374-M0 | 78.0 | 22.0 | 86.0 | 14.0 |
| 2687 | siRNA375-M0 | 79.6 | 20.4 | 79.1 | 20.9 |
| 2690 | siRNA376-M0 | 79.8 | 20.2 | 81.5 | 18.5 |
| 2691 | siRNA377-M0 | 99.8 | 0.2 | 79.3 | 20.7 |
| 2692 | siRNA378-M0 | 78.3 | 21.7 | 79.0 | 21.0 |
| 2833 | siRNA379-M0 | 78.2 | 21.8 | 82.1 | 17.9 |
| 2834 | siRNA380-M0 | 193.4 | -93.4 | 88.9 | 11.1 |
| 2835 | siRNA381-M0 | 94.4 | 5.6 | 82.8 | 17.2 |
| 2838 | siRNA382-M0 | 81.5 | 18.5 | 158.5 | -58.5 |
| 2839 | siRNA383-M0 | 76.9 | 23.1 | 80.2 | 19.8 |
| 2858 | siRNA384-M0 | 85.5 | 14.5 | 75.3 | 24.7 |

*'*siRNA# in Table 6 correspond to the siRNA# in Table 5B*

**Example 4: *In vivo* Screening of siRNA Agents in Mouse**

Initial Screening

Based in the *in vitro* screening results, 30 target sequences were selected for further study. siRNAs were then prepared as fully chemically modified siRNA conjugated to a targeting moiety comprising a structure of Formula ($Z^6$). A combination of 2'-O-methyl-RNA phosphoramidites and 2'-Deoxy-2'-fluoro-RNA phosphoramidites were used to obtain siRNA for *in vivo* study (FIG. 1, Table 7B). Monomer phosphoramidites were used to incorporate the targeting moiety at the 3'-end of the sense strand. Table 7A provides the nucleobase sequences of the siRNAs. It is to be understood that any chemical modification pattern may be applied to the nucleobase sequences of the siRNAs in Table 7A. For the initial screening, modification pattern MOD3 (see FIG. 1) was used. Table 7B below lists the exemplary siRNAs used for the initial *in vivo* screening in mouse.

Chemically Modified Duplex siRNA Preparation

Oligoribonucleotides were synthesized according to the phosphoramidite technology on solid phase employing a Mermade 12 synthesizer (LGC Bioautomation). Syntheses were performed on a solid support made of controlled pore glass (CPG). Specifically, oligonucleotides were assembled on a 500 Å universal solid support with a loading of 89.3 µmol/g, purchased from Chemgenes. All 2'-modified RNA phosphoramidites as well as ancillary reagents were purchased from commercially available resources. Specifically, the following 2'-O-Methyl phosphoramidites were used: (5'-O-dimethoxytrityl-N6-(benzoyl)-2'-O-methyl-adenosine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, 5'-O-dimethoxytrityl-N4-(acetyl)-2'-O-methyl-cytidine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, (5'-

O-dimethoxytrityl-N2-(isobutyryl)-2'-O-methyl-guanosine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, and 5'-O-dimethoxytrityl-2'-O-methyl-uridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite. The 2'-Deoxy-2'-fluoro-phosphoramidites carried the same protecting groups as the 2'-O-methyl RNA amidites. For introduction of the targeting moiety, 1-O-GalNAc- 5-O-dimethoxytrityl-2-O-methyl-ribose-3-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite was used. All phosphoramidites were dissolved in anhydrous acetonitrile (100 mM) besides 5'-O-dimethoxytrityl-2'-O-methyl-uridine-3'-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite which was dissolved in a 20% dimethylformamide/acetonitrile solution (100 mM). Molecular sieves (3Å) were added to all amidite solutions and reagent solutions 6 hours before the synthesis. 5-Ethyl thiotetrazole (ETT, 500 mM in acetonitrile) was used as an activator. Coupling times were 6 minutes. In order to introduce phosphorothioate linkages a 50 mM solution of 3-((Dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from Chemgenes, Wimington, MA, USA) in anhydrous acetonitrile/pyridine (1:1 v/v) was employed. Oligonucleotides were synthesized with removal of the final DMT protecting group ("DMT off").

Cleavage And Deprotection of Support Bound Oligomer

After finalization of the solid phase synthesis, oligonucleotides were cleaved from the solid support by the addition a mixture consisting of 5 wt % diethylamine in concentrated aqueous ammonia, both available from Sigma Aldrich). To achieve quantitative removal of all protecting groups, the solutions were incubated with shaking at 35°C for 24 hours. The crude suspension was filtered through a 0.45 micron Millex PVDF filter, washed the solid support with additional water, then concentrated the filtrate in vacuo to remove the diethylamine and aqueous ammoniaresidues.

Purification Of Oligoribonucleotides

Crude oligomers were purified by anionic exchange HPLC using SourceQ column (GE Healthcare, Freiburg, Germany) on an AKTA Purifier system (GE Healthcare, Freiburg, Germany). Buffer A was 20 mM sodium phosphate, pH 8 and contained 10% acetonitrile and buffer B contained 1M sodium bromide in buffer A. A flow rate of 10 mL/min and a gradient starting from 25% to 65% buffer B was employed. UV traces at 260 and 280 nm were recorded to monitor product elution. Appropriate fractions were pooled and analyzed via mass spec. Pooled oligonucleotides were desalted via size exclusion chromatography on an AKTA Purifier

system (GE Healthcare, Freiburg, Germany) utilizing Milli-Q filtered water. Desalted oligonucleotides were then frozen over dry ice and lyophilized.

Annealing of Oligoribonucleotides to Generate siRNA

Complementary strands were dissolved in Milli-Q or DNAse/RNAse water, then mixed at an equimolar ratio. The mixtures were annealed using ThermoMixer C equipment (Eppendorf). It was shaked at 400 rpm and heated at 90 ˚C for 15 minutes, then slowly reduced to 15 ˚C. An aliquot was sampled and analyzed using UPLC/MS (QTOF or Orbitrap) to confirm completion and proper of annealing process.

Table 7A: Nucleobase sequences of exemplary siRNAs‡

| Position | siRNA#* | Sense Strand Sequence | SEQ ID NO: | Anti-Sense Strand Sequence | SEQ ID NO: |
|---|---|---|---|---|---|
| 223 | siRNA27' | UCUUGAGUUCCUCAGAGCAAA | 419 | UUUGCUCUGAGGAACUCAAGAAG | 1161 |
| 844 | siRNA125' | GCGCAUGUUUCUCAAUGACAA | 517 | UUGUCAUUGAGAAACAUGCGCAG | 1162 |
| 1417 | siRNA256' | UAUCCACGAAAUCAAGCAAUA | 648 | UAUUGCUUGAUUUCGUGGAUAAG | 1163 |
| 1039 | siRNA172' | AAAAGUCAGCUUGGAAGGCAA | 564 | UUGCCUUCCAAGCUGACUUUUAG | 1164 |
| 1227 | siRNA205' | UAGCUCUUUACCCACAGUUAA | 597 | UUAACUGUGGGUAAAGAGCUAAG | 1165 |
| 1084 | siRNA174' | ACUGAAUGACCUGCCAGUAUA | 566 | UAUACUGGCAGGUCAUUCAGUAG | 1166 |
| 1011 | siRNA161' | UGAAAAGAACAUUAGAGAAUA | 553 | UAUUCUCUAAUGUUCUUUUCAAG | 1167 |
| 1191 | siRNA190' | AGGACGGUUCCUACAACAUCA | 582 | UGAUGUUGUAGGAACCGUCCUAG | 1168 |
| 292 | siRNA47' | UGUCCAUUUCAUCACAAAUCA | 439 | UGAUUUGUGAUGAAAUGGACAAG | 1169 |
| 673 | siRNA118' | UCUAAACAAUCUUGACAACUA | 510 | UAGUUGUCAAGAUUGUUUAGAAG | 1170 |
| 1298 | siRNA231' | AGGUAUCUUGAUGAAAACGGA | 623 | UCCGUUUUCAUCAAGAUACCUAG | 1171 |
| 1561 | siRNA272' | AUAUAAAUUCAAGCAUUUGUA | 664 | UACAAAUGCUUGAAUUUAUAUAG | 1172 |
| 1237 | siRNA212' | CCCACAGUUAAUGCACUUAGA | 604 | UCUAAGUGCAUUAACUGUGGGAG | 1173 |
| 1228 | siRNA206' | AGCUCUUUACCCACAGUUAAA | 598 | UUUAACUGUGGGUAAAGAGCUAG | 1174 |
| 1386 | siRNA250' | GAGCUACAAUAUGUCCUGGAA | 642 | UUCCAGGACAUAUUGUAGCUCAG | 1175 |
| 1217 | siRNA203' | GAUGACAUCAUAGCUCUUUAA | 595 | UUAAAGAGCUAUGAUGUCAUCAG | 1176 |
| 478 | siRNA73' | CUUGAAUUCCCUCACGGAAAA | 465 | UUUUCCGUGAGGGAAUUCAAGAG | 1177 |
| 595 | siRNA100' | CCGAGUGAUGUUUGAAGCUGA | 492 | UCAGCUUCAAACAUCACUCGGAG | 1178 |
| 1291 | siRNA226' | AUAUGAUAGGUAUCUUGAUGA | 618 | UCAUCAAGAUACCUAUCAUAUAG | 1179 |
| 115 | siRNA12' | UUGUCUAUGGCUUAUUCUUGA | 404 | UCAAGAAUAAGCCAUAGACAAAG | 1180 |
| 477 | siRNA72' | CCUUGAAUUCCCUCACGGAAA | 464 | UUUCCGUGAGGGAAUUCAAGGAG | 1181 |
| 1005 | siRNA158' | AAGAAGUGAAAAGAACAUUAA | 550 | UUAAUGUUCUUUUCACUUCUUAG | 1182 |
| 251 | siRNA38' | AAACAUGGUCAUGUUUUUACA | 430 | UGUAAAAAACAUGACCAUGUUUAG | 1183 |
| 303 | siRNA51'- | UCACAAAUCCCUUGUCAUACA | 443 | UGUAUGACAAGGGAUUUGUGAAG | 1184 |
| 1209 | siRNA199' | UCCGAAAAGAUGACAUCAUAA | 591 | UUAUGAUGUCAUCUUUUCGGAAG | 1185 |
| 841 | siRNA124' | CCUGCGCAUGUUUCUCAAUGA | 516 | UCAUUGAGAAACAUGCGCAGGAG | 1186 |
| 781 | siRNA122' | GGCAGAGAGCUUGAGGCACGA | 514 | UCGUGCCUCAAGCUCUCUGCCAG | 1187 |
| 602 | siRNA101' | AUGUUUGAAGCUGGGUAUUUA | 493 | UAAAUACCCAGCUUCAAACAUAG | 1188 |

| | | | | | |
|---|---|---|---|---|---|
| 1433 | siRNA260' | CAAUUUUUGAUUCUGAUGCUA | 652 | UAGCAUCAGAAUCAAAAAUUGAG | 1189 |
| 506 | siRNA81' | GAAAACCUCCAACGUAUCAUA | 473 | UAUGAUACGUUGGAGGUUUUCAG | 1190 |

**The ' after an siRNA# (e.g., siRNA#') indicates that the siRNA correspond to the same siRNA# in Table 5A, where the siRNA# and siRNA#' has the same sense strand nucleobase sequence, but the siRNA#' has the last two nucleobases of the antisense strand (counting 5'- 3') changed from "UU" to "AG", relative to siRNA#.*
*‡Each uracil base (U) in any one of the sequences provided in Table 7A may independently and optionally be replaced with a thymine base (T).*

Table 7B: List of Chemically Modified Duplexes Prepared for the initial *in vivo* Screening‡

| Position | siRNA#* | Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified sense strand: | Anti-Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified antisense strand: |
|---|---|---|---|---|---|---|---|
| 223 | siRNA27'-M3^ | [mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] | 419 | 1219 | [mUs][fUs][fU][mG][fC][fU][fC][fU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] | 1161 | 1989 |
| 844 | siRNA125'-M3 | [mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] | 517 | 1329 | [mUs][fUs][fG][mU][fC][fA][fU][fU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] | 1162 | 1993 |
| 1417 | siRNA256'-M3 | [mUs][mAs][mU][mC][mC][mA][mC][mG][fA][fA][fA][fU][mC][mA][mA][mG][mC][mA][mA][mU][mAs] | 648 | 1474 | [mUs][fAs][fU][mU][fG][fC][fU][fU][mG][fA][mU][mU][mU][fC][mG][mU][mG][mG][mA][mU][mAs][mAs][mG] | 1163 | 1997 |
| 1039 | siRNA172'-M3 | [mAs][mAs][mA][mA][mG][mU][mC][mA][fG][fC][fU][fU][mG][mG][mA][mA][mG][mG][mC][mA][mAs] | 564 | 1379 | [mUs][fUs][fG][mC][fC][fU][fU][fC][mC][fA][mA][mG][mC][fU][mG][mA][mC][mU][mU][mU][mUs][mAs][mG] | 1164 | 1998 |
| 1227 | siRNA205'-M3 | [mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] | 597 | 1417 | [mUs][fUs][fA][mA][fC][fU][fG][fU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] | 1165 | 1999 |
| 1084 | siRNA174'-M3 | [mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] | 566 | 1382 | [mUs][fAs][fU][mA][fC][fU][fG][fG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] | 1166 | 2003 |
| 1011 | siRNA161'-M3 | [mUs][mGs][mA][mA][mA][mA][mG][mA][fA][fC][fA][fU][mU][mA][mG][mA][mG][mA][mA][mU][mAs] | 553 | 1367 | [mUs][fAs][fU][mU][fC][fU][fC][fU][mA][fA][mU][mG][mU][fU][mC][mU][mU][mU][mU][mC][mAs][mAs][mG] | 1167 | 2007 |
| 1191 | siRNA190'-M3 | [mAs][mGs][mG][mA][mC][mG][mG][mU][fU][fC][fC][fU][mA][mC][mA][mA][mC][mA][mU][mC][mAs] | 582 | 1399 | [mUs][fGs][fA][mU][fG][fU][fU][fG][mU][fA][mG][mG][mA][fA][mC][mC][mG][mU][mC][mC][mUs][mAs][mG] | 1168 | 2008 |
| 292 | siRNA47'-M3 | [mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] | 439 | 1241 | [mUs][fGs][fA][mU][fU][fU][fG][fU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] | 1169 | 2009 |
| 673 | siRNA118'-M3 | [mUs][mCs][mU][mA][mA][mA][mC][mA][fA][fU][fC][fU][mU][mG][mA][mC][mA][mA][mC][mU][mAs] | 510 | 1319 | [mUs][fAs][fG][mU][fU][fG][fU][fC][mA][fA][mG][mA][mU][fU][mG][mU][mU][mU][mA][mG][mAs][mAs][mG] | 1170 | 2013 |
| 1298 | siRNA231'-M3 | [mAs][mGs][mG][mU][mA][mU][mC][mU][fU][fG][fA][fU][mG][mA][mA][mA][mA][mC][mG][mG][mAs] | 623 | 1447 | [mUs][fCs][fC][mG][fU][fU][fU][fU][mC][fA][mU][mC][mA][fA][mG][mA][mU][mA][mC][mC][mUs][mAs][mG] | 1171 | 2014 |
| 1561 | siRNA272'-M3 | [mAs][mUs][mA][mU][mA][mA][mA][mU][fU][fC][fA][fA][mG][ | 664 | 1492 | [mUs][fAs][fC][mA][fA][fA][fU][fG][mC][fU][mU][mG][mA][fA | 1172 | 2015 |

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|---|---|---|---|---|---|---|---|
| | | mC][mA][mU][mU][mU][mG][mU][mAs] | | | ][mU][mU][mU][mA][mU][mA][mUs][mAs][mG] | | |
| 1237 | siRNA212’-M3 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][fA][fG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2017 |
| 1228 | siRNA206’-M3 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][fA][fC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2021 |
| 1386 | siRNA250’-M3 | [mGs][mAs][mG][mC][mU][mA][mC][mA][fA][fU][fA][fU][mG][mU][mC][mC][mU][mG][mG][mA][mAs] | 642 | 1467 | [mUs][fUs][fC][mC][fA][fG][fG][fA][mC][fA][mU][mA][mU][fU][mG][mU][mA][mG][mC][mU][mCs][mAs][mG] | 1175 | 2024 |
| 1217 | siRNA203’-M3 | [mGs][mAs][mU][mG][mA][mC][mA][mU][fC][fA][fU][fA][mG][mC][mU][mC][mU][mU][mU][mA][mAs] | 595 | 1414 | [mUs][fUs][fA][mA][fA][fG][fA][fG][mC][fU][mA][mU][mG][fA][mU][mG][mU][mC][mA][mU][mCs][mAs][mG] | 1176 | 2025 |
| 478 | siRNA73’-M3 | [mCs][mUs][mU][mG][mA][mA][mU][mU][fC][fC][fC][fU][mC][mA][mC][mG][mG][mA][mA][mA][mAs] | 465 | 1270 | [mUs][fUs][fU][mU][fC][fC][fG][fU][mG][fA][mG][mG][mG][fA][mA][mU][mU][mC][mA][mA][mGs][mAs][mG] | 1177 | 2026 |
| 595 | siRNA100’-M3 | [mCs][mCs][mG][mA][mG][mU][mG][mA][fU][fG][fU][fU][mU][mG][mA][mA][mG][mC][mU][mG][mAs] | 492 | 1299 | [mUs][fCs][fA][mG][fC][fU][fU][fC][mA][fA][mA][mC][mA][fU][mC][mA][mC][mU][mC][mG][mGs][mAs][mG] | 1178 | 2027 |
| 1291 | siRNA226’-M3 | [mAs][mUs][mA][mU][mG][mA][mU][mA][fG][fG][fU][fA][mU][mC][mU][mU][mG][mA][mU][mG][mAs] | 618 | 1441 | [mUs][fCs][fA][mU][fC][fA][fA][fG][mA][fU][mA][mC][mC][fU][mA][mU][mC][mA][mU][mA][mUs][mAs][mG] | 1179 | 2028 |
| 115 | siRNA12’-M3 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2029 |
| 477 | siRNA72’-M3 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][fC][fG][fU][fG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2034 |
| 1005 | siRNA158’-M3 | [mAs][mAs][mG][mA][mA][mG][mU][mG][fA][fA][fA][fA][mG][mA][mA][mC][mA][mU][mU][mA][mAs] | 550 | 1363 | [mUs][fUs][fA][mA][fU][fG][fU][fU][mC][fU][mU][mU][mU][fC][mA][mC][mU][mU][mC][mU][mUs][mAs][mG] | 1182 | 2037 |
| 251 | siRNA38’-M3 | [mAs][mAs][mA][mC][mA][mU][mG][mG][fU][fC][fA][fU][mG][mU][mU][mU][mU][mU][mA][mC][mAs] | 430 | 1231 | [mUs][fGs][fU][mA][fA][fA][fA][fA][mC][fA][mU][mG][mA][fC][mC][mA][mU][mG][mU][mU][mUs][mAs][mG] | 1183 | 2038 |
| 303 | siRNA51’-M3 | [mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] | 443 | 1246 | [mUs][fGs][fU][mA][fU][fG][fA][fC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] | 1184 | 2039 |
| 1209 | siRNA199’-M3 | [mUs][mCs][mC][mG][mA][mA][mA][mA][fG][fA][fU][fG][mA][mC][mA][mU][mC][mA][mU][mA][mAs] | 591 | 1409 | [mUs][fUs][fA][mU][fG][fA][fU][fG][mU][fC][mA][mU][mC][fU][mU][mU][mU][mC][mG][mG][mAs][mAs][mG] | 1185 | 2043 |
| 841 | siRNA124’-M3 | [mCs][mCs][mU][mG][mC][mG][mC][mA][fU][fG][fU][fU][mU][mC][mU][mC][mA][mA][mU][mG][mAs] | 516 | 1327 | [mUs][fCs][fA][mU][fU][fG][fA][fG][mA][fA][mA][mC][mA][fU][mG][mC][mG][mC][mA][mG][mGs][mAs][mG] | 1186 | 2044 |
| 781 | siRNA122’-M3 | [mGs][mGs][mC][mA][mG][mA][mG][mA][fG][fC][fU][fU][mG][mA][mG][mG][mC][mA][mC][mG][mAs] | 514 | 1324 | [mUs][fCs][fG][mU][fG][fC][fC][fU][mC][fA][mA][mG][mC][fU][mC][mU][mC][mU][mG][mC][mCs][mAs][mG] | 1187 | 2045 |
| 602 | siRNA101’-M3 | [mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] | 493 | 1301 | [mUs][fAs][fA][mA][fU][fA][fC][fC][mC][fA][mG][mC][mU][fU][mC][mA][mA][mA][mC][mA][mUs][mAs][mG] | 1188 | 2046 |
| 1433 | siRNA260’-M3 | [mCs][mAs][mA][mU][mU][mU][mU][mU][fG][fA][fU][fU][mC][ | 652 | 1479 | [mUs][fAs][fG][mC][fA][fU][fC][fA][mG][fA][mA][mU][mC][fA | 1189 | 2050 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | mU][mG][mA][mU][mG][mC][mU][mAs] | | | ][mA][mA][mA][mA][mU][mU][mGs][mAs][mG] | | |
| 506 | siRNA81'-M3 | [mGs][mAs][mA][mA][mA][mC][mC][mU][fC][fC][fA][fA][mC][mG][mU][mA][mU][mC][mA][mU][mAs] | 473 | 1279 | [mUs][fAs][fU][mG][fA][fU][fA][fC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] | 1190 | 2051 |

**siRNA# in Table 7B correspond to the siRNA# in Table 7A, but with "M3" added to indicate modification pattern*
*^ M3 refers to modification pattern MOD3 as shown in FIG. 1; mA, mC, mG, and mU are 2'-O-methyl modified adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro modified adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate internucleoside linkage.*
*‡Each uracil base (U) in any one of the sequences provided in Table 7B may independently and optionally be replaced with a thymine base (T).*
*All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$).*
*It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand) of a targeting moiety comprising a structure of Formula ($Z^6$).*

**AAV8-human CYP7A1 mouse model**

All animal studies were approved by the Institutional Animal Care and Use Committee at Takeda Development Center Americas. Mice were housed in a pathogen-free animal facility at 22ºC under controlled 12-hour light/12-hour dark cycle. All mice were kept on standard chow diet (PicoLab Rodent Diet 20 – 5053). Male C57Bl/6J mice, 8-10 weeks of age, were purchased from Jackson Laboratories (Sacrament, CA, USA). On day -7, animals received a single intravenous dose of 1 x $10^{11}$ vector genome copies of adeno-associated virus (AAV8 serotype) containing the gene encoding for human CYP7A1. On day 0, animals received a single subcutaneous dose of conjugated siRNA (conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$)). On day 7, day 14, or day 28, 30 mg of liver tissue was collected and snap frozen for RNA isolation and quantitative polymerase chain reaction (qPCR).

RNA Extraction from liver tissue and Real Time PCR

Total RNA was isolated from 30 mg pieces of frozen liver tissues using MagMAX mirVana Total RNA isolation kit (ThermoFisher). Quantitative PCR was carried out in duplicate with 50ng of total RNA in 10uL volume per well in 384-well plates using QuantiTect Multiplex RT-PCR (Qiagen) master mix and predesigned TaqMan primer and probes (ThermoFisher). Human CYP7A1 (Catalog # Hs00928359_g1) was used for target gene knockdown and endogenous control 18S (4319413E) was used as the reference gene. Relative gene expression was determined by using the delta-delta CT method, also known as the $2^{-\Delta\Delta Ct}$ method.

Relative fold gene expression was calculated by the delta-delta Ct method, also known as the $2^{-\Delta\Delta Ct}$ method. Fold change was multiplied by 100 to represent the data as percent (%) mRNA remaining. Results are presented in Table 8.

**Statistical Analysis**

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA or two-way ANOVA followed, if significant, by post-hoc Dunnett's test. Non parametric tests like Mann-Whitney were used when the N was too small, or data did not follow Gaussian distribution. The difference was considered significant when $p< 0.05$.

Table 8: Results of the Initial *in vivo* Screening in Mouse

| Position | siRNA#* | Mean % mRNA Remaining hAAV (3 mg/kg dose level, day 7) | Mean % Knockdown hAAV (3 mg/kg dose level, day 7) |
|---|---|---|---|
| 223 | siRNA27'-M3 | 26.2 | 73.8 |
| 844 | siRNA125'-M3 | 23.4 | 76.6 |
| 1417 | siRNA256'-M3 | 38.6 | 61.4 |
| 1039 | siRNA172'-M3 | 34.7 | 65.3 |
| 1227 | siRNA205'-M3 | 25.5 | 74.5 |
| 1084 | siRNA174'-M3 | 22 | 78 |
| 1011 | siRNA161'-M3 | 50.6 | 49.4 |
| 1191 | siRNA190'-M3 | 38.5 | 61.5 |
| 292 | siRNA47'-M3 | 34.6 | 65.4 |
| 673 | siRNA118'-M3 | 35.8 | 64.2 |
| 1298 | siRNA231'-M3 | 38.3 | 61.7 |
| 1561 | siRNA272'-M3 | 44.2 | 55.8 |
| 1237 | siRNA212'-M3 | 33.6 | 66.4 |
| 1228 | siRNA206'-M3 | 24 | 76 |
| 1386 | siRNA250'-M3 | 53.5 | 46.5 |
| 1217 | siRNA203'-M3 | 58 | 42 |
| 478 | siRNA73'-M3 | 48.7 | 51.3 |
| 595 | siRNA100'-M3 | 45.7 | 54.3 |
| 1291 | siRNA226'-M3 | 41.3 | 58.7 |
| 115 | siRNA12'-M3 | 29.4 | 70.6 |
| 477 | siRNA72'-M3 | 28.3 | 71.7 |
| 1005 | siRNA158'-M3 | 40.9 | 59.1 |
| 251 | siRNA38'-M3 | 42.8 | 57.2 |
| 303 | siRNA51'-M3 | 34.3 | 65.7 |
| 1209 | siRNA199'-M3 | 52 | 48 |
| 841 | siRNA124'-M3 | 35.2 | 64.8 |
| 781 | siRNA122'-M3 | 45.3 | 54.7 |
| 602 | siRNA101'-M3 | 35.3 | 64.7 |
| 1433 | siRNA260'-M3 | 56.2 | 43.8 |
| 506 | siRNA81'-M3 | 13.8 | 86.2 |

** The siRNA# in Table 8 corresponds to the siRNA# in Table 7B.*
*All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$).*

Second Screening

Twelve-siRNAs were selected based on their RNAi activity for further optimization. All these siRNA showed greater than 60% knockdown in a AAV model. Three (3) new modification patterns (MOD1, MOD2, and MOD4, FIG. 1) were applied to these sequences (in addition to the modification pattern, MOD3, used for the initial screening) to obtain 48-unique siRNAs (shown in Table 9).

Table 9: List of Chemically Modified Duplexes Prepared for the second *in vivo* Screening‡

| Position | siRNA# | Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified sense strand: | Anti-Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified antisense strand: |
|---|---|---|---|---|---|---|---|
| 223 | siRNA27'-M3^ | [mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] | 419 | 1219 | [mUs][fUs][fU][mG][fC][fU][fC][fU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] | 1161 | 1989 |
| 844 | siRNA125'-M3 | [mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] | 517 | 1329 | [mUs][fUs][fG][mU][fC][fA][fU][fU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] | 1162 | 1993 |
| 1227 | siRNA205'-M3 | [mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] | 597 | 1417 | [mUs][fUs][fA][mA][fC][fU][fG][fU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] | 1165 | 1999 |
| 1084 | siRNA174'-M3 | [mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] | 566 | 1382 | [mUs][fAs][fU][mA][fC][fU][fG][fG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] | 1166 | 2003 |
| 292 | siRNA47'-M3 | [mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] | 439 | 1241 | [mUs][fGs][fA][mU][fU][fU][fG][fU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] | 1169 | 2009 |
| 1237 | siRNA212'-M3 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][fA][fG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2017 |
| 1228 | siRNA206'-M3 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][fA][fC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2021 |
| 115 | siRNA12'-M3 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2029 |
| 477 | siRNA72'-M3 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][fC][fG][fU][fG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2034 |
| 303 | siRNA51'-M3 | [mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] | 443 | 1246 | [mUs][fGs][fU][mA][fU][fG][fA][fC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] | 1184 | 2039 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 602 | siRNA101’-M3 | [mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] | 493 | 1301 | [mUs][fAs][fA][mA][fU][fA][fC][fC][mC][fA][mG][mC][mU][fU][mC][mA][mA][mA][mC][mA][mUs][mAs][mG] | 1188 | 2046 |
| 506 | siRNA81’-M3 | [mGs][mAs][mA][mA][mA][mC][mC][mU][fC][fC][fA][fA][mC][mG][mU][mA][mU][mC][mA][mU][mAs] | 473 | 1279 | [mUs][fAs][fU][mG][fA][fU][fA][fC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] | 1190 | 2051 |
| 844 | siRNA125’-M4 | [mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] | 517 | 1329 | [mUs][fUs][fG][mU][mC][mA][fU][mU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] | 1162 | 1994 |
| 1227 | siRNA205’-M4 | [mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] | 597 | 1417 | [mUs][fUs][fA][mA][mC][mU][fG][mU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] | 1165 | 2000 |
| 1084 | siRNA174’-M4 | [mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] | 566 | 1382 | [mUs][fAs][fU][mA][mC][mU][fG][mG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] | 1166 | 2004 |
| 292 | siRNA47’-M4 | [mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] | 439 | 1241 | [mUs][fGs][fA][mU][mU][mU][fG][mU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] | 1169 | 2010 |
| 1237 | siRNA212’-M4 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][mA][mG][fU][mG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2018 |
| 1228 | siRNA206’-M4 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][mA][mC][fU][mG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2022 |
| 115 | siRNA12’-M4 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][mG][mA][fA][mU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2030 |
| 477 | siRNA72’-M4 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][mC][mG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2035 |
| 303 | siRNA51’-M4 | [mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] | 443 | 1246 | [mUs][fGs][fU][mA][mU][mG][fA][mC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] | 1184 | 2040 |
| 602 | siRNA101’-M4 | [mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] | 493 | 1301 | [mUs][fAs][fA][mA][mU][mA][fC][mC][mC][fA][mG][mC][mU][fU][mC][mA][mA][mA][mC][mA][mUs][mAs][mG] | 1188 | 2047 |
| 506 | siRNA81’-M4 | [mGs][mAs][mA][mA][mA][mC][mC][mU][fC][fC][fA][fA][mC][mG][mU][mA][mU][mC][mA][mU][mAs] | 473 | 1279 | [mUs][fAs][fU][mG][mA][mU][fA][mC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] | 1190 | 2052 |
| 223 | siRNA27’-M4 | [mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] | 419 | 1219 | [mUs][fUs][fU][mG][mC][mU][fC][mU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] | 1161 | 1990 |
| 844 | siRNA125’-M1 | [mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] | 517 | 1329 | [mUs][fUs][fG][mU][fC][mA][fU][fU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] | 1162 | 1995 |
| 1227 | siRNA205’-M1 | [mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] | 597 | 1417 | [mUs][fUs][fA][mA][fC][mU][fG][fU][mG][fG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] | 1165 | 2001 |
| 1084 | siRNA174’-M1 | [mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] | 566 | 1382 | [mUs][fAs][fU][mA][fC][mU][fG][fG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] | 1166 | 2005 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 292 | siRNA47'-M1 | [mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] | 439 | 1241 | [mUs][fGs][fA][mU][fU][mU][fG][fU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] | 1169 | 2011 |
| 1237 | siRNA212'-M1 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2016 |
| 1228 | siRNA206'-M1 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2020 |
| 115 | siRNA12'-M1 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][fG][mA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2031 |
| 477 | siRNA72'-M1 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][fC][mG][fU][fG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2036 |
| 303 | siRNA51'-M1 | [mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] | 443 | 1246 | [mUs][fGs][fU][mA][fU][mG][fA][fC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] | 1184 | 2041 |
| 602 | siRNA101'-M1 | [mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] | 493 | 1301 | [mUs][fAs][fA][mA][fU][mA][fC][fC][mC][fA][mG][mC][mU][fU][mC][mA][mA][mA][mC][mA][mUs][mAs][mG] | 1188 | 2048 |
| 506 | siRNA81'-M1 | [mGs][mAs][mA][mA][mA][mC][mC][mU][fC][fC][fA][fA][mC][mG][mU][mA][mU][mC][mA][mU][mAs] | 473 | 1279 | [mUs][fAs][fU][mG][fA][mU][fA][fC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] | 1190 | 2053 |
| 223 | siRNA27'-M1 | [mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] | 419 | 1219 | [mUs][fUs][fU][mG][fC][mU][fC][fU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] | 1161 | 1991 |
| 844 | siRNA125'-M2 | [mGs][mCs][mG][mC][mA][mU][mG][mU][fU][fU][fC][fU][mC][mA][mA][mU][mG][mA][mC][mA][mAs] | 517 | 1329 | [mUs][fUs][fG][mU][fC][fA][fU][mU][mG][fA][mG][mA][mA][fA][mC][mA][mU][mG][mC][mG][mCs][mAs][mG] | 1162 | 1996 |
| 1227 | siRNA205'-M2 | [mUs][mAs][mG][mC][mU][mC][mU][mU][fU][fA][fC][fC][mC][mA][mC][mA][mG][mU][mU][mA][mAs] | 597 | 1417 | [mUs][fUs][fA][mA][fC][fU][fG][mU][mG][fG][mG][mU][mA][fA][mA][mG][mA][mG][mC][mU][mAs][mAs][mG] | 1165 | 2002 |
| 1084 | siRNA174'-M2 | [mAs][mCs][mU][mG][mA][mA][mU][mG][fA][fC][fC][fU][mG][mC][mC][mA][mG][mU][mA][mU][mAs] | 566 | 1382 | [mUs][fAs][fU][mA][fC][fU][fG][mG][mC][fA][mG][mG][mU][fC][mA][mU][mU][mC][mA][mG][mUs][mAs][mG] | 1166 | 2006 |
| 292 | siRNA47'-M2 | [mUs][mGs][mU][mC][mC][mA][mU][mU][fU][fC][fA][fU][mC][mA][mC][mA][mA][mA][mU][mC][mAs] | 439 | 1241 | [mUs][fGs][fA][mU][fU][fU][fG][mU][mG][fA][mU][mG][mA][fA][mA][mU][mG][mG][mA][mC][mAs][mAs][mG] | 1169 | 2012 |
| 1237 | siRNA212'-M2 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][fA][fG][fU][mG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2019 |
| 1228 | siRNA206'-M2 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][fA][fC][fU][mG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2023 |
| 115 | siRNA12'-M2 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][fG][fA][fA][mU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2032 |
| 477 | siRNA72'-M2 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2033 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 303 | siRNA51'-M2 | [mUs][mCs][mA][mC][mA][mA][mA][mU][fC][fC][fC][fU][mU][mG][mU][mC][mA][mU][mA][mC][mAs] | 443 | 1246 | [mUs][fGs][fU][mA][fU][fG][fA][mC][mA][fA][mG][mG][mG][fA][mU][mU][mU][mG][mU][mG][mAs][mAs][mG] | 1184 | 2042 |
| 602 | siRNA101'-M2 | [mAs][mUs][mG][mU][mU][mU][mG][mA][fA][fG][fC][fU][mG][mG][mG][mU][mA][mU][mU][mU][mAs] | 493 | 1301 | [mUs][fAs][fA][mA][fU][fA][fC][mC][mC][fA][mG][mC][mU][fU][mC][mA][mA][mA][mC][mA][mUs][mAs][mG] | 1188 | 2049 |
| 506 | siRNA81'-M2 | [mGs][mAs][mA][mA][mA][mC][mC][mU][fC][fC][fA][fA][mC][mG][mU][mA][mU][mC][mA][mU][mAs] | 473 | 1279 | [mUs][fAs][fU][mG][fA][fU][fA][mC][mG][fU][mU][mG][mG][fA][mG][mG][mU][mU][mU][mU][mCs][mAs][mG] | 1190 | 2054 |
| 223 | siRNA27'-M2 | [mUs][mCs][mU][mU][mG][mA][mG][mU][fU][fC][fC][fU][mC][mA][mG][mA][mG][mC][mA][mA][mAs] | 419 | 1219 | [mUs][fUs][fU][mG][fC][fU][fC][mU][mG][fA][mG][mG][mA][fA][mC][mU][mC][mA][mA][mG][mAs][mAs][mG] | 1161 | 1992 |

** The siRNA# corresponds to the siRNA# in Table 7A with different M# to indicate different modification patterns applied to the same nucleobase sequences.*

*All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$). It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand) of a targeting moiety comprising a structure of Formula ($Z^6$).*

*^M1, M2, M3, and M4 refer to modification patterns MOD1, MOD2, MOD3, and MOD4, respectively, as shown in FIG. 1; mA, mC, mG, and mU are 2'-O-methyl modified adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro modified adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate internucleoside linkage.*

*‡Each uracil base (U) in any one of the sequences provided in Table 9 may independently and optionally be replaced with a thymine base (T).*

Table 10 summarizes the second *in vivo* screening results.

Table 10: Results of the Second *in vivo* Screening in Mouse

| | siRNA # | Mean % mRNA Remaining hAAV (2 mg/kg dose level, day 14) | Mean % mRNA Remaining hAAV (2 mg/kg dose level, day 28) |
|---|---|---|---|
| 844 | siRNA125'-M3 | 53.3 | 42.8 |
| 1227 | siRNA205'-M3 | 44.9 | 57.5 |
| 1084 | siRNA174'-M3 | 93.6 | 56.8 |
| 292 | siRNA-47'-M3 | 63.2 | 40.2 |
| 1237 | siRNA212'-M3 | 38.1 | 31.8 |
| 1228 | siRNA206'-M3 | 49.8 | 39.2 |
| 115 | siRNA12'-M3 | 57.0 | 49.1 |
| 477 | siRNA72'-M3 | 44.2 | 19.7 |
| 303 | siRNA51'-M3 | 48.5 | 32.5 |
| 602 | siRNA101'-M3 | 71.4 | 62.8 |
| 506 | siRNA81'-M3 | 43.4 | 61.4 |
| 223 | siRNA27'-M3 | 40.1 | 37.5 |
| 844 | siRNA125'-M4 | 51.4 | 50.9 |
| 1227 | siRNA205'-M4 | 96.0 | 63.9 |
| 1084 | siRNA174'-M4 | 85.5 | 52.2 |
| 292 | siRNA47'-M4 | 39.0 | 37.7 |
| 1237 | siRNA212'-M4 | 46.9 | 23.4 |
| 1228 | siRNA206'-M4 | 61.7 | 70.3 |
| 115 | siRNA12'-M4 | 57.9 | 31.8 |
| 477 | siRNA72'-M4 | 61.3 | 39.5 |
| 303 | siRNA51'-M4 | 49.0 | 33.5 |
| 602 | siRNA101'-M4 | 76.7 | 69.4 |
| 506 | siRNA81'-M4 | 51.1 | 41.7 |

| | | | |
|---|---|---|---|
| 223 | siRNA27'-M4 | 69.5 | 51.8 |
| 844 | siRNA125'-M1 | 35.5 | 45.4 |
| 1227 | siRNA205'-M1 | 70.1 | 76.7 |
| 1084 | siRNA174'-M1 | 57.6 | 89.2 |
| 292 | siRNA47'-M1 | 49.3 | 63.5 |
| 1237 | siRNA212'-M1 | 36.9 | 35.3 |
| 1228 | siRNA206'-M1 | 72.8 | 88.2 |
| 115 | siRNA12'-M1 | 35.9 | 38.8 |
| 477 | siRNA72'-M1 | 31.3 | 28.3 |
| 303 | siRNA51'-M1 | 27.5 | 35.8 |
| 602 | siRNA101'-M1 | 57.3 | 85.3 |
| 506 | siRNA81'-M1 | 46.4 | 52.8 |
| 223 | siRNA27'-M1 | 51.5 | 73.1 |
| 844 | siRNA125'-M2 | 32.8 | 50.2 |
| 1227 | siRNA205'-M2 | 76.5 | 86.1 |
| 1084 | siRNA174'-M2 | 77.0 | 90.7 |
| 292 | siRNA47'-M2 | 31.1 | 65.3 |
| 1237 | siRNA212'-M2 | 45.3 | 67.7 |
| 1228 | siRNA206'-M2 | 39.4 | 87.4 |
| 115 | siRNA12'-M2 | 29.3 | 42.6 |
| 477 | siRNA72'-M2 | 29.9 | 71.5 |
| 303 | siRNA51'-M2 | 28.3 | 43.7 |
| 602 | siRNA101'-M2 | 42.5 | 81.8 |
| 506 | siRNA81'-M2 | 53.1 | 53.9 |
| 223 | siRNA27'-M2 | 44.7 | 50.4 |

** The siRNA# corresponds to the siRNA# in Table 9. All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$).*

**Example 5: *In vivo* Efficacy in ANIT Cholestasis Model**

Mouse cross-reactive sequence siRNA206'-M1 was used to demonstrate efficacy in a mouse model of cholestasis. α-naphthylisothiocyanate (ANIT) is a commonly used chemical to induce intrahepatic cholestasis modeling human disease. C57Bl/6 mice were administered siRNA206'-M1 on day 1, 8, and 15. ANIT was administered at 60 mg/kg by oral gavage on day 18. Serum chemistry, gene expression, and 7α-hydroxy-4-cholesten-3-one (C4) biomarker analyses were performed on day 21. Gene expression data was represented as fold change over vehicle group using the delta Ct method.

The results showed that the inhibition of CYP7A1 expression in hepatocytes leads to reduction in cholestasis. As shown in Tables 11 and 12, treatment with siRNA206'-M1 resulted in the reduction in serum bile acids and liver injury markers ALP, AST, ALT, and bilirubin, indicating the decrease in liver injury. Cholestasis induced the expression of pro-fibrotic genes *col1a1*, *timp1*, and *ccl2* as well as bile acid transport genes *ostb*, *ntcp*, and *bsep* while treatment with siRNA206'-M1 normalized expression of these genes to near healthy control levels. Reduction of circulating CYP7A1 biomarker, serum C4, indicated inhibition of the target gene.

Table 11: Serum Chemistry of ANIT Cholestasis Mice under Treatment

| Group | ALP (U/L) ± sem | AST (U/L) ± sem | ALT (U/L) ± sem | Bilirubin (mg/dL) ± sem | C4 (ng/mL) ± sem | Bile Acids (umol/L) ± sem |
|---|---|---|---|---|---|---|
| Vehicle | 289.3 ± 39.93 | 4512 ± 1068 | 2432 ± 818.8 | 19.78 ± 2.17 | 60.0 ± 14.13 | 1769 ± 306.8 |
| siRNA206'-M1 | 38.5 ± 8.36 | 178.8 ± 52.0 | 142.5 ± 47.22 | 0.28 ± 0.75 | 15.66 ± 2.3 | 21.97 ± 8.50 |
| Healthy control | 23.6 ± 5.87 | 79.8 ± 18.2 | 27 ± 2.83 | 0.16 ± 0.02 | 41.2 ± 5.6 | 8.7 ± 0.96 |

Table 12: Gene Express Profile of ANIT Cholestasis Mice under Treatment

| Group | Col1a1 ± sem | Timp1 ± sem | Ccl2 ± sem | Ostb ± sem | Ntcp ± sem | Bsep ± sem |
|---|---|---|---|---|---|---|
| Vehicle | 1.11 ± 0.22 | 1.04 ± 0.13 | 1.008 ± 0.06 | 1.01 ± 0.08 | 1.04 ± 0.12 | 1.04 ± 0.14 |
| siRNA206'-M1 | 0.50 ± 0.21 | 0.19 ± 0.11 | 0.12 ± 0.05 | 0.04 ± 0.03 | 2.94 ± 0.32 | 0.78 ± 0.08 |
| Healthy control | 0.09 ± 0.02 | 0.005 ± 0.0008 | 0.014 ± 0.003 | 0.006 ± 0.001 | 3.39 ± 0.17 | 0.87 ± 0.05 |

**Example 6: *In vitro* RNAi activity in HepaRG human hepatic cell line**

To assess the efficacy and potency of sequences against the human CYP7A1 transcript, an *in vitro* dose response study was performed in the HepaRG human hepatic cell line. HepaRG cells are widely considered a robust surrogate for primary human hepatocytes due to similar expression profile, unlike other hepatic cell lines which have lost major liver-like functions.

Four (4) siRNA constructs, conjugated siRNA212'-M1, conjugated siRNA206'-M1, conjugated siRNA72'-M2, or conjugated siRNA12'-M3 (shown in Table 13), were tested in HepaRG cells. HepaRG cells were plated in a 96-well plate at 60,000 cells/well containing antibiotic-free medium and placed at 37°C in an atmosphere with 5% $CO_2$ in a humidified incubator. Within 24 hours after seeding, cells were transfected with biological triplicates for each treatment. A 5-fold, 11-point dose response with a top dose of 1µM of CYP7A1 siRNA constructs were applied to the cells following the manufacturer's recommendation (Thermofisher) for Lipofectamine RNAiMax. Cells were returned to the incubator for 48 hours with a medium change at 24 hours. Cell lysates were prepared 48 hours post-transfection by removing medium and washing with cold PBS and the addition of lysis buffer (following the manufacturer's recommendation for Cells-to-CT 1-step TaqMan Kit, Thermofisher). Reverse transcription was performed on cell lysates which were then used for qPCR. qPCR was performed in duplicate for each sample in a 10 µl reaction mix, multiplexing the probe for CYP7A1 with the housekeeping gene probe (GAPDH): Nuclease-free water 5.5 µl + qRT-PCR Master Mix 2.5 µl + TaqMan Primer-1, 0.5 µl + TaqMan Primer-2, 0.5 µl.

For each well, the target mRNA level was normalized to the respective GAPDH mRNA level and relative expression was calculated based on mock transfection control wells. Dose

response curves were generated in GraphPad Prism and used to calculate half-maximal inhibitory concentrations (IC50) and maximal gene knockdown of a given siRNA construct. Table 14 summarizes the *in vitro* screening results.

Table 13: Chemically Modified Duplexes Prepared for the in vitro HepaRG Cell Study‡

| Position | siRNA #* | Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified sense strand: | Anti-Sense Strand Sequence | Nucleobase SEQ ID NO: | SEQ ID NO of modified antisense strand: |
|---|---|---|---|---|---|---|---|
| 1237 | siRNA212'-M1 | [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] | 604 | 1425 | [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] | 1173 | 2016 |
| 1228 | siRNA206'-M1 | [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] | 598 | 1418 | [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] | 1174 | 2020 |
| 477 | siRNA72'-M2 | [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] | 464 | 1267 | [mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] | 1181 | 2033 |
| 115 | siRNA12'-M3 | [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] | 404 | 1202 | [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] | 1180 | 2029 |

**The siRNA# corresponds to the siRNA# in Table 9.*
*All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$). It is to be understood that the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand) of a targeting moiety comprising a structure of Formula ($Z^6$).*
*‡Each uracil base (U) in any one of the sequences provided in Table 12 may independently and optionally be replaced with a thymine base (T).*

Table 14: *In vitro* Screening of siRNAs in HepaRG Human Hepatic Cell Line

| Position | siRNA#* | IC50 (nM) | Maximum mRNA knockdown (%) |
|---|---|---|---|
| 1237 | siRNA212'-M1 | 0.16 | 69 |
| 1228 | siRNA206'-M1 | 0.24 | 78 |
| 115 | siRNA12'-M3 | 0.34 | 72 |
| 477 | siRNA72'-M2 | 0.52 | 61 |

** The siRNA# corresponds to the siRNA# in Table 13. All siRNAs tested in this experiment are conjugated at the 3'terminal nucleoside of the sense strand to a targeting moiety comprising a structure of Formula ($Z^6$).*

**Example 7: *In vivo* Study of siRNA Agents in Non-Human Primate (NHP)**

The same four (4) siRNAs from Example 6 were tested in a single injection, single dose level (20 mg/kg), 56-day duration study in cynomolgus monkeys (*Macaca fascicularis*). The research protocol and animal housing were approved by the IACUC of Huazhen Laboratory Animal Breeding Center, Guangzhou China. Briefly, healthy naïve male cynomolgus monkeys 3-5 years of age were individually housed in a controlled environment with 12:12-h light:darkness cycle, controlled humidity range of 60–80% and temperature maintained in the

range of 18–26°C. Animals were fed twice a day (BID) with a snack in between meals and had free access to drinking water. Animals were acclimated to all experimental procedures prior to study initiation. Vehicle, conjugated siRNA212'-M1, conjugated siRNA206'-M1, conjugated siRNA72'-M2, or conjugated siRNA12'-M3 (shown in Table 13) was administered in a single subcutaneous injection on day 0. Blood samples were collected at pre-dose day -7 and on days 7, 14, 21, 28, 35, 42, 49, and 56. All samples were analyzed for serum 7α-hydroxy-4-cholesten-3-one (C4), total bile acid, and cholic acid levels by mass spectrometry. C4 data are represented as percent reduction relative to vehicle group while total bile acid and cholic acid are represented percent reduction relative to pre-treatment samples.

Target engagement and pathway modulation were assessed by quantitation of serum C4 and the primary bile acid, cholic acid. Around 40-60% decrease in plasma C4 level was observed. Around 35-90% decrease in total bile acid, and around 40 – 97% decrease in cholic acid was observed.

## ADDITIONAL EMBODIMENTS

1. An RNAi agent for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleosides to a CYP7A1 target sequence in Table 2, wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

2. An RNAi agent for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleosides to a CYP7A1 target sequence of nucleotides 113-133, 221-241, 249-269, 290-310, 301-321, 475-495, 476-496, 504-524, 593-613, 600-620, 671-691, 779-799, 839-859, 842-862, 1003-1023, 1009-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1227, 1215-1235, 1225-1245, 1226-1246, 1235-1255, 1289-1309, 1296-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1, wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

3. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises at least 15 consecutive nucleobases of any one of SEQ ID NOs: 777-1190.

4. The RNAi agent of any one of embodiments 1-3, wherein the sense strand comprises at least 15 consecutive nucleobases of any one of SEQ ID NOs: 393-776.

5. The RNAi agent of any one of embodiments 1-4, wherein the sense strand is 21 nucleosides in length and the antisense strand is 23 nucleosides in length, optionally wherein the RNAi agent comprises a 3' overhang of at least 1 nucleoside on a least one strand.

6. The RNAi agent of any one of embodiments 1-4, wherein the antisense strand comprises nucleobases 2-21 (counting 5' → 3') of any one of SEQ ID NOs: 777-1190 and the sense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776.

7. The RNAi agent of any one of embodiments 1-6, wherein the antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190 and the sense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776.

8. The RNAi agent of any one of embodiments 1-7, wherein the RNAi agent comprises the nucleobases sequences of an siRNA selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272'.

9. The RNAi agent of any one of embodiments 1-7, wherein the antisense strand comprises a nucleobase sequence selected from (5'→3'):
(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)
(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)
(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)
(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)
(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)
(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)
(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)
(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)

(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)
(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)
(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and
(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

10. The RNAi agent of embodiment 9, wherein the sense strand comprises a nucleobase sequence selected from (5'→3'):
(i) UCUUGAGUUCCUCAGAGCAAA (SEQ ID NO: 419);
(ii) GCGCAUGUUUCUCAAUGACAA (SEQ ID NO: 517);
(iii) UAGCUCUUUACCCACAGUUAA (SEQ ID NO: 597);
(iv) ACUGAAUGACCUGCCAGUAUA (SEQ ID NO: 566);
(v) UGUCCAUUUCAUCACAAAUCA (SEQ ID NO: 439);
(vi) CCCACAGUUAAUGCACUUAGA (SEQ ID NO: 604);
(vii) AGCUCUUUACCCACAGUUAAA (SEQ ID NO: 598);
(viii) UUGUCUAUGGCUUAUUCUUGA (SEQ ID NO: 404);
(ix) CCUUGAAUUCCCUCACGGAAA (SEQ ID NO: 464);
(x) UCACAAAUCCCUUGUCAUACA (SEQ ID NO: 443);
(xi) AUGUUUGAAGCUGGGUAUUUA (SEQ ID NO: 493); and
(xii) GAAAACCUCCAACGUAUCAUA (SEQ ID NO: 473).

11. The RNAi agent of any one of embodiments 1-10, wherein the RNAi agent comprises one or more modified nucleosides, optionally wherein each nucleoside of the antisense strand is a modified nucleoside and each nucleoside of the sense strand is a modified nucleoside.

12. The RNAi agent of embodiment 11, wherein the one or more modified nucleosides are 2' modified nucleosides.

13. The RNAi agent of embodiment 12, wherein the 2'-modified nucleoside is selected from 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside and combinations thereof, optionally wherein the 2'-modified nucleoside is selected from a 2'-O-methyl (2'-O-Me) modified nucleoside, a 2'-fluoro (2'-F) modified nucleoside, and combinations thereof.

14. The RNAi agent of any one of embodiments 11-13, wherein each nucleoside of the antisense strand is selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside, and each nucleoside of the sense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside.

15. The RNAi agent of any one of embodiments 11-14, wherein the nucleosides at one or more positions 9, 10, 11, and 12 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides, optionally wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides.

16. The RNAi agent of any one of embodiments 11-15, wherein the nucleosides at one or more positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides, optionally wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides.

17. The RNAi agent of any one of embodiments 11-16, wherein the nucleosides at one or more positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides.

18. The RNAi agent of any one of embodiments 11-17, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

19. The RNAi agent of any one of embodiments 11-17, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

20. The RNAi agent of any one of embodiments 11-17, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

21. The RNAi agent of any one of embodiments 11-17, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

22. The RNAi agent of any one of embodiments 11-21, wherein the nucleosides at one or more positions 1, 4, 5, 6, 8, 9, 11-13, and 15-23 (counting 5' → 3') of the antisense strand are

2'-O-Me modified nucleosides, optionally wherein the nucleosides at positions 1, 4, 9, 11-13, and 15-23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides.

23. The RNAi agent of any one of embodiments 1-22, wherein the RNAi agent comprises one or more modified internucleoside linkages, optionally wherein the RNAi agent comprises one or more phosphorothioate internucleoside linkages in at least one strand.

24. The RNAi agent of any one of embodiments 1-23, wherein the RNAi agent comprises two phosphorothioate internucleoside linkages in the sense strand, optionally wherein the two phosphorothioate internucleoside linkages are the first two internucleoside linkages in the sense strand from 5'→3'.

25. The RNAi agent of any one of embodiments 1-24, wherein the RNAi agent comprises four phosphorothioate internucleoside linkages in the antisense strand, wherein the four phosphorothioate internucleoside linkages are the first two internucleoside linkages and the last two internucleoside linkages in the antisense strand from 5'→3'.

26. The RNAi agent of any one of embodiments 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

27. The RNAi agent of any one of embodiments 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

28. The RNAi agent of any one of embodiments 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

29. The RNAi agent of any one of embodiments 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

30. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand and the sense strand comprise a structure as provided in Table 5B, Table 7B, or Table 9.

31. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1173 and a structure (5'→3') of [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

32. The RNAi agent of embodiment 31, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 604 and a structure (5'→3') of [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425), wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

33. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1174 and a structure (5'→3') of [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

34. The RNAi agent of embodiment 33, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 598 and a structure (5'→3') of [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

35.0. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1180 and a structure (5'→3') selected from the group consisting of
[mUs][fCs][mA][fA][mG][fA][mA][fU][mA][fA][mG][mC][mC][fA][mU][fA][mG][fA][mC][fA][mAs][mAs][mG] (SEQ ID NO: 2055),
[mUs][fCs][fA][fA][mG][fA][mA][fU][mA][fA][mG][fC][mC][fA][mU][fA][mG][fA][mC][fA][mAs][mAs][mG] (SEQ ID NO: 2056),
[mUs][fCs][mA][mA][mG][fA][mA][fU][fA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2057),

[mUs][fCs][mA][mA][mG][fA][mA][mU][mA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2058), and
[mUs][fCs][mA][mA][mG][fA][gA][fU][fA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2059),
wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively;
gA is GNA adenosine; and
s is a phosphorothioate linkage.

35. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1180 and a structure (5'→3') of
[mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

36.0. The RNAi agent of embodiment 35.0, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 404 and a structure (5'→3') of
[fUs][mUs][fG][mU][fC][mU][fA][mU][fG][fG][fC][mU][fU][mA][fU][mU][fC][mU][fU][mG][fAs] (SEQ ID NO: 2060),
[mUs][mUs][mG][mU][mC][mU][fA][mU][fG][mG][fC][mU][fU][mA][fU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2062), and
[mUs][mUs][mG][mU][mC][mU][fA][mU][fG][fG][fC][mU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2061),
wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and
s is a phosphorothioate linkage.

36. The RNAi agent of embodiment 35, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 404 and a structure (5'→3') of [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

37. The RNAi agent of embodiment 1 or embodiment 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1181 and a structure (5'→3') of [mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

38. The RNAi agent of embodiment 37, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 464 and a structure (5'→3') of [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

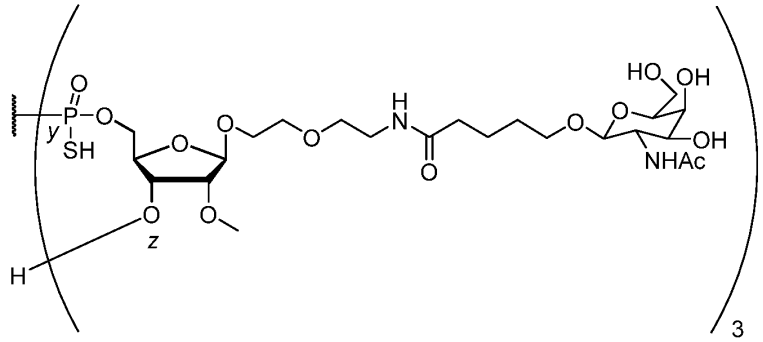
39. The RNAi agent of any one of embodiments 1-38, further comprising a targeting moiety.

40. The RNAi agent of embodiment 39, wherein the targeting moiety is conjugated to the 3' end of the sense strand of the RNAi agent.

41. The RNAi agent of embodiment 39 or embodiment 40, wherein the targeting moiety comprises N-acetyl-galactosamine (GalNAc), optionally wherein the targeting moiety comprises a GalNAc trimer.

42. The RNAi agent of any one of embodiments 39-41, wherein the targeting moiety comprises one or more instances of GalNAc attached through a monovalent, bivalent, trivalent, or tetravalent branched linker.

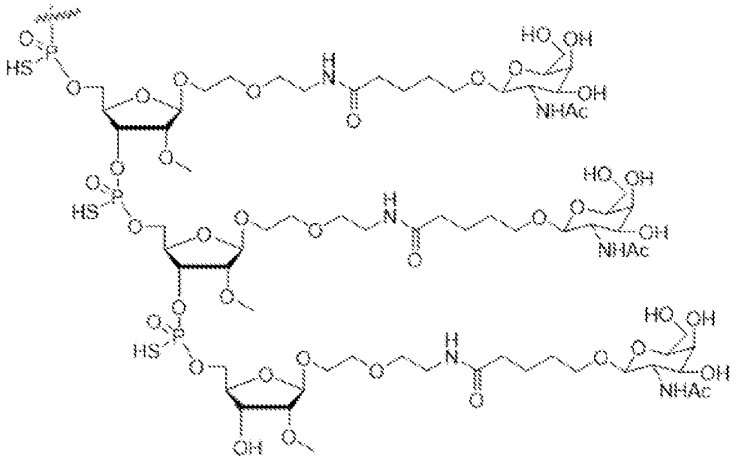
43. The RNAi agent of embodiment 39, wherein the targeting moiety is of formula:




($Z^6$);

or a pharmaceutically acceptable salt thereof, wherein the  indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand.

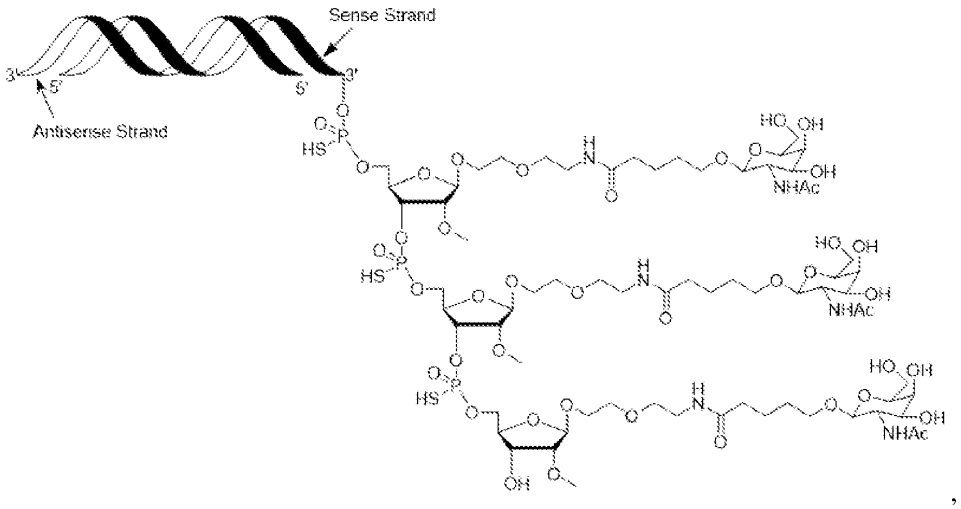
43.1. The RNAi agent of embodiment 39, wherein the targeting moiety is of formula:




;

or a pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand.

44. The RNAi agent of embodiment 43 or 43.1, wherein the resulting conjugate comprises a structure of:




,

or a pharmaceutically acceptable salt thereof.

45. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure (5' → 3') of

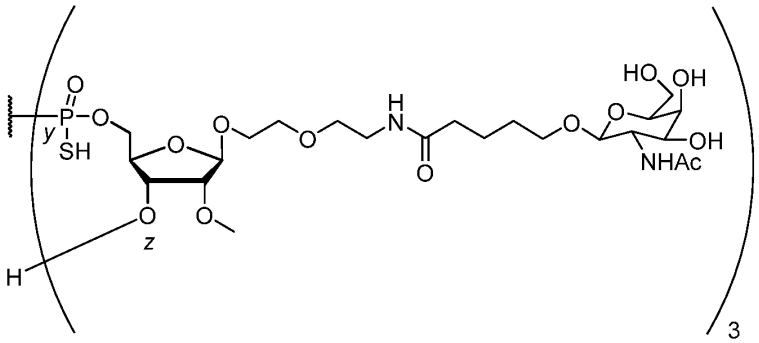
[mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure (5' → 3') of

[mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



3 ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

46. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure (5' → 3') of

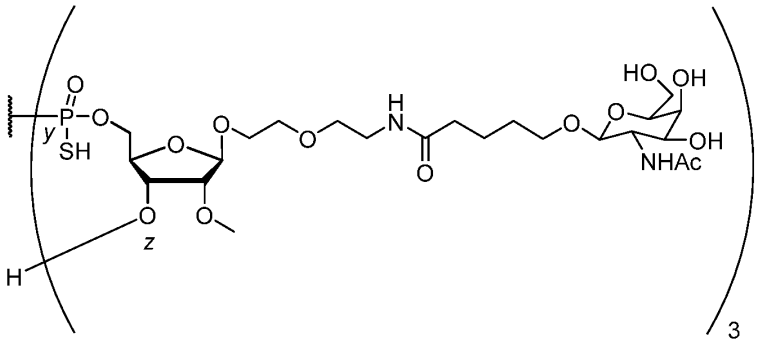
[mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure (5' → 3') of

[mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



$_3$ ($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

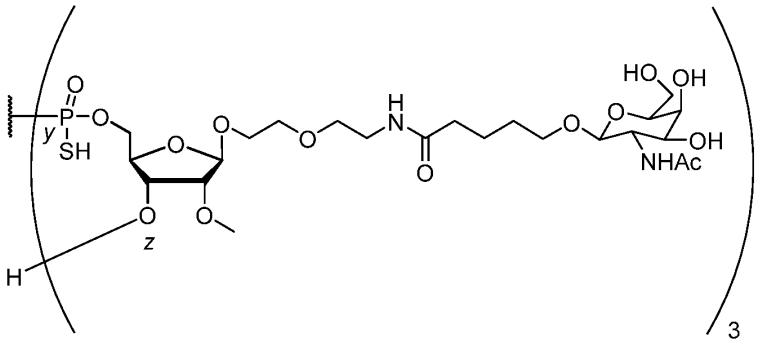
47. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

47.1. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of

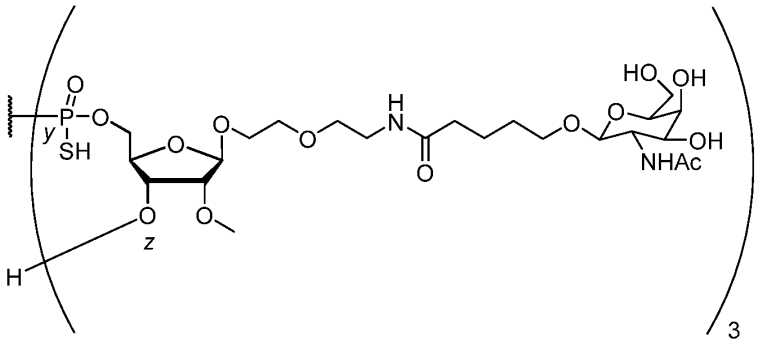
[mUs][fCs][mA][fA][mG][fA][mA][fU][mA][fA][mG][mC][mC][fA][mU][fA][mG][fA][mC][fA][mAs][mAs][mG] (SEQ ID NO: 2055); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of

[fUs][mUs][fG][mU][fC][mU][fA][mU][fG][fG][fC][mU][fU][mA][fU][mU][fC][mU][fU][mG][fAs] (SEQ ID NO: 2060);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula (Z$^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

47.2. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of

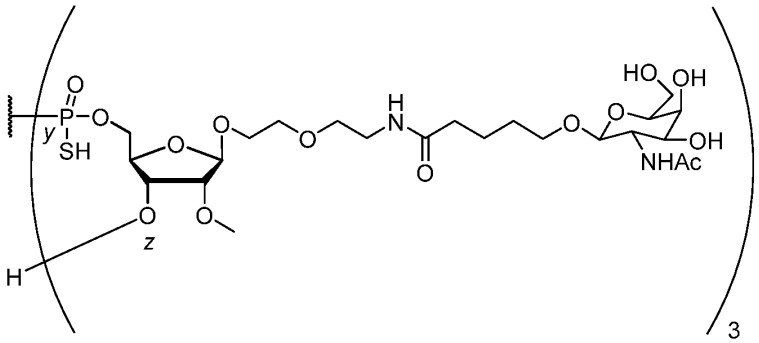
[mUs][fCs][fA][fA][mG][fA][mA][fU][mA][fA][mG][fC][mC][fA][mU][fA][mG][fA][mC][fA][mAs][mAs][mG] (SEQ ID NO: 2056); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of

[mUs][mUs][mG][mU][mC][mU][fA][mU][fG][mG][fC][mU][fU][mA][fU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2062);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula (Z$^6$)




($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

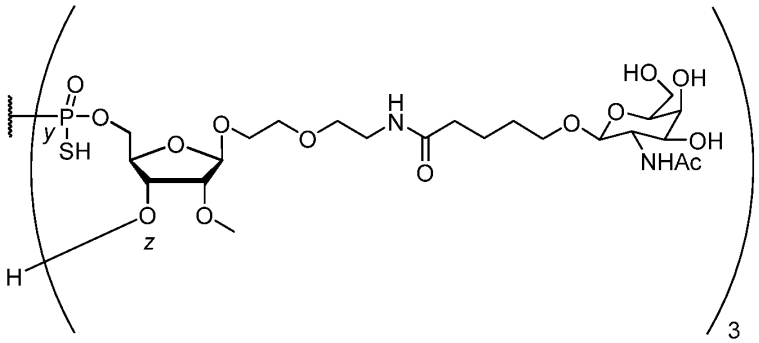
47.3. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of [mUs][fCs][mA][mA][mG][fA][mA][fU][fA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2057); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of [mUs][mUs][mG][mU][mC][mU][fA][mU][fG][fG][fC][mU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2061);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)




($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

47.4. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of

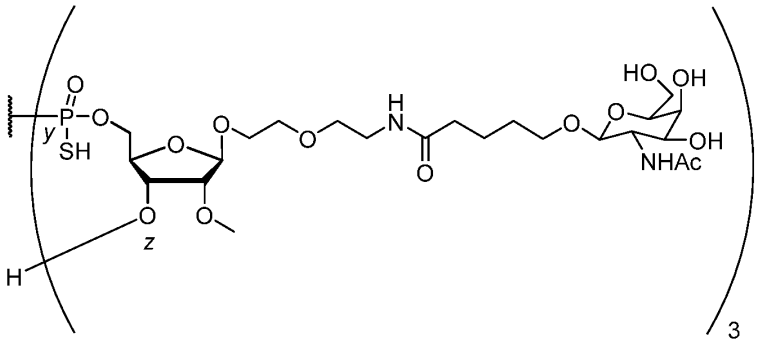
[mUs][fCs][mA][mA][mG][fA][mA][mU][mA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2058); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of

[mUs][mUs][mG][mU][mC][mU][fA][mU][fG][fG][fC][mU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2061);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)




($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

47.5. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of

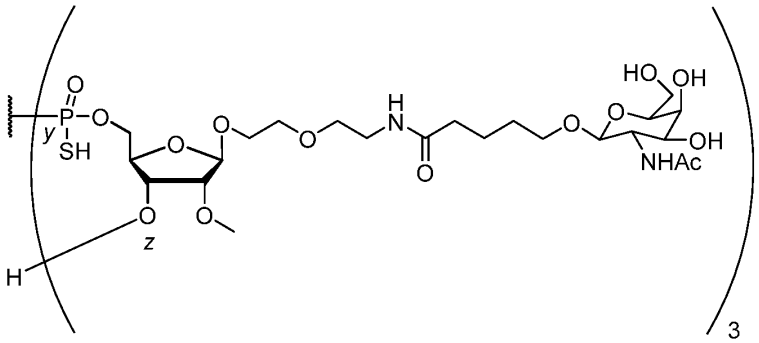
[mUs][fCs][mA][mA][mG][fA][gA][fU][fA][mA][mG][mC][mC][fA][mU][fA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2059); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of

[mUs][mUs][mG][mU][mC][mU][fA][mU][fG][fG][fC][mU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 2061);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; gA is GNA adenosine; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

48. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure (5' → 3') of

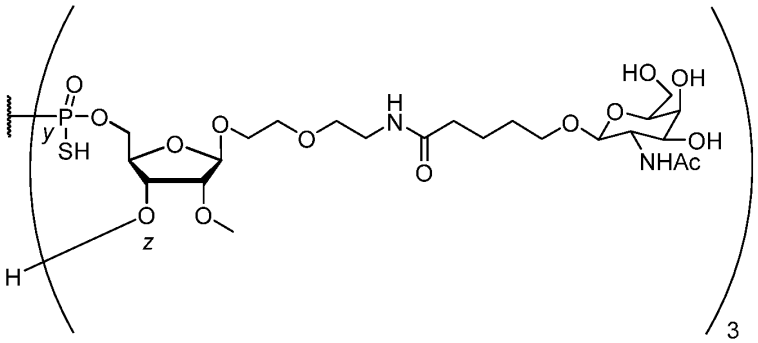
[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure (5' → 3') of

[mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula (Z$^6$)



$_3$ ($Z^6$),

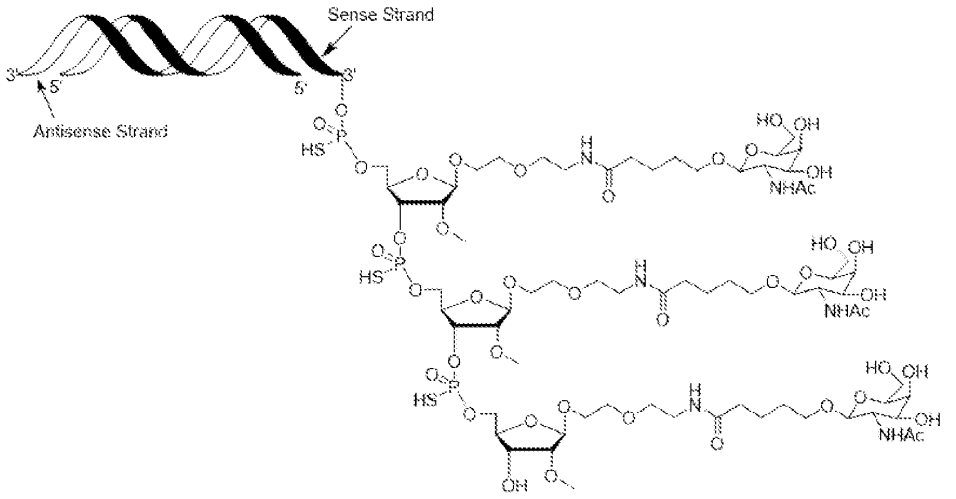
or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

49. A conjugate comprising a Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) RNAi agent covalently linked to a targeting moiety, wherein the CYP7A1 RNAi agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1180 and a structure (5'→3') of [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029); and

wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 404 and a structure (5'→3') of [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202); wherein:

mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

wherein the targeting moiety is covalently linked to the 3' terminal nucleoside of the sense strand and the s of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of a first repeat unit of the targeting moiety, wherein the conjugate comprises a structure of:




,

or a pharmaceutically acceptable salt thereof.

50. A pharmaceutical composition for inhibiting expression of a gene encoding CYP7A1 comprising the RNAi agent of any one of embodiments 1-48 or the conjugate of embodiment 49.

51. A method of inhibiting expression of a CYP7A1 gene in a cell, the method comprising contacting the cell with the RNAi agent of any one of embodiments 1-48, the conjugate of embodiment 49, or the pharmaceutical composition of embodiment 50, thereby inhibiting expression of the CYP7A1 gene in the cell.

52. The method of embodiment 51, wherein the cell is within a subject, optionally wherein the subject is human.

53. The method of embodiment 52, wherein the subject has a CYP7A1 disease or CYP7A1-associated disease.

54. A method of treating a subject having a CYP7A1 disease or CYP7A1-associated disease, comprising administering to the subject a therapeutically effective amount of the RNAi agent of any one of embodiments 1-48, the conjugate of embodiment 49, or the pharmaceutical composition of embodiment 50, thereby treating the subject having the CYP7A1 disease or CYP7A1-associated disease.

55. A method of treating at least one symptom in a subject having a CYP7A1 disease or

CYP7A1-associated disease, comprising administering to the subject an effective amount of the RNAi agent of any one of embodiments 1-48, the conjugate of embodiment 49, or the pharmaceutical composition of embodiment 50, thereby treating at least one symptom in the subject having the CYP7A1 disease or CYP7A1-associated disease.

56. The method of embodiment 54 or embodiment 55, wherein the disease is a CYP7A1 disease or CYP7A1-associated disease, optionally wherein the CYP7A1 disease or CYP7A1-associated disease is primary sclerosing cholangitis (PSC).

57. The method of any one of embodiments 54-56, wherein the subject is human.

58. The method of any one of embodiments 54-57, further comprising administering an agent for the treatment of a CYP7A1 disease or CYP7A1-associated disease.

## EQUIVALENTS

The disclosure may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting of the disclosure. Scope of the disclosure is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

## CLAIMS

What is claimed is:

1. An RNAi agent for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleosides to a CYP7A1 target sequence in Table 2, wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

2. An RNAi agent for inhibiting expression of Cytochrome P450 family 7 subfamily A member 1 (CYP7A1) in a cell, wherein the RNAi agent comprises a sense strand and an antisense strand forming a duplex region, wherein the antisense strand comprises a region of complementarity of at least 15 nucleosides to a CYP7A1 target sequence of nucleotides 113-133, 221-241, 249-269, 290-310, 301-321, 475-495, 476-496, 504-524, 593-613, 600-620, 671-691, 779-799, 839-859, 842-862, 1003-1023, 1009-1029, 1037-1057, 1082-1102, 1189-1209, 1207-1227, 1215-1235, 1225-1245, 1226-1246, 1235-1255, 1289-1309, 1296-1316, 1384-1404, 1415-1435, 1431-1451, or 1559-1579 of SEQ ID NO: 1, wherein the region of complementarity comprises a nucleoside sequence that contains no more than 3 mismatches to the CYP7A1 target sequence, and wherein the sense strand is at least substantially complementary to the antisense strand.

3. The RNAi agent of claim 1 or claim 2, wherein the antisense strand comprises at least 15 consecutive nucleobases of any one of SEQ ID NOs: 777-1190.

4. The RNAi agent of any one of claims 1-3, wherein the sense strand comprises at least 15 consecutive nucleobases of any one of SEQ ID NOs: 393-776.

5. The RNAi agent of any one of claims 1-4, wherein the sense strand is 21 nucleosides in length and the antisense strand is 23 nucleosides in length, optionally wherein the RNAi agent comprises a 3' overhang of at least 1 nucleoside on a least one strand.

6. The RNAi agent of any one of claims 1-4, wherein the antisense strand comprises nucleobases 2-21 (counting 5' → 3') of any one of SEQ ID NOs: 777-1190 and the sense strand

comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776.

7. The RNAi agent of any one of claims 1-6, wherein the antisense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 777-1190 and the sense strand comprises the nucleobase sequence of any one of SEQ ID NOs: 393-776.

8. The RNAi agent of any one of claims 1-7, wherein the RNAi agent comprises the nucleobases sequences of an siRNA selected from siRNA1-siRNA384, siRNA12', siRNA27', siRNA38', siRNA47', siRNA51', siRNA72', siRNA73', siRNA81', siRNA100', siRNA101', siRNA118', siRNA122', siRNA124', siRNA125', siRNA158', siRNA161', siRNA172', siRNA174', siRNA190', siRNA199', siRNA203', siRNA205', siRNA206', siRNA212', siRNA226', siRNA231', siRNA250', siRNA256', siRNA260', and siRNA272'.

9. The RNAi agent of any one of claims 1-7, wherein the antisense strand comprises a nucleobase sequence selected from (5'→3'):
(i) UUUGCUCUGAGGAACUCAAGAAG (SEQ ID NO: 1161)
(ii) UUGUCAUUGAGAAACAUGCGCAG (SEQ ID NO: 1162)
(iii) UUAACUGUGGGUAAAGAGCUAAG (SEQ ID NO: 1165)
(iv) UAUACUGGCAGGUCAUUCAGUAG (SEQ ID NO: 1166)
(v) UGAUUUGUGAUGAAAUGGACAAG (SEQ ID NO: 1169)
(vi) UCUAAGUGCAUUAACUGUGGGAG (SEQ ID NO: 1173)
(vii) UUUAACUGUGGGUAAAGAGCUAG (SEQ ID NO: 1174)
(viii) UCAAGAAUAAGCCAUAGACAAAG (SEQ ID NO: 1180)
(ix) UUUCCGUGAGGGAAUUCAAGGAG (SEQ ID NO: 1181)
(x) UGUAUGACAAGGGAUUUGUGAAG (SEQ ID NO: 1184)
(xi) UAAAUACCCAGCUUCAAACAUAG (SEQ ID NO: 1188); and
(xii) UAUGAUACGUUGGAGGUUUUCAG (SEQ ID NO: 1190).

10. The RNAi agent of claim 9, wherein the sense strand comprises a nucleobase sequence selected from (5'→3'):
(i) UCUUGAGUUCCUCAGAGCAAA (SEQ ID NO: 419);
(ii) GCGCAUGUUUCUCAAUGACAA (SEQ ID NO: 517);
(iii) UAGCUCUUUACCCACAGUUAA (SEQ ID NO: 597);
(iv) ACUGAAUGACCUGCCAGUAUA (SEQ ID NO: 566);
(v) UGUCCAUUUCAUCACAAAUCA (SEQ ID NO: 439);

(vi) CCCACAGUUAAUGCACUUAGA (SEQ ID NO: 604);
(vii) AGCUCUUUACCCACAGUUAAA (SEQ ID NO: 598);
(viii) UUGUCUAUGGCUUAUUCUUGA (SEQ ID NO: 404);
(ix) CCUUGAAUUCCCUCACGGAAA (SEQ ID NO: 464);
(x) UCACAAAUCCCUUGUCAUACA (SEQ ID NO: 443);
(xi) AUGUUUGAAGCUGGGUAUUUA (SEQ ID NO: 493); and
(xii) GAAAACCUCCAACGUAUCAUA (SEQ ID NO: 473).

11. The RNAi agent of any one of claims 1-10, wherein the RNAi agent comprises one or more modified nucleosides, optionally wherein each nucleoside of the antisense strand is a modified nucleoside and each nucleoside of the sense strand is a modified nucleoside.

12. The RNAi agent of claim 11, wherein the one or more modified nucleosides are 2' modified nucleosides.

13. The RNAi agent of claim 12, wherein the 2'-modified nucleoside is selected from 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside and combinations thereof, optionally wherein the 2'-modified nucleoside is selected from a 2'-O-methyl (2'-O-Me) modified nucleoside, a 2'-fluoro (2'-F) modified nucleoside, and combinations thereof.

14. The RNAi agent of any one of claims 11-13, wherein each nucleoside of the antisense strand is selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside, and each nucleoside of the sense strand is a 2'-modified nucleoside selected from a 2'-F modified nucleoside and a 2'-O-Me modified nucleoside.

15. The RNAi agent of any one of claims 11-14, wherein the nucleosides at one or more positions 9, 10, 11, and 12 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides, optionally wherein the nucleosides at positions 9, 10, 11, and 12 (counting 5' → 3') of the sense strand are 2'-F modified nucleosides.

16. The RNAi agent of any one of claims 11-15, wherein the nucleosides at one or more positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the

sense strand are 2'-O-Me modified nucleosides, optionally wherein the nucleosides at positions 1, 2, 3, 4, 5, 6, 7, 8 and 13, 14, 15, 16, 17, 18, 19, 20, 21 (counting 5' → 3') of the sense strand are 2'-O-Me modified nucleosides.

17. The RNAi agent of any one of claims 11-16, wherein the nucleosides at one or more positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5' → 3') of the antisense strand are 2'-F modified nucleosides.

18. The RNAi agent of any one of claims 11-17, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

19. The RNAi agent of any one of claims 11-17, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

20. The RNAi agent of any one of claims 11-17, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

21. The RNAi agent of any one of claims 11-17, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides.

22. The RNAi agent of any one of claims 11-21, wherein the nucleosides at one or more positions 1, 4, 5, 6, 8, 9, 11-13, and 15-23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides, optionally wherein the nucleosides at positions 1, 4, 9, 11-13, and 15-23 (counting 5' → 3') of the antisense strand are 2'-O-Me modified nucleosides.

23. The RNAi agent of any one of claims 1-22, wherein the RNAi agent comprises one or more modified internucleoside linkages, optionally wherein the RNAi agent comprises one or more phosphorothioate internucleoside linkages in at least one strand.

24. The RNAi agent of any one of claims 1-23, wherein the RNAi agent comprises two phosphorothioate internucleoside linkages in the sense strand, optionally wherein the two phosphorothioate internucleoside linkages are the first two internucleoside linkages in the sense strand from 5'→3'.

25. The RNAi agent of any one of claims 1-24, wherein the RNAi agent comprises four

phosphorothioate internucleoside linkages in the antisense strand, wherein the four phosphorothioate internucleoside linkages are the first two internucleoside linkages and the last two internucleoside linkages in the antisense strand from 5'→3'.

26. The RNAi agent of any one of claims 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

27. The RNAi agent of any one of claims 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

28. The RNAi agent of any one of claims 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 5, 6, 7, 8, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

29. The RNAi agent of any one of claims 1-25, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the nucleosides at positions 9, 10, 11, 12 (counting 5'→3') of the sense strand are 2'-F modified nucleosides, wherein the nucleosides at positions 2, 3, 7, 10, and 14 (counting 5'→3') of the antisense strand are 2'-F modified nucleosides, wherein

all nucleosides of the antisense strand and the sense strand that are not 2'-F modified nucleosides are 2'-O-Me modified nucleosides, and wherein the first two internucleoside linkages of the sense strand (from 5'→3') and the first two and the last two internucleoside linkages of the antisense strand (from 5'→3') are phosphorothioate internucleoside linkages.

30. The RNAi agent of claim 1 or 2, wherein the antisense strand and the sense strand comprise a structure as provided in Table 5B, Table 7B, or Table 9.

31. The RNAi agent of claim 1 or 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1173 and a structure (5'→3') of [mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

32. The RNAi agent of claim 31, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 604 and a structure (5'→3') of [mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

33. The RNAi agent of claim 1 or 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1174 and a structure (5'→3') of [mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro

uridine, respectively; and
s is a phosphorothioate linkage.

34. The RNAi agent of claim 33, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 598 and a structure (5'→3') of [mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

35. The RNAi agent of claim 1 or 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1180 and a structure (5'→3') of [mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

36. The RNAi agent of claim 35, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 404 and a structure (5'→3') of [mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

37. The RNAi agent of claim 1 or 2, wherein the antisense strand comprises the nucleobase sequence of SEQ ID NO: 1181 and a structure (5'→3') of

[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

38. The RNAi agent of claim 37, wherein the sense strand comprises the nucleobase sequence of SEQ ID NO: 464 and a structure (5'→3') of [mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267), wherein:
mA, mC, mG, and mU are 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, respectively;
fA, fC, fG, and fU are 2'-fluoro adenosine, 2'-fluoro cytidine, 2'-fluoro guanosine, and 2'-fluoro uridine, respectively; and
s is a phosphorothioate linkage.

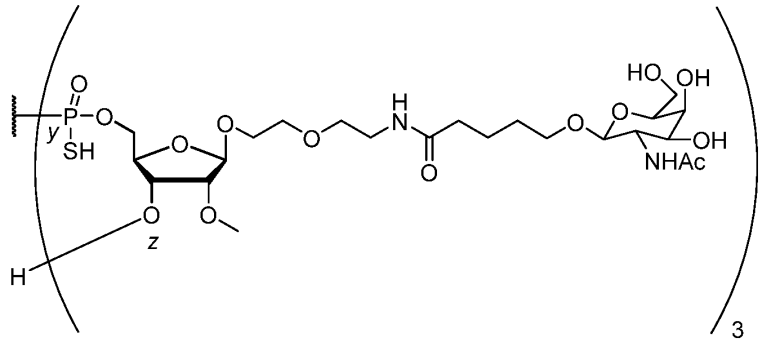
39. The RNAi agent of any one of claims 1-38, further comprising a targeting moiety.

40. The RNAi agent of claim 39, wherein the targeting moiety is conjugated to the 3' end of the sense strand of the RNAi agent.

41. The RNAi agent of claim 39 or claim 40, wherein the targeting moiety comprises N-acetyl-galactosamine (GalNAc), optionally wherein the targeting moiety comprises a GalNAc trimer.

42. The RNAi agent of any one of claims 39-41, wherein the targeting moiety comprises one or more instances of GalNAc attached through a monovalent, bivalent, trivalent, or tetravalent branched linker.

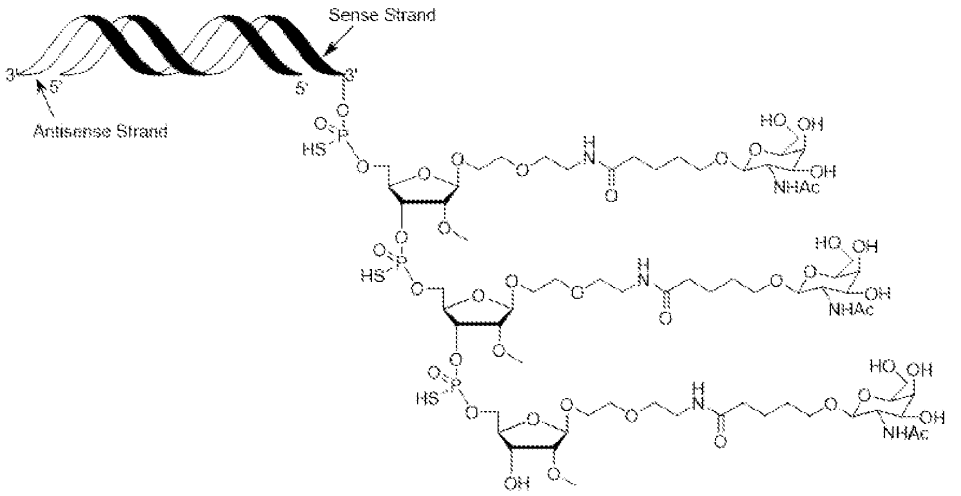
43. The RNAi agent of claim 39, wherein the targeting moiety is of formula:



$(Z^6)$;

or a pharmaceutically acceptable salt thereof, wherein the indicates the attachment point that is covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand.

44. The RNAi agent of claim 43, wherein the resulting conjugate comprises a structure of:




,

or a pharmaceutically acceptable salt thereof.

45. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1173 and a structure (5' → 3') of

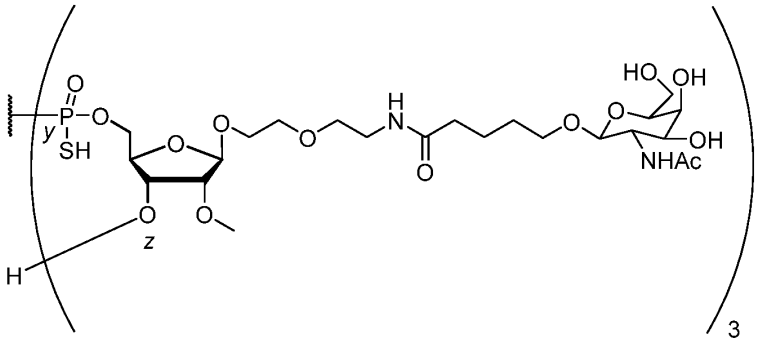
[mUs][fCs][fU][mA][fA][mG][fU][fG][mC][fA][mU][mU][mA][fA][mC][mU][mG][mU][mG][mG][mGs][mAs][mG] (SEQ ID NO: 2016); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 604 and a structure (5' → 3') of

[mCs][mCs][mC][mA][mC][mA][mG][mU][fU][fA][fA][fU][mG][mC][mA][mC][mU][mU][mA][mG][mAs] (SEQ ID NO: 1425);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)




($Z^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula ($Z^6$).

46. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1174 and a structure (5' → 3') of

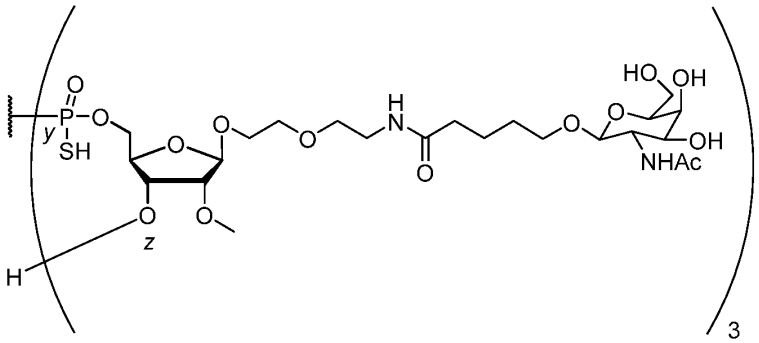
[mUs][fUs][fU][mA][fA][mC][fU][fG][mU][fG][mG][mG][mU][fA][mA][mA][mG][mA][mG][mC][mUs][mAs][mG] (SEQ ID NO: 2020); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 598 and a structure (5' → 3') of

[mAs][mGs][mC][mU][mC][mU][mU][mU][fA][fC][fC][fC][mA][mC][mA][mG][mU][mU][mA][mA][mAs] (SEQ ID NO: 1418);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula ($Z^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

47. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1180 and a structure (5' → 3') of

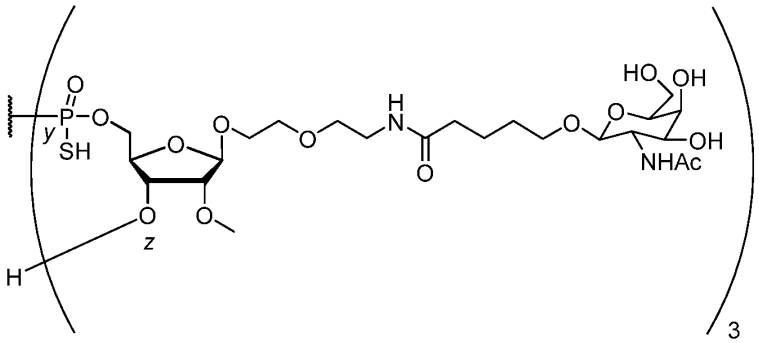
[mUs][fCs][fA][mA][fG][fA][fA][fU][mA][fA][mG][mC][mC][fA][mU][mA][mG][mA][mC][mA][mAs][mAs][mG] (SEQ ID NO: 2029); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 404 and a structure (5' → 3') of

[mUs][mUs][mG][mU][mC][mU][mA][mU][fG][fG][fC][fU][mU][mA][mU][mU][mC][mU][mU][mG][mAs] (SEQ ID NO: 1202);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula (Z$^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

48. An RNAi agent comprising:

an antisense strand comprising the nucleobase sequence of SEQ ID NO: 1181 and a structure (5' → 3') of

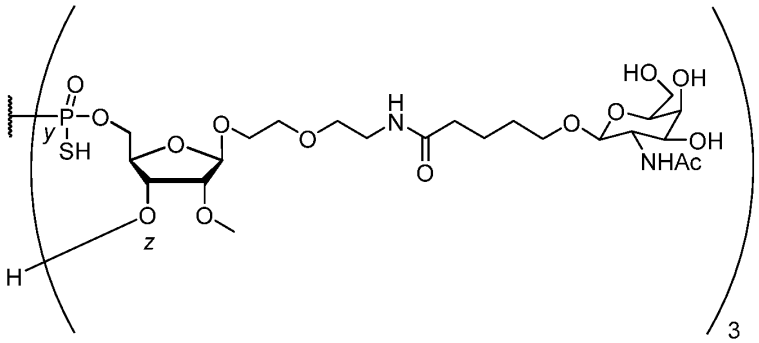
[mUs][fUs][fU][mC][fC][fG][fU][mG][mA][fG][mG][mG][mA][fA][mU][mU][mC][mA][mA][mG][mGs][mAs][mG] (SEQ ID NO: 2033); and

a sense strand comprising the nucleobase sequence of SEQ ID NO: 464 and a structure (5' → 3') of

[mCs][mCs][mU][mU][mG][mA][mA][mU][fU][fC][fC][fC][mU][mC][mA][mC][mG][mG][mA][mA][mAs] (SEQ ID NO: 1267);

wherein mA, mC, mG, and mU are 2'-O-methyl adenosine, cytidine, guanosine, and uridine, respectively; fA, fC, fG, and fU are 2'-fluoro adenosine, cytidine, guanosine, and uridine, respectively; and s is a phosphorothioate linkage;

and wherein the 3' terminal nucleoside of the sense strand is covalently linked to a structure of Formula (Z$^6$)



(Z$^6$),

or a pharmaceutically acceptable salt thereof, wherein the "s" of the 3'-terminal [mAs] of the sense strand corresponds to the phosphorothioate linkage of the first repeat unit of a targeting moiety (i.e., the repeat unit that is directly covalently linked to the 3'-O of the sugar moiety of the 3' terminal nucleoside of the sense strand), wherein the targeting moiety comprises a structure of Formula (Z$^6$).

49. A pharmaceutical composition for inhibiting expression of a gene encoding CYP7A1 comprising the RNAi agent of any one of claims 1-48.

50. A method of inhibiting expression of a CYP7A1 gene in a cell, the method comprising contacting the cell with the RNAi agent of any one of claims 1-48, or the pharmaceutical composition of claim 49, thereby inhibiting expression of the CYP7A1 gene in the cell.

51. The method of claim 50, wherein the cell is within a subject, optionally wherein the subject is human.

52. The method of claim 51, wherein the subject has a CYP7A1 disease or CYP7A1-associated disease.

53. A method of treating a subject having a CYP7A1 disease or CYP7A1-associated disease, comprising administering to the subject a therapeutically effective amount of the RNAi agent of any one of claims 1-48, or the pharmaceutical composition of claim 49, thereby treating the subject having the CYP7A1 disease or CYP7A1-associated disease.

54. A method of treating at least one symptom in a subject having a CYP7A1 disease or CYP7A1-associated disease, comprising administering to the subject an effective amount of the RNAi agent of any one of claims 1-48, or the pharmaceutical composition of claim 49, thereby

treating at least one symptom in the subject having the CYP7A1 disease or CYP7A1-associated disease.

55. The method of claim 53 or claim 54, wherein the disease is a CYP7A1 disease or CYP7A1-associated disease, optionally wherein the CYP7A1 disease or CYP7A1-associated disease is primary sclerosing cholangitis (PSC).

56. The method of any one of claims 53-55, wherein the subject is human.

57. The method of any one of claims 53-56, further comprising administering an agent for the treatment of a CYP7A1 disease or CYP7A1-associated disease.