RNAi Agents And Compositions For Inhibiting Expression Of Angiopoietin-Like 3 (ANGPTL3), And Methods Of Use

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Provisional Patent Application Serial No. 62/694,976, filed on July 7, 2018, United States Provisional Patent Application Serial No. 62/651,284, filed on April 2, 2018, United States Provisional Patent Application Serial No. 62/583,919, filed on November 9, 2017, and United States Provisional Patent Application Serial No. 62/558,819, filed on September 14, 2017, the contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

This application contains a Sequence Listing which has been submitted in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy is named 30658_SequenceListing and is 111 kb in size.

FIELD OF THE INVENTION

The present disclosure relates to RNA interference (RNAi) agents, e.g., double stranded RNAi agents, for inhibition of angiopoietin-like 3 gene expression, compositions that include angiopoietin-like 3 RNAi agents, and methods of use thereof.

BACKGROUND

Angiopoietin-like 3 (also called ANGPTL3, ANGPL3, ANG3, or angiopoietin-like protein 3) is an angiopoietin protein encoded by the human angiopoietin-like 3 gene that is reported to be involved in regulating lipid metabolism. ANGPTL3 is a 460-amino acid polypeptide that consists of a signal peptide, N-terminal coiled-coil domain, and a C-terminal fibrinogen (FBN)-like domain. ANGPTL3 is known to be primarily produced in hepatocytes in humans, and after synthesis is secreted into circulation. ANGPTL3 acts as an inhibitor of lipoprotein lipase, which catalyzes hydrolysis of triglycerides, and endothelial lipase, which hydrolyzes lipoprotein phospholipids. Inhibition of these enzymes can cause increases in plasma levels of triglycerides, high-density lipoproteins (HDL), and phospholipids. Further, loss-of-function mutations in ANGPTL3 lead to familial hypobetalipoproteinemia, which is characterized by

low levels of triglycerides and low-density lipoprotein (LDL-C) in plasma. In humans, loss-of-function in ANGPTL3 is also correlated with a decreased risk of atherosclerotic cardiovascular disease.

An effective therapeutic that targets ANGPTL3 could provide a beneficial impact in the treatment (including prophylactic treatment) of cardiometabolic diseases such as hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic-related disorders and diseases. While certain double-stranded RNA-based compounds have been identified as being capable of inhibiting the expression of an ANGPTL3 gene (*see, e.g.*, International Patent Application Publication Nos. WO 2012/177784, WO 2016/168286, and WO 2016/154127), the ANGPTL3 RNAi agents disclosed herein were not previously disclosed or known, and provide for highly potent and highly efficient ANGPTL3-specific inhibition of expression of an ANGTPL3 gene.

SUMMARY

There exists a need for novel ANGPTL3-specific RNA interference (RNAi) agents (also herein termed RNAi agent, RNAi trigger, or trigger), *e.g.*, double stranded RNAi agents, that are able to selectively and efficiently inhibit the expression of an ANGPTL3 gene. Further, there exists a need for compositions that include novel ANGPTL3-specific RNAi agents for the treatment of diseases associated with, among other things, elevated triglyceride (TG) levels.

In general, the present disclosure features ANGPTL3 gene-specific RNAi agents, compositions that include ANGPTL3 RNAi agents, and methods for inhibiting expression of an ANGPTL3 gene *in vitro* and/or *in vivo* using the ANGPTL3 RNAi agents and compositions that include ANGPTL3 RNAi agents described herein. The ANGPTL3 RNAi agents described herein can selectively and efficiently decrease or inhibit expression of an ANGPTL3 gene, and thereby reduce TG levels and/or cholesterol levels in a subject, e.g., a human or animal subject.

The described ANGPTL3 RNAi agents can be used in methods for therapeutic treatment (including the prophylactic and preventative treatment) of symptoms and diseases associated

with elevated TG levels and/or elevated cholesterol levels, including, but not limited to hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic-related disorders and diseases. The ANGPTL3 RNAi agents disclosed herein can selectively reduce ANGPTL3 gene expression, which can lead to a reduction in, among other things, TG levels and/or cholesterol levels, in a subject. The methods disclosed herein include the administration of one or more ANGPTL3 RNAi agents to a subject, e.g., a human or animal subject, using any suitable methods known in the art, such as subcutaneous injection or intravenous administration.

In one aspect, the disclosure features RNAi agents for inhibiting expression of the human ANGPTL3 gene, wherein the RNAi agent includes a sense strand and an antisense strand. Also described herein are compositions that include or consist of an RNAi agent capable of inhibiting the expression of an ANGPTL3 gene, wherein the RNAi agent includes or consists of a sense strand and an antisense strand, and the composition further comprises at least one pharmaceutically acceptable excipient. The compositions described herein that include one or more of the disclosed ANGPTL3 RNAi agents are able to selectively and efficiently decrease expression of an ANGPTL3 gene. The compositions that include one or more ANGPTL3 RNAi agents can be administered to a subject, such as a human or animal subject, for the treatment (including prophylactic treatment or inhibition) of symptoms and diseases associated with elevated TG, elevated cholesterol, and/or enhanced ANGPTL3 expression.

An ANGPTL3 RNAi agent described herein includes a sense strand (also referred to as a passenger strand), and an antisense strand (also referred to as a guide strand). The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the RNAi agent sense and antisense strands described herein each can be 16 to 30 nucleotides in length. In some embodiments, the sense and antisense strands are independently 17 to 26 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. In some embodiments, the sense and antisense strands are independently 21 to 26 nucleotides in length. In some embodiments, the sense and antisense strands are independently 21 to 24 nucleotides in length. In some embodiments, both the sense strand and the antisense strand are 21 nucleotides in length. In some embodiments, the sense

and/or antisense strands are independently 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. The RNAi agents described herein, upon delivery to a cell expressing ANGPTL3, inhibit the expression of one or more ANGPTL3 genes *in vivo* or *in vitro*.

A sense strand of the ANGPTL3 RNAi agents described herein includes at least 16 consecutive nucleotides that have at least 85% identity to a core stretch sequence (also referred to herein as a "core stretch" or "core sequence") of the same number of nucleotides in an ANGPTL3 mRNA. In some embodiments, this sense strand core stretch is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, this sense strand core stretch is 17 nucleotides in length. In some embodiments, this sense strand core stretch is 19 nucleotides in length.

An antisense strand of an ANGPTL3 RNAi agent described herein includes at least 16 consecutive nucleotides that have at least 85% complementarity to a core stretch of the same number of nucleotides in an ANGPTL3 mRNA and to a core stretch of the same number of nucleotides in the corresponding sense strand. In some embodiments, this antisense strand core stretch is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, this antisense strand core stretch is 19 nucleotides in length. In some embodiments, this antisense strand core stretch is 17 nucleotides in length

In some embodiments, the ANGPTL3 RNAi agents disclosed herein target the portion of an ANGPTL3 gene having the sequence of any of the sequences disclosed in Table 1.

Examples of ANGPTL3 RNAi agent sense strands and antisense strands that can be included in the ANGPTL3 RNAi agents disclosed herein are provided in Table 3 and Table 4. Examples of ANGPTL3 RNAi agent duplexes are provided in Table 5. Examples of 19-nucleotide core stretch sequences that consist of or are included in the sense strands and antisense strands of ANGPTL3 RNAi agents disclosed herein, are provided in Table 2.

In another aspect, the disclosure features methods for delivering ANGPTL3 RNAi agents to liver cells in a subject, such as a mammal, *in vivo*. Also described herein are compositions for use in such methods. The one or more ANGPTL3 RNAi agents can be delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. Nucleic acid delivery methods include, but are not limited to, by encapsulation in liposomes, by iontophoresis, or by

incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, proteinaceous vectors, or Dynamic Polyconjugates™ (DPCs) (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, each of which is incorporated herein by reference).

In some embodiments, an ANGPTL3 RNAi agent is delivered to target cells or tissues by covalently linking or conjugating the RNAi agent to a targeting group, such as an asialoglycoprotein receptor ligand (i.e., a ligand that includes a compound having affinity for the asialoglycoprotein receptor). In some embodiments, an asialoglycoprotein receptor ligand includes, consists of, or consists essentially of, a galactose or galactose derivative cluster. In some embodiments, an ANGPTL3 RNAi agent is linked to a targeting ligand comprising the galactose derivative N-acetyl-galactosamine. In some embodiments, a galactose derivative cluster includes an N-acetyl-galactosamine trimer or an N-acetyl-galactosamine tetramer. In some embodiments, a galactose derivative cluster is an N-acetyl-galactosamine trimer or an N-acetyl-galactosamine tetramer. In some embodiments, the ANGPTL3 RNAi agents that are conjugated to targeting ligands that include N-acetyl-galactosamine are selectively internalized by liver cells, and hepatocytes in particular, either through receptor-mediated endocytosis or by other means. Examples of targeting groups useful for delivering RNAi agents are disclosed, for example, in International Patent Application Publication Nos. WO 2018/044350 and WO 2017/156012, which are incorporated by reference herein in their entirety.

A targeting group can be linked to the 3′ or 5′ end of a sense strand or an antisense strand of an ANGPTL3 RNAi agent. In some embodiments, a targeting group is linked to the 3′ or 5′ end of the sense strand. In some embodiments, a targeting group is linked to the 5′ end of the sense strand. In some embodiments, a targeting group is linked internally to a nucleotide on the sense strand and/or the antisense strand of the RNAi agent. In some embodiments, a targeting group is linked to the RNAi agent via a linker.

A targeting group, with or without a linker, can be linked to the 5′ or 3′ end of any of the sense and/or antisense strands disclosed in Tables 2, 3, and 4. A linker, with or without a targeting group, can be attached to the 5′ or 3′ end of any of the sense and/or antisense strands disclosed in Tables 2, 3, and 4.

In some embodiments, described herein are compositions that include one or more ANGPTL3 RNAi agents that have the duplex structures disclosed in Table 5.

In a further aspect, described herein are pharmaceutical compositions that include one or more described ANGPTL3 RNAi agent(s), optionally combined with one or more additional (i.e., second, third, etc.) therapeutics. In some embodiments, the pharmaceutical compositions that include one or more described ANGPTL3 RNAi agent(s), optionally combined with one or more additional (i.e., second, third, etc.) therapeutics, can be formulated in a pharmaceutically acceptable carrier or diluent. In some embodiments, these compositions can be administered to a subject, such as a mammal. In some embodiments, the mammal is a human.

In some embodiments, described herein are compositions that include a combination or cocktail of at least two ANGPTL3 RNAi agents having different nucleotide sequences. In some embodiments, the two or more different ANGPTL3 RNAi agents are each separately and independently linked to targeting groups. In some embodiments, the two or more different ANGPTL3 RNAi agents are each linked to targeting groups that include or consist of targeting ligands that include one or more moieties that target the asialoglycoprotein receptor. In some embodiments, the two or more different ANGPTL3 RNAi agents are each linked to targeting groups that include or consist of targeting ligands that include one or more galactose-derivatives. In some embodiments, the two or more different ANGPTL3 RNAi agents are each linked to targeting groups that include or consist of targeting ligands that include one or more N-acetyl-galactosamines.

In another aspect, the disclosure features methods for inhibiting expression of an ANGPTL3 gene, wherein the methods include administering to a subject or to a cell of a subject an amount of an ANGPTL3 RNAi agent capable of inhibiting the expression of an ANGPTL3 gene, wherein the ANGPTL3 RNAi agent comprises a sense strand and an antisense strand, and wherein the antisense strand includes the sequence of any one of the antisense strand nucleotide sequences in Table 2 or Table 3. In some embodiments, disclosed herein are methods of inhibiting expression of an ANGPTL3 gene, wherein the methods include administering to a subject or to a cell an amount of an ANGPTL3 RNAi agent capable of inhibiting the expression of an ANGPTL3 gene, wherein the ANGPTL3 RNAi agent comprises a sense strand and an antisense strand, and wherein the sense strand includes the sequence of any one of the sense

strand nucleotide sequences in Tables 2 or 4. Also described herein are compositions for use in such methods.

In a further aspect, the disclosure features methods of treatment (including preventative or prophylactic treatment) of diseases or symptoms caused by elevated TG levels and/or elevated cholesterol levels, wherein the methods include administering to a subject in need thereof an ANGPTL3 RNAi agent having an antisense strand that includes the sequence of any of the sequences in Tables 2 or 3. In some embodiments, described herein are methods of treatment (including preventative treatment) of diseases or symptoms caused by elevated TG levels and/or elevated cholesterol levels, wherein the methods include administering to a subject in need thereof an ANGPTL3 RNAi agent having a sense strand comprising the sequence of any of the sequences in Tables 2 or 4. Also described herein are compositions for use in such methods.

Also described are methods of treating a human subject having a pathological state (such as a condition or disease), or being at risk of developing a pathological state, that is mediated at least in part by ANGPTL3 gene expression, the methods comprising the step of administering to the subject a therapeutically effective amount of a ANGPTL3 RNAi agent and/or ANGPTL3 RNAi agent-containing composition. The method of treating a subject with an ANGPTL3 RNAi agent and/or ANGPTL3 RNAi agent-containing composition can optionally be combined with one or more steps of administering one or more additional (i.e., second, third, etc.) therapeutics or treatments. An additional therapeutic can be another ANGPTL3 RNAi agent (e.g., an ANGPTL3 RNAi agent that targets a different sequence within the ANGPTL3 gene). An additional therapeutic can also be a small molecule drug, antibody, antibody fragment, and/or aptamer. In some embodiments, the one or more additional therapeutics is a statin, such as atorvastatin, fluvastatin, pravastatin, pitavastatin, rosuvastatin, or simvastatin.

In some embodiments, the described ANGPTL3 RNAi agent(s) are optionally combined with one or more additional therapeutics. The ANGPTL3 RNAi agent and additional therapeutic(s) can be administered in a single composition or they can be administered separately. In some embodiments, the one or more additional therapeutics is administered separately in separate dosage forms from the RNAi agent (e.g., the ANGPTL3 RNAi agent is administered by subcutaneous injection, while the additional therapeutic involved in the method of treatment dosing regimen is administered orally). In some embodiments, the described ANGPTL3 RNAi

agent(s) are administered to a subject in need thereof via subcutaneous injection, and the one or more optional additional therapeutics are administered orally, which together provide for a treatment regimen for diseases and conditions associated with elevated TG and/or cholesterol levels. In some embodiments, the described ANGPTL3 RNAi agent(s) are administered to a subject in need thereof via subcutaneous injection, and the one or more optional additional therapeutics are administered via a separate subcutaneous injection. In some embodiments, the ANGPTL3 RNAi agent and one or more additional therapeutics are combined into a single dosage form (e.g., a "cocktail" formulated into a single composition for subcutaneous injection). The ANGPTL3 RNAi agents, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

In some embodiments, disclosed herein are methods for inhibiting expression of an ANGPTL3 gene in a cell or a subject, wherein the methods include administering to the cell or subject an ANGPTL3 RNAi agent having a sense strand comprising the sequence of any of the sequences in Table 4, and an antisense strand comprising the sequence of any of the sequences in Table 3.

In some embodiments, compositions for delivering an ANGPTL3 RNAi agent to a liver cell, particularly hepatocytes, *in vivo*, are described, the compositions comprising: an ANGPTL3 RNAi agent conjugated to a targeting group. In some embodiments, the targeting group is an asialoglycoprotein receptor ligand.

In some embodiments, disclosed herein are methods for inhibiting expression of an ANGPTL3 gene in a cell, the methods comprising administering one or more ANGPTL3 RNAi agents having the duplex structure of a duplex set forth in Table 5.

In some embodiments, disclosed herein are methods of treatment (including prophylactic or preventative treatment) of diseases, disorders, or symptoms caused by elevated TG levels and/or elevated cholesterol levels, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an ANGPTL3 RNAi agent that includes an antisense strand that is at least partially complementary to the portion of the ANGPTL3 mRNA having the sequence in Table 1. In some embodiments, disclosed herein are methods of treatment (including prophylactic or preventative treatment) of diseases or symptoms caused

by elevated TG levels and/or elevated cholesterol levels, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an ANGPTL3 RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Tables 2 or 3, and a sense strand that comprises any of the sequences in Tables 2 or 4 that is at least partially complementary to the antisense strand. In some embodiments, disclosed herein are methods of treatment (including prophylactic or preventative treatment) of diseases or symptoms caused by elevated TG levels and/or elevated cholesterol levels, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an ANGPTL3 RNAi agent that includes a sense strand that comprises any of the sequences in Tables 2 or 4, and an antisense strand comprising the sequence of any of the sequences in Tables 2 or 3 that is at least partially complementary to the sense strand.

In some embodiments, disclosed herein are methods for inhibiting expression of an ANGPTL3 gene in a cell, wherein the methods include administering to the cell an ANGPTL3 RNAi agent that includes an antisense strand that is at least partially complementary to the portion of the ANGPTL3 mRNA having the sequence in Table 1. In some embodiments, disclosed herein are methods of inhibiting expression of an ANGPTL3 gene in a cell, wherein the methods include administering to a cell an ANGPTL3 RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Tables 2 or 3, and a sense strand that comprises any of the sequences in Tables 2 or 4 that is at least partially complementary to the antisense strand. In some embodiments, disclosed herein are methods of inhibiting expression of an ANGPTL3 gene in a cell, wherein the methods include administering an ANGPTL3 RNAi agent that includes a sense strand that comprises any of the sequences in Tables 2 or 4, and an antisense strand that includes the sequence of any of the sequences in Tables 2 or 3 that is at least partially complementary to the sense strand.

In some embodiments, disclosed herein are compositions for inhibiting expression of an ANGPTL3 gene in a cell, wherein the methods include administering a composition that comprises an ANGPTL3 RNAi agent having the duplex structure of a duplex set forth in Table 5.

In some embodiments, disclosed herein are compositions for delivering an ANGPTL3 RNAi agent to a liver cell *in vivo*, the composition including an ANGPTL3 RNAi agent conjugated or linked to a targeting group. In some embodiments, the targeting group is an

asialoglycoprotein receptor ligand. In some embodiments, compositions for delivering an ANGPTL3 RNAi agent to a liver cell *in vivo* are described, the composition including an ANGPTL3 RNAi agent linked to an N-acetyl-galactosamine targeting ligand.

The ANGPTL3 RNAi agents disclosed herein are designed to target specific positions on an ANGPTL3 gene (SEQ ID NO:1). As defined herein, an antisense strand sequence is designed to target an ANGPTL3 gene at a given position on the gene when the 5′ terminal nucleobase of the antisense strand is aligned with a position that is 19 nucleotides downstream (towards the 3′ end) from the position on the gene when base pairing to the gene. For example, as illustrated in Tables 1 and 2 herein, an antisense strand sequence designed to target an ANGPTL3 gene at position 304 requires that when base pairing to the gene, the 5′ terminal nucleobase of the antisense strand is aligned with position 322 of the ANGPTL3 gene.

As provided herein, an ANGPTL3 RNAi agent does not require that the nucleobase at position 1 (5′ → 3′) of the antisense strand be complementary to the gene, provided that there is at least 85% complementarity (e.g., at least 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% complementarity) of the antisense strand and the gene across a core stretch sequence of at least 16 consecutive nucleotides. For example, for an ANGPTL3 RNAi agent disclosed herein that is designed to target position 304 of an ANGPTL3 gene, the 5′ terminal nucleobase of the antisense strand of the of the ANGPTL3 RNAi agent must be aligned with position 322 of the gene; however, the 5′ terminal nucleobase of the antisense strand may be, but is not required to be, complementary to position 322 of an ANGPTL3 gene, provided that there is at least 85% complementarity (e.g., at least 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% complementarity) of the antisense strand and the gene across a core stretch sequence of at least 16 consecutive nucleotides. As shown by, among other things, the various examples disclosed herein, the specific site of binding of the gene by the antisense strand of the ANGPTL3 RNAi agent (e.g., whether the ANGPTL3 RNAi agent is designed to target an ANGPTL3 gene at position 304, at position 921, at position 922, or at some other position) is important to the level of inhibition achieved by the ANGPTL3 RNAi agent.

The use of ANGPTL3 RNAi agents provides methods for therapeutic (including prophylactic) treatment of diseases/disorders associated with elevated TG and/or cholesterol levels and/or enhanced or elevated ANGPTL3 expression. The described ANGPTL3 RNAi agents mediate RNA interference to inhibit the expression of one or more genes necessary for production of

ANGPTL3. ANGPTL3 RNAi agents can also be used to treat or prevent various diseases or disorders, including hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, diabetes, cardiovascular disease, coronary artery disease, and other metabolic-related disorders and diseases. Furthermore, compositions for delivery of ANGPTL3 RNAi agents to liver cells *in vivo* are described.

The pharmaceutical compositions including one or more ANGPTL3 RNAi agents can be administered in a number of ways depending upon whether local or systemic treatment is desired. Administration can be, but is not limited to, intravenous, intraarterial, subcutaneous, intraperitoneal, subdermal (e.g., via an implanted device), and intraparenchymal administration. In some embodiments, the pharmaceutical compositions described herein are administered by subcutaneous injection.

In some embodiments, disclosed herein are compositions for delivering an ANGPTL3 RNAi agent to a liver cell *in vivo*, wherein the composition includes an ANGPTL3 RNAi agent conjugated or linked to a targeting group. In some embodiments, the targeting group is an asialoglycoprotein receptor ligand. In some embodiments, compositions for delivering an ANGPTL3 RNAi agent to a liver cell *in vivo* are described, wherein the composition includes an ANGPTL3 RNAi agent linked to a targeting ligand that includes N-acetyl-galactosamine.

In some embodiments, the ANGPTL3 RNAi agents described herein can include one or more targeting groups having the structure of (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s, each as defined herein in Table 6.

In some embodiments, the ANGPTL3 RNAi agents described herein include one targeting group at the 5' end of the sense strand having the structure of (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s, each as defined herein in Table 6.

The described ANGPTL3 RNAi agents and/or compositions that include ANGPTL3 RNAi agents can be used in methods for therapeutic treatment of diseases or conditions caused by elevated TG and/or cholesterol levels. Such methods include administration of an ANGPTL3 RNAi agent as described herein to a subject, e.g., a human or animal subject.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3), wherein SEQ ID NO:3 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. As the person of ordinary skill in the art would clearly understand, the inclusion of a phosphorothioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides (*see, e.g.*, Figs. 5A through 5K showing all internucleoside linkages). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a

phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. As the person of ordinary skill in the art would clearly understand, the inclusion of a phosphorothioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides (*see, e.g.*, Figs. 5A through 5K showing all internucleoside linkages). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6), wherein SEQ ID NO:5 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. As the person of ordinary skill in the art would clearly understand, the inclusion of a phosphorothioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides (*see, e.g.*, Figs. 5A through 5K showing all internucleoside linkages). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8), wherein SEQ ID NO:8 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10), wherein SEQ ID NO:10 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a

phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12), wherein SEQ ID NO:12 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s

represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15), wherein all or substantially all of the nucleotides are modified nucleotides. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15), wherein SEQ ID NO:14 is located at positions 1-21 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence differing

by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the nucleotide sequence (5′ → 3′) asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage, and wherein the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) GCUCAACAUAUUUGAUCAGUA (SEQ ID NO:17). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) GCUCAACAUAUUUGAUCAGUA (SEQ ID NO:17), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) GCUCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:19), wherein (A^{2N}) represents a 2-aminoadenine nucleotide. In some embodiments, an ANGPTL3 RNAi agent disclosed herein

includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) GCUCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:19), wherein (A^{2N}) represents a 2-aminoadenine nucleotide, and wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) GCUCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:21), wherein (A^{2N}) represents a 2-aminoadenine nucleotide. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) GCUCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:21), wherein (A^{2N}) represents a 2-aminoadenine nucleotide, and wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) ACUCAACAUAUUUGAUCAGUA (SEQ ID NO:24). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6), wherein

all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) ACUCAACAUAUUUGAUCAGUA (SEQ ID NO:24), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) GCCCAUGGACAUUAAUUCAAA (SEQ ID NO:26). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) GCCCAUGGACAUUAAUUCAAA (SEQ ID NO:26), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) ACCCAUGGACAUUAAUUCAAA (SEQ ID NO:28). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) ACCCAUGGACAUUAAUUCAAA (SEQ ID NO:28), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) UCCAUGGACAUUAAUUCAACA (SEQ ID NO:30). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) UCCAUGGACAUUAAUUCAACA (SEQ ID NO:30), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15) and a sense strand that consists of, consists essentially of, or comprises a nucleobase sequence differing by 0 or 1 nucleobases from the nucleotide sequence (5′ → 3′) GGUUGCUAUGUUAGACGAUGU (SEQ ID NO:32). In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15), wherein all or substantially all of the nucleotides are modified nucleotides, and a sense strand that consists of, consists essentially of, or comprises a nucleotide sequence differing by no more than 1 nucleotide from the nucleotide sequence (5′ → 3′) GGUUGCUAUGUUAGACGAUGU (SEQ ID NO:32), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfAfUfuugaucagua (SEQ ID NO:16), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro

adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfAfUfuugaucagua (SEQ ID NO:16), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfa_2NUfuugaucagua (SEQ ID NO:18), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; a_2N represents 2′-O-methyl-2-aminoadenosine (see Table 6); Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfa_2NUfuugaucagua (SEQ ID NO:18), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfAfUfuugaucagua (SEQ ID NO:16), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate

linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaacaUfAfUfuugaucagua (SEQ ID NO:16), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaaca_2NUfa_2NUfuugaucagua (SEQ ID NO:20), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; a_2N represents 2′-O-methyl-2-aminoadenosine (see Table 6); Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcucaaca_2NUfa_2NUfuugaucagua (SEQ ID NO:20), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcUfcAfaCfaUfAfUfuugaucagua (SEQ ID NO:22), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; a_2N represents 2′-O-methyl-2-aminoadenosine (see Table 6); Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some

embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcUfcAfaCfaUfAfUfuugaucagua (SEQ ID NO:22), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) acucaacaUfAfUfuugaucagua (SEQ ID NO:23), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) acucaacaUfAfUfuugaucagua (SEQ ID NO:23), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcccauggAfCfAfuuaauucaaa (SEQ ID NO:25), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide

sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gcccauggAfCfAfuuaaauucaaa (SEQ ID NO:25), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) acccauggAfCfAfuuaaauucaaa (SEQ ID NO:27), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) acccauggAfCfAfuuaaauucaaa (SEQ ID NO:27), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) uccauggaCfAfUfuaauucaaca (SEQ ID NO:29), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ →

3′) uccauggaCfAfUfuaauucaaca (SEQ ID NO:29), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) uccauggaCfAfUfuaauucaaca (SEQ ID NO:29), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) uccauggaCfAfUfuaauucaaca (SEQ ID NO:29), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) gguugcuaUfGfUfuagacgaugu (SEQ ID NO:31), wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s represents a phosphorothioate linkage. In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14), and a sense strand that consists of, consists essentially of, or comprises the modified nucleotide sequence (5′ → 3′) cccuaaaaGfGfGfacaguauucu (SEQ ID NO:31), and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide

sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′):

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3);
UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6);
UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8);
UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10);
UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12); or
ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15);

wherein the ANGPTL3 RNAi agent further includes a sense strand that is at least partially complementary to the antisense strand; and wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′):

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3);
UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6);
UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8);
UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10);
UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12); or
ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15);

wherein the ANGPTL3 RNAi agent further includes a sense strand that is at least partially complementary to the antisense strand; wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides; and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′):

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3);

UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6);

UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8);

UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10);

UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12); or

ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15);

wherein the ANGPTL3 RNAi agent further includes a sense strand that is at least partially complementary to the antisense strand; wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides; and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine; and wherein the respective antisense strand sequence is located at positions 1-21 of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand, wherein the antisense strand and the sense strand consist of, consist essentially of, or comprise nucleotide sequences that differ by 0 or 1 nucleotides from one of the following nucleotide sequence (5′ → 3′) pairs:

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAACAUAUUUGAUCAGUA (SEQ ID NO:17);

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:19), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:21), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6) and ACUCAACAUAUUUGAUCAGUA (SEQ ID NO:24);

UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8) and GCCCAUGGACAUUAAUUCAAA (SEQ ID NO:26);

UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10) and ACCCAUGGACAUUAAUUCAAA (SEQ ID NO:28);

UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12) and UCCAUGGACAUUAAUUCAACA (SEQ ID NO:30); or

ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15) and GGUUGCUAUGUUAGACGAUGU (SEQ ID NO:32);

wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand, wherein the antisense strand and the sense strand consist of, consist essentially of, or comprise nucleotide sequences that differ by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′) pairs:

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAACAUAUUUGAUCAGUA (SEQ ID NO:17);

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:19), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGAGC (SEQ ID NO:3) and GCUCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:21), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGAGU (SEQ ID NO:6) and ACUCAACAUAUUUGAUCAGUA (SEQ ID NO:24);

UUUGAAUUAAUGUCCAUGGGC (SEQ ID NO:8) and GCCCAUGGACAUUAAUUCAAA (SEQ ID NO:26);

UUUGAAUUAAUGUCCAUGGGU (SEQ ID NO:10) and ACCCAUGGACAUUAAUUCAAA (SEQ ID NO:28);

UGUUGAAUUAAUGUCCAUGGA (SEQ ID NO:12) and UCCAUGGACAUUAAUUCAACA (SEQ ID NO:30); or

ACAUCGUCUAACAUAGCAACC (SEQ ID NO:15) and GGUUGCUAUGUUAGACGAUGU (SEQ ID NO:32);

wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides; and wherein the sense strand further includes inverted abasic residues at the 3’ terminal end and at the 5’ end of the nucleotide sequence, and the

sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′):

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2);
usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4);
usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5);
usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7);
usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9);
usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11);
usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13);
asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14);

wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s represents a phosphorothioate linkage; and wherein the ANGPTL3 RNAi agent further includes the sense strand that is at least partially complementary to the antisense strand; and wherein all or substantially all of the nucleotides on the sense strand are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that consists of, consists essentially of, or comprises a modified nucleotide sequence that differs by 0 or 1 nucleotides from one of the following nucleotide sequences (5′ → 3′):

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2);
usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4);
usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5);
usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7);
usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9);
usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11);
usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13);
asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14);

wherein the ANGPTL3 RNAi agent further includes the sense strand that is at least partially complementary to the antisense strand; wherein all or substantially all of the nucleotides on the

sense strand are modified nucleotides; wherein all or substantially all of the nucleotides on both the antisense strand and the sense strand are modified nucleotides; and wherein the sense strand further includes inverted abasic residues at the 3' terminal end and at the 5' end of the nucleotide sequence, and the sense strand also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand that consists of, consists essentially of, or comprises modified nucleotide sequences that differs by 0 or 1 nucleotides from one of the following nucleotide sequence pairs (5′ → 3′):

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaacaUfAfUfuugaucagua (SEQ ID NO:16);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaacaUfa_2NUfuugaucagua (SEQ ID NO:18);

usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4) and
gcucaacaUfAfUfuugaucagua (SEQ ID NO:16);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaaca_2NUfa_2NUfuugaucagua (SEQ ID NO:20);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcUfcAfaCfaUfAfUfuugaucagua (SEQ ID NO:22);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5) and
acucaacaUfAfUfuugaucagua (SEQ ID NO:23);

usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7) and
gcccauggAfCfAfuuaauucaaa (SEQ ID NO:25);

usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9) and
acccauggAfCfAfuuaauucaaa (SEQ ID NO:27);

usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11) and
uccauggaCfAfUfuaauucaaca (SEQ ID NO:29);

usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13) and
uccauggaCfAfUfuaauucaaca (SEQ ID NO:29); or

asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14) and
gguugcuaUfGfUfuagacgaugu (SEQ ID NO:31);

wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine,

respectively; a_2N represents 2′-O-methyl-2-aminoadenosine (see Table 6); and s represents a phosphorothioate linkage.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand that consists of, consists essentially of, or comprises one of the following nucleotide sequence pairs (5′ → 3′):

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaacaUfAfUfuugaucagua (SEQ ID NO:16);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaacaUfa_2NUfuugaucagua (SEQ ID NO:18);

usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc (SEQ ID NO:4) and
gcucaacaUfAfUfuugaucagua (SEQ ID NO:16);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcucaaca_2NUfa_2NUfuugaucagua (SEQ ID NO:20);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2) and
gcUfcAfaCfaUfAfUfuugaucagua (SEQ ID NO:22);

usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu (SEQ ID NO:5) and
acucaacaUfAfUfuugaucagua (SEQ ID NO:23);

usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc (SEQ ID NO:7) and
gcccauggAfCfAfuuaauucaaa (SEQ ID NO:25);

usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu (SEQ ID NO:9) and
acccauggAfCfAfuuaauucaaa (SEQ ID NO:27);

usGfsusugaauuaaUfgUfcCfaUfgGfsa (SEQ ID NO:11) and
uccauggaCfAfUfuaauucaaca (SEQ ID NO:29);

usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa (SEQ ID NO:13) and
uccauggaCfAfUfuaauucaaca (SEQ ID NO:29); or

asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc (SEQ ID NO:14) and
gguugcuaUfGfUfuagacgaugu (SEQ ID NO:31);

wherein a, c, g, and u represent 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf represent 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; a_2N represents 2′-O-methyl-2-aminoadenosine (see Table 6); s represents a phosphorothioate linkage; and wherein the sense strand further includes inverted abasic residues at the 3’ terminal end and at the 5’ end of the nucleotide sequence, and the sense strand

also includes a targeting ligand that is covalently linked to the 5' terminal end, wherein the targeting ligand includes N-acetyl-galactosamine.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that includes a nucleobase sequence that differs by 0 or 1 nucleobases from the nucleotide sequences selected from the group consisting of (5′ → 3′):

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50);
UGUUGAAUUAAUGUCCAUG (SEQ ID NO:55);
UUUGAAUUAAUGUCCAUGG (SEQ ID NO:60); or
ACAUCGUCUAACAUAGCAA (SEQ ID NO:64).

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that includes a nucleobase sequence that differs by 0 or 1 nucleobases from the nucleotide sequences selected from the group consisting of (5′ → 3′):

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50);
UGUUGAAUUAAUGUCCAUG (SEQ ID NO:55);
UUUGAAUUAAUGUCCAUGG (SEQ ID NO:60); or
ACAUCGUCUAACAUAGCAA (SEQ ID NO:64); and

wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand that includes a nucleobase sequence that differs by 0 or 1 nucleobases from the nucleotide sequences selected from the group consisting of (5′ → 3′):

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50);
UGUUGAAUUAAUGUCCAUG (SEQ ID NO:55);
UUUGAAUUAAUGUCCAUGG (SEQ ID NO:60); or
ACAUCGUCUAACAUAGCAA (SEQ ID NO:64); and

wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO:50, SEQ ID NO:55, SEQ ID NO:60, or SEQ ID NO:64, respectively, is located at nucleotide positions 1-19 (5′ → 3′) of the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand that each include a nucleobase sequences that differs by 0 or 1 nucleobases from the nucleotide sequence pairs selected from the group consisting of (5′ → 3′):

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAACAUAUUUGAUCAGUA (SEQ ID NO:130);

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:131), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:132), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UGUUGAAUUAAUGUCCAUG (SEQ ID NO:55) and CAUGGACAUUAAUUCAACA (SEQ ID NO:145);

UUUGAAUUAAUGUCCAUGG (SEQ ID NO:60) and CCAUGGACAUUAAUUCAAA (SEQ ID NO:150);

ACAUCGUCUAACAUAGCAA (SEQ ID NO:64) and UUGCUAUGUUAGACGAUGU (SEQ ID NO:154).

In some embodiments, an ANGPTL3 RNAi agent disclosed herein includes an antisense strand and a sense strand that each include a nucleobase sequences that differs by 0 or 1 nucleobases from the nucleotide sequence pairs selected from the group consisting of (5′ → 3′):

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAACAUAUUUGAUCAGUA (SEQ ID NO:130);

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAACAU(A^{2N})UUUGAUCAGUA (SEQ ID NO:131), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UACUGAUCAAAUAUGUUGA (SEQ ID NO:50) and UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA (SEQ ID NO:132), wherein (A^{2N}) represents a 2-aminoadenine nucleotide;

UGUUGAAUUAAUGUCCAUG (SEQ ID NO:55) and CAUGGACAUUAAUUCAACA (SEQ ID NO:145);

UUUGAAUUAAUGUCCAUGG (SEQ ID NO:60) and CCAUGGACAUUAAUUCAAA (SEQ ID NO:150);

ACAUCGUCUAACAUAGCAA (SEQ ID NO:64) and UUGCUAUGUUAGACGAUGU (SEQ ID NO:154); and

wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the compositions described herein comprising one or more ANGPTL3 RNAi agents are packaged in a kit, container, pack, dispenser, pre-filled syringes, or vials. In some embodiments, the compositions described herein are administered parenterally.

As used herein, the terms "oligonucleotide" and "polynucleotide" mean a polymer of linked nucleosides each of which can be independently modified or unmodified.

As used herein, an "RNAi agent" (also referred to as an "RNAi trigger") means a composition that contains an RNA or RNA-like (e.g., chemically modified RNA) oligonucleotide molecule that is capable of degrading or inhibiting (e.g., degrades or inhibits under appropriate conditions) 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. RNAi agents disclosed herein are comprised of a sense strand and an antisense strand, and include, but are not limited to: short (or small) interfering RNAs (siRNAs), double stranded RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), and dicer substrates. The antisense strand of the RNAi agents described herein is at least partially complementary to the mRNA being targeted (i.e. ANGPTL3 mRNA). RNAi agents can include one or more modified nucleotides and/or one or more non-phosphodiester linkages.

As used herein, the terms "silence," "reduce," "inhibit," "down-regulate," or "knockdown" when referring to expression of a given gene, 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 the RNAi agents described herein as compared to a second cell, group of cells, tissue, organ, or subject that has not or have not been so treated.

As used herein, the terms "sequence" and "nucleotide sequence" mean a succession or order of nucleobases or nucleotides, described with a succession of letters using standard nomenclature.

As used herein, a "base," "nucleotide base," or "nucleobase," is a heterocyclic pyrimidine or purine compound that is a component of a nucleotide, and includes the primary purine bases adenine and guanine, and the primary pyrimidine bases cytosine, thymine, and uracil. A nucleobase may further be modified to include, without limitation, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. (*See, e.g.*, Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008). The synthesis of such modified nucleobases (including phosphoramidite compounds that include modified nucleobases) is known in the art.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleobase or nucleotide sequence (e.g., RNAi agent sense strand or targeted mRNA) in relation to a second nucleobase or nucleotide sequence (e.g., RNAi agent antisense strand or a single-stranded antisense oligonucleotide), means the ability of an oligonucleotide or polynucleotide including the first nucleotide sequence to hybridize (form base pair hydrogen bonds under mammalian physiological conditions (or similar conditions in vitro)) and form a duplex or double helical structure under certain standard conditions with an oligonucleotide or polynucleotide including the second nucleotide sequence. Complementary sequences include Watson-Crick base pairs or non-Watson-Crick base pairs and include natural or modified nucleotides or nucleotide mimics, at least to the extent that the above hybridization requirements are fulfilled. Sequence identity or complementarity is independent of modification. For example, a and Af, as defined herein, are complementary to U (or T) and identical to A for the purposes of determining identity or complementarity.

As used herein, "perfectly complementary" or "fully complementary" means that in a hybridized pair of nucleobase or nucleotide sequence molecules, all (100%) of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

As used herein, "partially complementary" means that in a hybridized pair of nucleobase or nucleotide sequence molecules, at least 70%, but not all, of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

As used herein, "substantially complementary" means that in a hybridized pair of nucleobase or nucleotide sequence molecules, at least 85%, but not all, of the bases in a contiguous sequence of a first oligonucleotide will hybridize with the same number of bases in a contiguous sequence of a second oligonucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

As used herein, the terms "complementary," "fully complementary," "partially complementary," and "substantially complementary" are used with respect to the nucleobase 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 sequence of an ANGPTL3 mRNA.

As used herein, the term "substantially identical" or "substantial identity," as applied to a nucleic acid sequence means the nucleotide sequence (or a portion of a nucleotide sequence) has at least about 85% sequence identity or more, e.g., at least 90%, at least 95%, or at least 99% identity, compared to a reference sequence. Percentage of sequence identity is determined by comparing two optimally aligned sequences over a comparison window. The percentage is calculated by determining the number of positions at which the same type of nucleic acid base 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 and multiplying the result by 100 to yield the percentage of sequence identity. The inventions disclosed herein encompass nucleotide sequences substantially identical to those disclosed herein.

As used herein, the terms "treat," "treatment," and the like, 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 in a subject. As used herein, "treat" and "treatment" may include the preventative treatment, management, prophylactic treatment, and/or inhibition or reduction of the number, severity, and/or frequency of one or more symptoms of a disease in a subject.

As used herein, the phrase "introducing into a cell," when referring to an RNAi agent, means functionally delivering the RNAi agent into a cell. The phrase "functional delivery," means delivering the RNAi agent to the cell in a manner that enables the RNAi agent to have the expected biological activity, e.g., sequence-specific inhibition of gene expression.

Unless stated otherwise, use of the symbol  as used herein means that any group or groups may be linked thereto that is in accordance with the scope of the inventions described herein.

As used herein, the term "isomers" refers to compounds that have identical molecular formulae, but that differ in the nature or the sequence of bonding of their atoms or in the arrangement of their atoms in space. 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 "diastereoisomers," and stereoisomers that are non-superimposable mirror images are termed "enantiomers," or sometimes optical isomers. A carbon atom bonded to four non-identical substituents is termed a "chiral center."

As used herein, unless specifically identified in a structure as having a particular conformation, for each structure in which asymmetric centers are present and thus give rise to enantiomers, diastereomers, or other stereoisomeric configurations, each structure disclosed herein is intended to represent all such possible isomers, including their optically pure and racemic forms. For example, the structures disclosed herein are intended to cover mixtures of diastereomers as well as single stereoisomers.

As used in a claim herein, the phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. When used in a claim herein, the phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the claimed invention.

The person of ordinary skill in the art would readily understand and appreciate that the compounds and compositions disclosed herein may have certain atoms (e.g., N, O, or S atoms) in a protonated or deprotonated state, depending upon the environment in which the compound or composition is placed. Accordingly, as used herein, the structures disclosed herein envisage

that certain functional groups, such as, for example, OH, SH, or NH, may be protonated or deprotonated. The disclosure herein is intended to cover the disclosed compounds and compositions regardless of their state of protonation based on the environment (such as pH), as would be readily understood by the person of ordinary skill in the art.

As used herein, the term "linked" or "conjugated" when referring to the connection between two compounds or molecules means that two compounds or molecules are joined by a covalent bond. Unless stated, the terms "linked" and "conjugated" as used herein may refer to the connection between a first compound and a second compound either with or without any intervening atoms or groups of atoms.

As used herein, the term "including" is used to herein mean, and is used interchangeably with, the phrase "including but not limited to." The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless the context clearly indicates otherwise.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other objects, features, aspects, and advantages of the invention will be apparent from the following detailed description, accompanying figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A to 1D. Chemical structure representation of ANGPTL3 RNAi agent AD05488, conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6) at the 5' terminal end of the sense strand, shown in a sodium salt form.

FIG. 2A to 2D. Chemical structure representation of ANGPTL3 RNAi agent AD05488, conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6) at the 5' terminal end of the sense strand, shown in a free acid form.

FIG. 3A to 3D. Chemical structure representation of ANGPTL3 RNAi agent AD05775, conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6) at the 5' terminal end of the sense strand, shown in a sodium salt form.

FIG. 4A to 4D. Chemical structure representation of ANGPTL3 RNAi agent AD05775, conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6) at the 5' terminal end of the sense strand, shown in a free acid form.

FIG. 5A. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05488 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5A discloses SEQ ID NOs: 2 and 300.

The following abbreviations are used in Figures 3A to 3B: a, c, g, and u are 2′-O-methyl modified nucleotides; Af, Cf, Gf, and Uf are 2′-fluoro modified nucleotides; p is a phosphodiester linkage; s is a phosphorothioate linkage; invAb is an inverted abasic residue; a_2N is a 2'-O-methyl-2-aminoadenosine modified nucleotide (*see* Table 6); and (NAG37)s is a tridentate N-acetyl-galactosamine targeting ligand having the structure depicted in Table 6.

FIG. 5B. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05775 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5B discloses SEQ ID NOs: 2 and 334.

FIG. 5C. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05791 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5C discloses SEQ ID NOs: 4 and 300.

FIG. 5D. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05777 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5D discloses SEQ ID NOs: 2 and 336.

FIG. 5E. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05743 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5E discloses SEQ ID NOs: 2 and 326.

FIG. 5F. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05487 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5F discloses SEQ ID NOs: 5 and 299.

FIG. 5G. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05307 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5G discloses SEQ ID NOs: 7 and 278.

FIG. 5H. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05418 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5H discloses SEQ ID NOs: 9 and 292.

FIG. 5I. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05577 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5I discloses SEQ ID NOs: 11 and 279.

FIG. 5J. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05308 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5J discloses SEQ ID NOs: 13 and 279.

FIG. 5K. Schematic diagram of the modified sense and antisense strands of ANGPTL3 RNAi agent AD05840 (*see* Tables 3-5), conjugated to an N-acetyl-galactosamine tridentate ligand having the structure of (NAG37)s (*see* Table 6). Fig. 5K discloses SEQ ID NOs: 15 and 357.

DETAILED DESCRIPTION

RNAi Agents

Described herein are RNAi agents for inhibiting expression of an ANGPTL3 gene (referred to herein as ANGPTL3 RNAi agents or ANGPTL3 RNAi triggers). Each ANGPTL3 RNAi agent comprises a sense strand and an antisense strand. The sense strand and the antisense strand each can be 16 to 30 nucleotides in length. The sense and antisense strands can be either the same length or they can be different lengths. In some embodiments, the sense and antisense strands are each independently 17 to 27 nucleotides in length. In some embodiments, the sense and antisense strands are each independently 17-21 nucleotides in length. In some embodiments, both the sense and antisense strands are each 21-26 nucleotides in length. In some embodiments, the sense and antisense strands are each 21-24 nucleotides in length. In some embodiments, the sense strand is about 19 nucleotides in length while the antisense strand is about 21 nucleotides in length. In some embodiments, the sense strand is about 21 nucleotides in length while the antisense strand is about 23 nucleotides in length. In some embodiments, a sense strand is 23 nucleotides in length and an antisense strand is 21 nucleotides in length. In some embodiments, both the sense and antisense strands are each 21 nucleotides in length. In some embodiments, the RNAi agent sense and antisense strands are each independently 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, or 27 nucleotides in length. In some embodiments, a double-stranded RNAi agent has a duplex length of about 16, 17, 18, 19, 20, 21, 22, 23 or 24 nucleotides.

In some embodiments, the region of perfect, substantial, or partial complementarity between the sense strand and the antisense strand is 16-26 (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26) nucleotides in length and occurs at or near the 5′ end of the antisense strand (e.g., this region may be separated from the 5′ end of the antisense strand by 0, 1, 2, 3, or 4 nucleotides that are not perfectly, substantially, or partially complementary).

The sense strand and antisense strand each contain a core stretch (also referred to herein as a "core sequence" or a "core stretch sequence") that is 16 to 23 nucleotides in length. An antisense strand core stretch is 100% (perfectly) complementary or at least about 85% (substantially) complementary to a nucleotide sequence (sometimes referred to, e.g., as a target sequence) present in the ANGPTL3 mRNA target. A sense strand core stretch sequence is 100% (perfectly) complementary or at least about 85% (substantially) complementary to a core

stretch sequence in the antisense strand, and thus the sense strand core stretch sequence is typically perfectly identical or at least about 85% identical to a nucleotide sequence (target sequence) present in the ANGPTL3 mRNA target. A sense strand core stretch sequence can be the same length as a corresponding antisense core sequence or it can be a different length. In some embodiments, the antisense strand core stretch sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, the sense strand core stretch sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

Examples of nucleotide sequences used in forming ANGPTL3 RNAi agents are provided in Tables 2, 3, and 4. Examples of RNAi agent duplexes, that include the sense strand and antisense strand sequences in Tables 2, 3, and 4, are shown in Table 5.

The ANGPTL3 RNAi agent sense and antisense strands anneal to form a duplex. A sense strand and an antisense strand of an ANGPTL3 RNAi agent can be partially, substantially, or fully complementary to each other. Within the complementary duplex region, the sense strand core stretch sequence is at least 85% complementary or 100% complementary to the antisense core stretch sequence. In some embodiments, the sense strand core stretch sequence contains a sequence of at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23 nucleotides that is at least 85% or 100% complementary to a corresponding 16, 17, 18, 19, 20, 21, 22, or 23 nucleotide sequence of the antisense strand core stretch sequence (i.e., the sense and antisense core stretch sequences of an ANGPTL3 RNAi agent have a region of at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, or at least 23 nucleotides that is at least 85% base paired or 100% base paired.)

In some embodiments, the antisense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2 or Table 3. In some embodiments, the sense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2 or Table 4.

The sense strand and/or the antisense strand can optionally and independently contain an additional 1, 2, 3, 4, 5, or 6 nucleotides (extension) at the 3′ end, the 5′ end, or both the 3′ and 5′ ends of the core stretch sequences. The antisense strand additional nucleotides, if present, may or may not be complementary to the corresponding sequence in the ANGPTL3 mRNA.

The sense strand additional nucleotides, if present, may or may not be identical to the corresponding sequence in the ANGPTL3 mRNA. The antisense strand additional nucleotides, if present, may or may not be complementary to the corresponding sense strand's additional nucleotides, if present.

As used herein, an extension comprises 1, 2, 3, 4, 5, or 6 nucleotides at the 5' and/or 3' end of the sense strand core stretch sequence and/or antisense strand core stretch sequence. The extension nucleotides on a sense strand may or may not be complementary to nucleotides, either core stretch sequence nucleotides or extension nucleotides, in the corresponding antisense strand. Conversely, the extension nucleotides on an antisense strand may or may not be complementary to nucleotides, either core stretch nucleotides or extension nucleotides, in the corresponding sense strand. In some embodiments, both the sense strand and the antisense strand of an RNAi agent contain 3′ and 5′ extensions. In some embodiments, one or more of the 3′ extension nucleotides of one strand base pairs with one or more 5′ extension nucleotides of the other strand. In other embodiments, one or more of 3′ extension nucleotides of one strand do not base pair with one or more 5′ extension nucleotides of the other strand. In some embodiments, an ANGPTL3 RNAi agent has an antisense strand having a 3′ extension and a sense strand having a 5′ extension. In some embodiments, the extension nucleotide(s) are unpaired and form an overhang. As used herein, an "overhang" refers to a stretch of one or more unpaired nucleotides located at a terminal end of either the sense strand or the antisense strand that does not form part of the hybridized or duplexed portion of an RNAi agent disclosed herein.

In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand having a 3′ extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In other embodiments, an ANGPTL3 RNAi agent comprises an antisense strand having a 3′ extension of 1, 2, or 3 nucleotides in length. In some embodiments, one or more of the antisense strand extension nucleotides comprise uracil or thymidine nucleotides or nucleotides that are complementary to the corresponding ANGPTL3 mRNA sequence.

In some embodiments, the 3′ end of the antisense strand can include abasic residues (Ab), which can also be referred to as an "abasic site" or "abasic nucleotide." An abasic residue (Ab) is a nucleotide or nucleoside that lacks a nucleobase at the 1′ position of the sugar moiety. (See,

e.g., U.S. Patent No. 5,998,203). In some embodiments, Ab or AbAb can be added to the 3′ end of the antisense strand.

In some embodiments, the sense strand or the antisense strand may include a "terminal cap," which as used herein is a non-nucleotide compound or other moiety that can be incorporated at one or more termini of a strand of an RNAi agent disclosed herein, and can provide the RNAi agent, in some instances, with certain beneficial properties, such as, for example, protection against exonuclease degradation. In some embodiments, inverted abasic residues (invAb) are added as terminal caps (see Table 6). (See, e.g., F. Czauderna, Nucleic Acids Res., 2003, 31(11), 2705-16). Terminal caps are generally known in the art, and include, for example, inverted abasic residues as well as carbon chains such as a terminal C3, C6, or C12 groups. In some embodiments, a terminal cap is present at either the 5′ terminal end, the 3′ terminal end, or both the 5′ and 3′ terminal ends of the sense strand.

In some embodiments, an ANGPTL3 RNAi agent comprises a sense strand having a 3′ extension of 1, 2, 3, 4, or 5 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprises adenosine, uracil, or thymidine nucleotides, AT dinucleotide, or nucleotides that correspond to nucleotides in the ANGPTL3 mRNA sequence. In some embodiments, the 3′ sense strand extension includes or consists of one of the following sequences, but is not limited to: T, UT, TT, UU, UUT, TTT, or TTTT (each listed 5′ to 3′).

In some embodiments, the 3′ end of the sense strand may include additional abasic residues or inverted abasic terminal caps. In some embodiments, UUAb, UAb, or Ab are added to the 3′ end of the sense strand.

In some embodiments, one or more inverted abasic residues (invAb) are added to the 3′ end of the sense strand. In some embodiments, one or more inverted abasic residues (invAb) are added to the 5′ end of the sense strand. In some embodiments, one or more inverted abasic residues or inverted abasic sites are inserted between the targeting ligand and the nucleobase sequence of the sense strand of the RNAi agent. In some embodiments, the inclusion of one or more inverted abasic residues or inverted abasic sites at or near the terminal end or terminal ends of the sense strand of an RNAi agent allows for enhanced activity or other desired properties of an RNAi agent.

In some embodiments, an ANGPTL3 RNAi agent comprises a sense strand having a 5′ extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In some embodiments, one or more of the sense strand extension nucleotides comprise uracil or adenosine nucleotides or nucleotides that correspond to nucleotides in the ANGPTL3 mRNA sequence. In some embodiments, the sense strand 5′ extension is one of the following sequences, but is not limited to: CA, AUAGGC, AUAGG, AUAG, AUA, A, AA, AC, GCA, GGCA, GGC, UAUCA, UAUC, UCA, UAU, U, UU (each listed 5′ to 3′). A sense strand can have a 3′ extension and/or a 5' extension.

In some embodiments, the 5′ end of the sense strand can include one or more additional abasic residues (e.g., (Ab) or (AbAb)). In some embodiments, one or more inverted abasic residues (invAb) are added to the 5′ end of the sense strand. In some embodiments, one or more inverted abasic residues can be inserted between the targeting ligand and the nucleobase sequence of the sense strand of the RNAi agent. In some embodiments, the inclusion of one or more inverted abasic residues at or near the terminal end or terminal ends of the sense strand of an RNAi agent may allow for enhanced activity or other desired properties of an RNAi agent. In some embodiments, an abasic (deoxyribose) residue can be replaced with a ribitol (abasic ribose) residue.

In some embodiments, the 3′ end of the antisense strand core stretch sequence, or the 3′ end of the antisense strand sequence, may include an inverted abasic residue (invAb (see Table 6)).

Examples of sequences used in forming ANGPTL3 RNAi agents are provided in Tables 2, 3, and 4. In some embodiments, an ANGPTL3 RNAi agent antisense strand includes a sequence of any of the sequences in Tables 2 or 3. In certain embodiments, an ANGPTL3 RNAi agent antisense strand comprises or consists of any one of the modified sequences in Table 3. In some embodiments, an ANGPTL3 RNAi agent antisense strand includes the sequence of nucleotides (from 5′ end → 3′ end) 1-17, 2-15, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, or 2-24 of any of the sequences in Tables 2 or 3. In some embodiments, an ANGPTL3 RNAi agent sense strand includes the sequence of any of the sequences in Tables 2 or 4. In some embodiments, an ANGPTL3 RNAi agent sense strand includes the sequence of nucleotides (from 5′ end → 3′ end) 1-18, 1-19, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-26, 2-19, 2-20, 2-21, 2-22, 2-23, 2-24, 3-20, 3-21, 3-22, 3-23, 3-24, 4-21, 4-22, 4-23, 4-24, 5-22, 5-23, or 5-24 of any of the sequences in Tables 2 or 4. In certain embodiments,

an ANGPTL3 RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 4.

In some embodiments, the sense and antisense strands of the RNAi agents described herein contain the same number of nucleotides. In some embodiments, the sense and antisense strands of the RNAi agents described herein contain different numbers of nucleotides. In some embodiments, the sense strand 5′ end and the antisense strand 3′ end of an RNAi agent form a blunt end. In some embodiments, the sense strand 3′ end and the antisense strand 5′ end of an RNAi agent form a blunt end. In some embodiments, both ends of an RNAi agent form blunt ends. In some embodiments, neither end of an RNAi agent is blunt-ended. As used herein a "blunt end" refers to an end of a double stranded RNAi agent in which the terminal nucleotides of the two annealed strands are complementary (form a complementary base-pair).

In some embodiments, the sense strand 5′ end and the antisense strand 3′ end of an RNAi agent form a frayed end. In some embodiments, the sense strand 3′ end and the antisense strand 5′ end of an RNAi agent form a frayed end. In some embodiments, both ends of an RNAi agent form a frayed end. In some embodiments, neither end of an RNAi agent is a frayed end. As used herein a frayed end refers to an end of a double stranded RNAi agent in which the terminal nucleotides of the two annealed strands from a pair (i.e., do not form an overhang) but are not complementary (i.e. form a non-complementary pair). In some embodiments, one or more unpaired nucleotides at the end of one strand of a double stranded RNAi agent form an overhang. The unpaired nucleotides may be on the sense strand or the antisense strand, creating either 3' or 5' overhangs. In some embodiments, the RNAi agent contains: a blunt end and a frayed end, a blunt end and 5′ overhang end, a blunt end and a 3′ overhang end, a frayed end and a 5′ overhang end, a frayed end and a 3′ overhang end, two 5′ overhang ends, two 3′ overhang ends, a 5′ overhang end and a 3′ overhang end, two frayed ends, or two blunt ends. Typically, when present, overhangs are located at the 3' terminal ends of the sense strand, the antisense strand, or both the sense strand and the antisense strand.

Modified nucleotides, when used in various polynucleotide or oligonucleotide constructs, can preserve activity of the compound in cells while at the same time increasing the serum stability of these compounds, and can also minimize the possibility of activating interferon activity in humans upon administering of the polynucleotide or oligonucleotide construct.

In some embodiments, an ANGPTL3 RNAi agent is prepared or provided as a salt, mixed salt, or a free-acid. In some embodiments, an ANGPTL3 RNAi agent is prepared as a sodium salt. Such forms that are well known in the art are within the scope of the inventions disclosed herein.

Modified Nucleotides

In some embodiments, an ANGPTL3 RNAi agent contains one or more modified nucleotides. As used herein, a "modified nucleotide" is a nucleotide other than a ribonucleotide (2′-hydroxyl nucleotide). In some embodiments, at least 50% (e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%) of the nucleotides are modified nucleotides. As used herein, modified nucleotides can include, but are not limited to, deoxyribonucleotides, nucleotide mimics, abasic nucleotides (represented herein as Ab), 2′-modified nucleotides, 3′ to 3′ linkages (inverted) nucleotides (represented herein as invdN, invN, invn), modified nucleobase-comprising nucleotides, bridged nucleotides, peptide nucleic acids (PNAs), 2′,3′-seco nucleotide mimics (unlocked nucleobase analogues, represented herein as N_{UNA} or NUNA), locked nucleotides (represented herein as N_{LNA} or NLNA), 3′-O-methoxy (2′ internucleoside linked) nucleotides (represented herein as 3′-OMen), 2′-F-Arabino nucleotides (represented herein as NfANA or Nf_{ANA}), 5'-Me, 2'-fluoro nucleotide (represented herein as 5Me-Nf), morpholino nucleotides, vinyl phosphonate deoxyribonucleotides (represented herein as vpdN), vinyl phosphonate containing nucleotides, and cyclopropyl phosphonate containing nucleotides (cPrpN). 2′-modified nucleotides (*i.e.*, a nucleotide with a group other than a hydroxyl group at the 2′ position of the five-membered sugar ring) include, but are not limited to, 2′-O-methyl nucleotides (represented herein as a lower case letter 'n' in a nucleotide sequence), 2′-deoxy-2′-fluoro nucleotides (also referred to herein as 2′-fluoro nucleotide, and represented herein as Nf), 2′-deoxy nucleotides (represented herein as dN), 2′-methoxyethyl (2′-O-2-methoxylethyl) nucleotides (also referred to herein as 2′-MOE, and represented herein as NM), 2′-amino nucleotides, and 2′-alkyl nucleotides. It is not necessary for all positions in a given compound to be uniformly modified. Conversely, more than one modification can be incorporated in a single ANGPTL3 RNAi agent or even in a single nucleotide thereof. The ANGPTL3 RNAi agent sense strands and antisense strands can be synthesized and/or modified by methods known in the art. Modification at one nucleotide is independent of modification at another nucleotide.

Modified nucleobases include synthetic and natural nucleobases, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, (e.g., 2-aminopropyladenine, 5-propynyluracil, or 5-propynylcytosine), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, inosine, xanthine, hypoxanthine, 2-aminoadenine, 6-alkyl (e.g., 6-methyl, 6-ethyl, 6-isopropyl, or 6-n-butyl) derivatives of adenine and guanine, 2-alkyl (e.g., 2-methyl, 2-ethyl, 2-isopropyl, or 2-n-butyl) and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine, 2-thiocytosine, 5-halouracil, cytosine, 5-propynyl uracil, 5-propynyl cytosine, 6-azo uracil, 6-azo cytosine, 6-azo thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-sulfhydryl, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo (e.g., 5-bromo), 5-trifluoromethyl, and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, and 3-deazaadenine.

In some embodiments, all or substantially all of the nucleotides of an RNAi agent are modified nucleotides. As used herein, an RNAi agent wherein substantially all of the nucleotides present are modified nucleotides is an RNAi agent having four or fewer (i.e., 0, 1, 2, 3, or 4) nucleotides in both the sense strand and the antisense strand being ribonucleotides (i.e., unmodified). As used herein, a sense strand wherein substantially all of the nucleotides present are modified nucleotides is a sense strand having two or fewer (i.e., 0, 1, or 2) nucleotides in the sense strand being unmodified ribonucleotides. As used herein, an antisense sense strand wherein substantially all of the nucleotides present are modified nucleotides is an antisense strand having two or fewer (i.e., 0, 1, or 2) nucleotides in the sense strand being unmodified ribonucleotides. In some embodiments, one or more nucleotides of an RNAi agent is an unmodified ribonucleotide.

Modified Internucleoside Linkages

In some embodiments, one or more nucleotides of an ANGPTL3 RNAi agent are linked by non-standard linkages or backbones (i.e., modified internucleoside linkages or modified backbones). Modified internucleoside linkages or backbones include, but are not limited to, phosphorothioate groups (represented herein as a lower case "s"), chiral phosphorothioates, thiophosphates, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, alkyl phosphonates (e.g., methyl phosphonates or 3′-alkylene phosphonates), chiral phosphonates, phosphinates, phosphoramidates (e.g., 3′-amino phosphoramidate, aminoalkylphosphoramidates, or thionophosphoramidates), thionoalkyl-phosphonates,

thionoalkylphosphotriesters, morpholino linkages, boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of boranophosphates, or boranophosphates having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′. In some embodiments, a modified internucleoside linkage or backbone lacks a phosphorus atom. Modified internucleoside linkages lacking a phosphorus atom include, but are not limited to, short chain alkyl or cycloalkyl inter-sugar linkages, mixed heteroatom and alkyl or cycloalkyl inter-sugar linkages, or one or more short chain heteroatomic or heterocyclic inter-sugar linkages. In some embodiments, modified internucleoside backbones include, but are not limited to, siloxane backbones, sulfide backbones, sulfoxide backbones, sulfone backbones, formacetyl and thioformacetyl backbones, methylene formacetyl and thioformacetyl backbones, alkene-containing backbones, sulfamate backbones, methyleneimino and methylenehydrazino backbones, sulfonate and sulfonamide backbones, amide backbones, and other backbones having mixed N, O, S, and CH_2 components.

In some embodiments, a sense strand of an ANGPTL3 RNAi agent can contain 1, 2, 3, 4, 5, or 6 phosphorothioate linkages, an antisense strand of an ANGPTL3 RNAi agent can contain 1, 2, 3, 4, 5, or 6 phosphorothioate linkages, or both the sense strand and the antisense strand independently can contain 1, 2, 3, 4, 5, or 6 phosphorothioate linkages. In some embodiments, a sense strand of an ANGPTL3 RNAi agent can contain 1, 2, 3, or 4 phosphorothioate linkages, an antisense strand of an ANGPTL3 RNAi agent can contain 1, 2, 3, or 4 phosphorothioate linkages, or both the sense strand and the antisense strand independently can contain 1, 2, 3, or 4 phosphorothioate linkages.

In some embodiments, an ANGPTL3 RNAi agent sense strand contains at least two phosphorothioate internucleoside linkages. In some embodiments, the at least two phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 3' end of the sense strand. In some embodiments, one phosphorothioate internucleoside linkage is at the 5’ end of the sense strand, and another phosphorothioate linkage is at the 3’ end of the sense strand. In some embodiments, two phosphorothioate internucleoside linkage are located at the 5’ end of the sense strand, and another phosphorothioate linkage is at the 3’ end of the sense strand. In some embodiments, the sense strand dose not include any phosphorothioate internucleoside linkages between the nucleotides, but contains one, two, or three phosphorothioate linkages between the terminal nucleotides on both the 5’ and 3’ ends

and the optionally present inverted abasic residue terminal caps. In some embodiments, the targeting ligand is linked to the sense strand via a phosphorothioate linkage.

In some embodiments, an ANGPTL3 RNAi agent antisense strand contains four phosphorothioate internucleoside linkages. In some embodiments, the four phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 5' end of the antisense strand and between the nucleotides at positions 19-21, 20-22, 21-23, 22-24, 23-25, or 24-26 from the 5' end. In some embodiments, three phosphorothioate internucleoside linkages are located between positions 1-4 from the 5' end of the antisense strand, and a fourth phosphorothioate internucleoside linkage is located between positions 20-21 from the 5' end of the antisense strand. In some embodiments, an ANGPTL3 RNAi agent contains at least three or four phosphorothioate internucleoside linkages in the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent contains one or more modified nucleotides and one or more modified internucleoside linkages. In some embodiments, a 2′-modified nucleoside is combined with modified internucleoside linkage.

ANGPTL3 RNAi Agents

In some embodiments, the ANGPTL3 RNAi agents disclosed herein target an ANGPTL3 gene at or near the positions of the ANGPTL3 gene sequence shown in Table 1. In some embodiments, the antisense strand of an ANGPTL3 RNAi agent disclosed herein includes a core stretch sequence that is fully, substantially, or at least partially complementary to a target ANGPTL3 19-mer sequence disclosed in Table 1.

Table 1. ANGPTL3 19-mer mRNA Target Sequences (taken from *homo sapiens* angiopoietin like 3 (ANGPTL3) transcript, GenBank NM_014495.3 (SEQ ID NO:1))

SEQ ID No.	ANGPTL3 19-mer Target Sequences (5′ → 3′)	Corresponding Positions on SEQ ID NO: 1
33	UCAACAUAUUUGAUCAGUC	304-322
34	CAUGGACAUUAAUUCAACA	922-940
35	CCAUGGACAUUAAUUCAAC	921-939
36	UUGCUAUGUUAGACGAUGU	190-208
37	AAGAUAUACUCCAUAGUGA	1035-1053
38	CAGAGCCAAAAUCAAGAUU	172-190
39	GACAUGGUCUUAAAGACUU	241-259

SEQ ID No.	ANGPTL3 19-mer Target Sequences (5′ → 3′)	Corresponding Positions on SEQ ID NO: 1
40	AGCACCAAGAACUACUCCC	743-761
41	GCACCAAGAACUACUCCCU	744-762
42	GAUGGAGAAUUUUGGUUGG	1008-1026
43	AUGGAGAAUUUUGGUUGGG	1009-1027
44	ACUCCAUAGUGAAGCAAUC	1042-1060
45	CACGAAACCAACUAUACGC	1140-1158
46	CUACUUGGGAUCACAAAGC	1225-1243
47	CUUGGGAUCACAAAGCAAA	1228-1246
48	UGUGGAGAAAACAACCUAA	1302-1320
49	UGGAGAAAACAACCUAAAU	1304-1322

In some embodiments, an ANGPTL3 RNAi agent includes an antisense strand wherein position 19 of the antisense strand (5′→3′) is capable of forming a base pair with position 1 of a 19-mer target sequence disclosed in Table 1. In some embodiments, an ANGPTL3 RNAi agent includes an antisense strand wherein position 1 of the antisense strand (5′→3′) is capable of forming a base pair with position 19 of the 19-mer target sequence disclosed in Table 1.

In some embodiments, an ANGPTL3 RNAi agent includes an antisense strand wherein position 2 of the antisense strand (5′ → 3′) is capable of forming a base pair with position 18 of the 19-mer target sequence disclosed in Table 1. In some embodiments, an ANGPTL3 RNAi agent includes an antisense strand wherein positions 2 through 18 of the antisense strand (5′ → 3′) are capable of forming base pairs with each of the respective complementary bases located at positions 18 through 2 of the 19-mer target sequence disclosed in Table 1.

For the RNAi agents disclosed herein, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) can be perfectly complementary to the ANGPTL3 gene, or can be non-complementary to the ANGPTL3 gene. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) is a U, A, or dT. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) forms an A:U or U:A base pair with the sense strand.

In some embodiments, an ANGPTL3 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3. In some embodiments, an ANGPTL3 RNAi sense strand comprises the

sequence of nucleotides (from 5′ end → 3′ end) 1-17, 1-18, or 2-18 of any of the sense strand sequences in Table 2 or Table 4.

In some embodiments, an ANGPTL3 RNAi agent is comprised of (i) an antisense strand comprising the sequence of nucleotides (from 5′ end → 3′ end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3, and (ii) a sense strand comprising the sequence of nucleotides (from 5′ end → 3′ end) 1-17 or 1-18 of any of the sense strand sequences in Table 2 or Table 4.

In some embodiments, the ANGPTL3 RNAi agents include core 19-mer nucleotide sequences shown in the following Table 2.

Table 2. ANGPTL3 RNAi Agent Antisense Strand and Sense Strand Core Stretch Base Sequences (N=any nucleobase)

SEQ ID No.	Antisense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID No.	Sense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions on SEQ ID NO: 1
50	UACUGAUCAAAUAUGUUGA	130	UCAACAUAUUUGAUCAGUA	304-322
50	UACUGAUCAAAUAUGUUGA	131	UCAACAU(A^{2N})UUUGAUCAGUA	304-322
50	UACUGAUCAAAUAUGUUGA	132	UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA	304-322
51	AACUGAUCAAAUAUGUUGA	133	UCAACAUAUUUGAUCAGUU	304-322
51	AACUGAUCAAAUAUGUUGA	134	UCAACAU(A^{2N})UUUGAUCAGUU	304-322
51	AACUGAUCAAAUAUGUUGA	135	UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUU	304-322
52	GACUGAUCAAAUAUGUUGA	136	UCAACAUAUUUGAUCAGUC	304-322
52	GACUGAUCAAAUAUGUUGA	137	UCAACAU(A^{2N})UUUGAUCAGUC	304-322
52	GACUGAUCAAAUAUGUUGA	138	UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUC	304-322
53	NACUGAUCAAAUAUGUUGA	139	UCAACAUAUUUGAUCAGUN	304-322
53	NACUGAUCAAAUAUGUUGA	140	UCAACAU(A^{2N})UUUGAUCAGUN	304-322
53	NACUGAUCAAAUAUGUUGA	141	UCAAC(A^{2N})U(A^{2N})UUUGAUCAGUN	304-322
54	NACUGAUCAAAUAUGUUGN	142	NCAACAUAUUUGAUCAGUN	304-322
54	NACUGAUCAAAUAUGUUGN	143	NCAACAU(A^{2N})UUUGAUCAGUN	304-322
54	NACUGAUCAAAUAUGUUGN	144	NCAAC(A^{2N})U(A^{2N})UUUGAUCAGUN	304-322
55	UGUUGAAUUAAUGUCCAUG	145	CAUGGACAUUAAUUCAACA	922-940
56	AGUUGAAUUAAUGUCCAUG	146	CAUGGACAUUAAUUCAACU	922-940
57	NGUUGAAUUAAUGUCCAUG	147	CAUGGACAUUAAUUCAACN	922-940
58	NGUUGAAUUAAUGUCCAUN	148	NAUGGACAUUAAUUCAACN	922-940
59	GUUGAAUUAAUGUCCAUGG	149	CCAUGGACAUUAAUUCAAC	921-939
60	UUUGAAUUAAUGUCCAUGG	150	CCAUGGACAUUAAUUCAAA	921-939
61	AUUGAAUUAAUGUCCAUGG	151	CCAUGGACAUUAAUUCAAU	921-939
62	NUUGAAUUAAUGUCCAUGG	152	CCAUGGACAUUAAUUCAAN	921-939
63	NUUGAAUUAAUGUCCAUGN	153	NCAUGGACAUUAAUUCAAN	921-939

SEQ ID No.	Antisense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID No.	Sense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions on SEQ ID NO: 1
64	ACAUCGUCUAACAUAGCAA	154	UUGCUAUGUUAGACGAUGU	190-208
65	UCAUCGUCUAACAUAGCAA	155	UUGCUAUGUUAGACGAUGA	190-208
66	NCAUCGUCUAACAUAGCAA	156	UUGCUAUGUUAGACGAUGN	190-208
67	NCAUCGUCUAACAUAGCAN	157	NUGCUAUGUUAGACGAUGN	190-208
68	UCACUAUGGAGUAUAUCUU	158	AAGAUAUACUCCAUAGUGA	1035-1053
69	ACACUAUGGAGUAUAUCUU	159	AAGAUAUACUCCAUAGUGU	1035-1053
70	NCACUAUGGAGUAUAUCUU	160	AAGAUAUACUCCAUAGUGN	1035-1053
71	NCACUAUGGAGUAUAUCUN	161	NAGAUAUACUCCAUAGUGN	1035-1053
72	AAUCUUGAUUUUUGGCUCUG	162	CAGAGCCAAAAUCAAGAUU	172-190
73	UAUCUUGAUUUUUGGCUCUG	163	CAGAGCCAAAAUCAAGAUA	172-190
74	NAUCUUGAUUUUUGGCUCUG	164	CAGAGCCAAAAUCAAGAUN	172-190
75	NAUCUUGAUUUUUGGCUCUN	165	NAGAGCCAAAAUCAAGAUN	172-190
76	AAGUCUUUAAGACCAUGUC	166	GACAUGGUCUUAAAGACUU	241-259
77	UAGUCUUUAAGACCAUGUC	167	GACAUGGUCUUAAAGACUA	241-259
78	NAGUCUUUAAGACCAUGUC	168	GACAUGGUCUUAAAGACUN	241-259
79	NAGUCUUUAAGACCAUGUN	169	NACAUGGUCUUAAAGACUN	241-259
80	GGGAGUAGUUCUUGGUGCU	170	AGCACCAAGAACUACUCCC	743-761
81	UGGAGUAGUUCUUGGUGCU	171	AGCACCAAGAACUACUCCA	743-761
82	AGGAGUAGUUCUUGGUGCU	172	AGCACCAAGAACUACUCCU	743-761
83	NGGAGUAGUUCUUGGUGCU	173	AGCACCAAGAACUACUCCN	743-761
84	NGGAGUAGUUCUUGGUGCN	174	NGCACCAAGAACUACUCCN	743-761
85	AGGGAGUAGUUCUUGGUGC	175	GCACCAAGAACUACUCCCU	744-762
86	UGGGAGUAGUUCUUGGUGC	176	GCACCAAGAACUACUCCCA	744-762
87	NGGGAGUAGUUCUUGGUGC	177	GCACCAAGAACUACUCCCN	744-762
88	NGGGAGUAGUUCUUGGUGN	178	NCACCAAGAACUACUCCCN	744-762
89	AGAGAGUAGUUCUUGGUGC	179	GCACCAAGAACUACUCUCU	744-762

SEQ ID No.	Antisense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID No.	Sense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions on SEQ ID NO: 1
90	UGAGAGUAGUUCUUGGUGC	180	GCACCAAGAACUACUCUCA	744-762
91	NGAGAGUAGUUCUUGGUGC	181	GCACCAAGAACUACUCUCN	744-762
92	NGAGAGUAGUUCUUGGUGN	182	NCACCAAGAACUACUCUCN	744-762
93	CCAACCAAAAUUCUCCAUC	183	GAUGGAGAAUUUUGGUUGG	1008-1026
94	UCAACCAAAAUUCUCCAUC	184	GAUGGAGAAUUUUGGUUGA	1008-1026
95	ACAACCAAAAUUCUCCAUC	185	GAUGGAGAAUUUUGGUUGU	1008-1026
96	NCAACCAAAAUUCUCCAUC	186	GAUGGAGAAUUUUGGUUGN	1008-1026
97	NCAACCAAAAUUCUCCAUN	187	NAUGGAGAAUUUUGGUUGN	1008-1026
98	CCCAACCAAAAUUCUCCAU	188	AUGGAGAAUUUUGGUUGGG	1009-1027
99	UCCAACCAAAAUUCUCCAU	189	AUGGAGAAUUUUGGUUGGA	1009-1027
100	ACCAACCAAAAUUCUCCAU	190	AUGGAGAAUUUUGGUUGGU	1009-1027
101	NCCAACCAAAAUUCUCCAU	191	AUGGAGAAUUUUGGUUGGN	1009-1027
102	NCCAACCAAAAUUCUCCAN	192	NUGGAGAAUUUUGGUUGGN	1009-1027
103	UAUUGCUUCACUAUGGAGU	193	ACUCCAUAGUGAAGCAAUA	1042-1060
104	AAUUGCUUCACUAUGGAGU	194	ACUCCAUAGUGAAGCAAUU	1042-1060
105	GAUUGCUUCACUAUGGAGU	195	ACUCCAUAGUGAAGCAAUC	1042-1060
106	NAUUGCUUCACUAUGGAGU	196	ACUCCAUAGUGAAGCAAUN	1042-1060
107	NAUUGCUUCACUAUGGAGN	197	ACUCCAUAGUGAAGCAAUN	1042-1060
108	UCGUAUAGUUGGUUUCGUG	198	CACGAAACCAACUAUACGA	1140-1158
109	ACGUAUAGUUGGUUUCGUG	199	CACGAAACCAACUAUACGU	1140-1158
110	GCGUAUAGUUGGUUUCGUG	200	CACGAAACCAACUAUACGC	1140-1158
111	NCGUAUAGUUGGUUUCGUG	201	CACGAAACCAACUAUACGN	1140-1158
112	NCGUAUAGUUGGUUUCGUN	202	NACGAAACCAACUAUACGN	1140-1158
113	UCUUUGUGAUCCCAAGUAG	203	CUACUUGGGAUCACAAAGA	1225-1243
114	ACUUUGUGAUCCCAAGUAG	204	CUACUUGGGAUCACAAAGU	1225-1243
115	GCUUUGUGAUCCCAAGUAG	205	CUACUUGGGAUCACAAAGC	1225-1243

SEQ ID No.	Antisense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID No.	Sense Strand Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	Corresponding Positions on SEQ ID NO: 1
116	NCUUUGUGAUCCCAAGUAG	206	CUACUUGGGAUCACAAAGN	1225-1243
117	NCUUUGUGAUCCCAAGUAN	207	NUACUUGGGAUCACAAAGN	1225-1243
118	UUUGCUUUGUGAUCCCAAG	208	CUUGGGAUCACAAAGCAAA	1228-1246
119	AUUGCUUUGUGAUCCCAAG	209	CUUGGGAUCACAAAGCAAU	1228-1246
120	NUUGCUUUGUGAUCCCAAG	210	CUUGGGAUCACAAAGCAAN	1228-1246
121	NUUGCUUUGUGAUCCCAAN	211	NUUGGGAUCACAAAGCAAN	1228-1246
122	UUAGGUUGUUUUCUCCACA	212	UGUGGAGAAAACAACCUAA	1302-1320
123	AUAGGUUGUUUUCUCCACA	213	UGUGGAGAAAACAACCUAU	1302-1320
124	NUAGGUUGUUUUCUCCACA	214	UGUGGAGAAAACAACCUAN	1302-1320
125	NUAGGUUGUUUUCUCCACN	215	NGUGGAGAAAACAACCUAN	1302-1320
126	AUUUAGGUUGUUUUCUCCA	216	UGGAGAAAACAACCUAAAU	1304-1322
127	UUUUAGGUUGUUUUCUCCA	217	UGGAGAAAACAACCUAAAA	1304-1322
128	NUUUAGGUUGUUUUCUCCA	218	UGGAGAAAACAACCUAAAN	1304-1322
129	NUUUAGGUUGUUUUCUCCN	219	NGGAGAAAACAACCUAAAN	1304-1322

(A^{2N}) = 2-aminoadenine nucleotide

The ANGPTL3 RNAi agent sense strands and antisense strands that comprise or consist of the sequences in Table 2 can be modified nucleotides or unmodified nucleotides. In some embodiments, the ANGPTL3 RNAi agents having the sense and antisense strand sequences that comprise or consist of the sequences in Table 2 are all or substantially all modified nucleotides.

In some embodiments, the antisense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2. In some embodiments, the sense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2.

As used herein, each N listed in a sequence disclosed in Table 2 may be independently selected from any and all nucleobases (including those found on both modified and unmodified nucleotides). In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is not complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is the same as the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is different from the N nucleotide at the corresponding position on the other strand.

Certain modified ANGPTL3 RNAi agent antisense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 3. Certain modified ANGPTL3 RNAi agent sense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 4. In forming ANGPTL3 RNAi agents, each of the nucleotides in each of the underlying base sequences listed in Tables 3 and 4, as well as in Table 2, above, can be a modified nucleotide.

The ANGPTL3 RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2 or Table 4, can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3,

provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

In some embodiments, an ANGPTL3 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2 or Table 3.

In some embodiments, an ANGPTL3 RNAi agent comprises or consists of a duplex having the nucleobase sequences of the sense strand and the antisense strand of any of the sequences in Table 2, Table 3 or Table 4.

Examples of antisense strands containing modified nucleotides are provided in Table 3. Examples of sense strands containing modified nucleotides are provided in Table 4.

As used in Tables 3 and 4, the following notations are used to indicate modified nucleotides, targeting groups, and linking groups:

A	=	adenosine-3′-phosphate;
C	=	cytidine-3′-phosphate;
G	=	guanosine-3′-phosphate;
U	=	uridine-3′-phosphate
I	=	inosine-3′-phosphate
n	=	any 2′-OMe modified nucleotide
a	=	2′-O-methyladenosine-3′-phosphate
as	=	2′-O-methyladenosine-3′-phosphorothioate
c	=	2′-O-methylcytidine-3′-phosphate
cs	=	2′-O-methylcytidine-3′-phosphorothioate
g	=	2′-O-methylguanosine-3′-phosphate
gs	=	2′-O-methylguanosine-3′-phosphorothioate
t	=	2′-O-methyl-5-methyluridine-3′-phosphate
ts	=	2′-O-methyl-5-methyluridine-3′-phosphorothioate
u	=	2′-O-methyluridine-3′-phosphate
us	=	2′-O-methyluridine-3′-phosphorothioate
i	=	2′-O-methylinosine-3′-phosphate
is	=	2′-O-methylinosine-3′-phosphorothioate
Nf	=	any 2′-fluoro modified nucleotide
Af	=	2′-fluoroadenosine-3′-phosphate
Afs	=	2′-fluoroadenosine-3′-phosporothioate

	Cf	=	2′-fluorocytidine-3′-phosphate
	Cfs	=	2′-fluorocytidine-3′-phosphorothioate
	Gf	=	2′-fluoroguanosine-3′-phosphate
	Gfs	=	2′-fluoroguanosine-3′-phosphorothioate
	Tf	=	2′-fluoro-5′-methyluridine-3′-phosphate
	Tfs	=	2′-fluoro-5′-methyluridine-3′-phosphorothioate
	Uf	=	2′-fluorouridine-3′-phosphate
	Ufs	=	2′-fluorouridine-3′-phosphorothioate
	dN	=	any 2′-deoxyribonucleotide
	dA	=	2′-deoxyadenosine-3′-phosphate
	dAs	=	2′-deoxyadenosine-3′-phosphorothioate
	dC	=	2′-deoxycytidine-3′-phosphate
	dCs	=	2′-deoxycytidine-3′-phosphorothioate
	dG	=	2′-deoxyguanosine-3′-phosphate
	dGs	=	2′-deoxyguanosine-3′-phosphorothioate
	dT	=	2′-deoxythymidine-3′-phosphate
	dTs	=	2′-deoxythymidine-3′-phosphorothioate
	dU	=	2′-deoxyuridine-3′-phosphate
	dUs	=	2′-deoxyuridine-3′-phosphorothioate
	N_{UNA}	=	2′,3′-seco nucleotide mimics (unlocked nucleobase analogs)-3′-Phosphate
	N_{UNAS}	=	2′,3′-seco nucleotide mimics (unlocked nucleobase analogs)-3′-phosphorothioate
	A_{UNA}	=	2′,3′-seco-adenosine-3′-phosphate
	A_{UNAS}	=	2′,3′-seco-adenosine-3′-phosphorothioate
	C_{UNA}	=	2′,3′-seco-cytidine-3′-phosphate
	C_{UNAS}	=	2′,3′-seco-cytidine-3′-phosphorothioate
	G_{UNA}	=	2′,3′-seco-guanosine-3′-phosphate
	G_{UNAS}	=	2′,3′-seco-guanosine-3′-phosphorothioate
	U_{UNA}	=	2′,3′-seco-uridine-3′-phosphate
	U_{UNAS}	=	2′,3′-seco-uridine-3′-phosphorothioate
	a_2N	=	see Table 6
	a_2Ns	=	see Table 6
	pu_2N	=	see Table 6
	pu_2Ns	=	see Table 6
	N_{LNA}	=	locked nucleotide
	Nf_{ANA}	=	2'-F-Arabino nucleotide
	NM	=	2′-O-methoxyethyl nucleotide
	AM	=	2′-O-methoxyethyladenosine-3′-phosphate
	AMs	=	2′-O-methoxyethyladenosine-3′-phosphorothioate

	GM	=	2′-O-methoxyethylguanosine-3′-phosphate
	GMs	=	2′-O-methoxyethylguanosine-3′-phosphorothioate
	TM	=	2′-O-methoxyethylthymidine-3′-phosphate
	TMs	=	2′-O-methoxyethylthymidine-3′-phosphorothioate
	mCM	=	see Table 6
	mCMs	=	see Table 6
	R	=	ribitol
	(invdN)	=	any inverted deoxyribonucleotide (3′-3′ linked nucleotide)
	(invAb)	=	inverted (3′-3′ linked) abasic deoxyribonucleotide, see Table 6
	(invAb)s	=	inverted (3′-3′ linked) abasic deoxyribonucleotide-5′-phosphorothioate, see Table 6
	(invn)	=	any inverted 2′-OMe nucleotide (3′-3′ linked nucleotide)
	s	=	phosphorothioate linkage
	sp	=	see Table 6
	D2u	=	see Table 6
	pD2u	=	see Table 6
	vpdN	=	vinyl phosphonate deoxyribonucleotide
	(5Me-Nf)	=	5'-Me, 2'-fluoro nucleotide
	cPrp	=	cyclopropyl phosphonate, see Table 6
	epTcPr	=	see Table 6
	epTM	=	see Table 6

As the person of ordinary skill in the art would readily understand, unless otherwise indicated by the sequence (such as, for example, by a phosphorothioate linkage "s"), when present in an oligonucleotide, the nucleotide monomers are mutually linked by 5'-3'-phosphodiester bonds. As the person of ordinary skill in the art would clearly understand, the inclusion of a phosphorothioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides (*see, e.g.*, Figs. 5A through 5K showing all internucleoside linkages). Further, the person of ordinary skill in the art would readily understand that the terminal nucleotide at the 3' end of a given oligonucleotide sequence would typically have a hydroxyl (-OH) group at the respective 3' position of the given monomer instead of a phosphate moiety *ex vivo*. Moreover, as the person of ordinary skill would readily understand and appreciate, while the phosphorothioate chemical structures depicted herein typically show the anion on the sulfur atom, the inventions disclosed herein encompass all phosphorothioate tautomers and/or diastereomers (e.g., where the sulfur atom has a double-bond and the anion is on an oxygen atom). Unless expressly indicated otherwise herein, such understandings of the

person of ordinary skill in the art are used when describing the ANGPTL3 RNAi agents and compositions of ANGPTL3 RNAi agents disclosed herein.

Certain examples of targeting groups and linking groups used with the ANGPTL3 RNAi agents disclosed herein are provided below in Table 6. More specifically, targeting groups and linking groups include the following, for which their chemical structures are provided below in Table 6: (PAZ), (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s . Each sense strand and/or antisense strand can have any targeting groups or linking groups listed herein, as well as other targeting or linking groups, conjugated to the 5′ and/or 3′ end of the sequence.

Table 3. ANGPTL3 RNAi Agent Antisense Strand Sequences

Antisense Strand ID:	Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM06999-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfgAfsc	220	UUUGAAUUAAUGUCCAUGGAC	363
AM07001-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfggsc	7	UUUGAAUUAAUGUCCAUGGGC	8
AM07003-AS	usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsa	13	UGUUGAAUUAAUGUCCAUGGA	12
AM07005-AS	usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsc	221	UGUUGAAUUAAUGUCCAUGGC	364
AM07007-AS	usUfsasGfgUfuGfuUfuUfcUfcCfaCfaCfsu	222	UUAGGUUGUUUUCUCCACACU	365
AM07009-AS	usUfsasGfgUfuGfuUfuUfcUfcCfaCfaCfsc	223	UUAGGUUGUUUUCUCCACACC	366
AM07011-AS	usUfsusUfaGfgUfuGfuUfuUfcUfcCfaCfsc	224	UUUUAGGUUGUUUUCUCCACC	367
AM07061-AS	usGfsgsAfgUfaguucUfuGfgUfgCfuCfsu	225	UGGAGUAGUUCUUGGUGCUCU	368
AM07062-AS	usGfsgsAfgUfaguucUfuGfgUfgCfuCfsc	226	UGGAGUAGUUCUUGGUGCUCC	369
AM07063-AS	asGfsgsGfaGfuaguuCfuUfgGfuGfcUfsc	227	AGGGAGUAGUUCUUGGUGCUC	370
AM07148-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfgAfsg	228	UUUGAAUUAAUGUCCAUGGAG	371
AM07149-AS	usUfsusGfaAfU$_{UNA}$UfaAfuGfuCfcAfuGfgAfsg	229	UUUGAAUUAAUGUCCAUGGAG	371
AM07155-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfgCfsg	230	UUUGAAUUAAUGUCCAUGGCG	372
AM07157-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsg	231	UUUGAAUUAAUGUCCAUGGGG	373
AM07159-AS	usUfsusGfaAfuUfaAfuGfuCfcAfuGfgGfsu	9	UUUGAAUUAAUGUCCAUGGGU	10
AM07161-AS	usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsg	232	UGUUGAAUUAAUGUCCAUGGG	374
AM07163-AS	usGfsusUfgAfaUfuAfaUfgUfcCfaUfgGfsu	233	UGUUGAAUUAAUGUCCAUGGU	375
AM07164-AS	usGfsusUfgAfA$_{UNA}$UfuAfaUfgUfcCfaUfgGfsu	234	UGUUGAAUUAAUGUCCAUGGU	375
AM07233-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsu	5	UACUGAUCAAAUAUGUUGAGU	6
AM07235-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc	2	UACUGAUCAAAUAUGUUGAGC	3
AM07237-AS	asAfsusCfuUfgAfuUfuUfgGfcUfcUfgGfsa	235	AAUCUUGAUUUUGGCUCUGGA	376
AM07239-AS	asAfsusCfuUfgAfuUfuUfgGfcUfcUfgGfsu	236	AAUCUUGAUUUUGGCUCUGGU	377

Antisense Strand ID:	Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07241-AS	usCfsasAfcCfaAfaAfuUfcUfcCfaUfcAfsc	237	UCAACCAAAAUUCUCCAUCAC	378
AM07243-AS	usCfsasAfcCfaAfaAfuUfcUfcCfaUfcGfsc	238	UCAACCAAAAUUCUCCAUCGC	379
AM07245-AS	usCfscsAfaCfcAfaAfaUfuCfuCfcAfuCfsa	239	UCCAACCAAAAUUCUCCAUCA	380
AM07347-AS	usGfsusUfgAfauuaaUfgUfcCfaUfgGfsa	240	UGUUGAAUUAAUGUCCAUGGA	12
AM07348-AS	usGfsusugAfauuaaUfgUfcCfaUfgGfsa	241	UGUUGAAUUAAUGUCCAUGGA	12
AM07349-AS	usGfsusUfgaauuaaUfgUfcCfaUfgGfsa	242	UGUUGAAUUAAUGUCCAUGGA	12
AM07350-AS	usGfsusugaauuaaUfgUfcCfaUfgGfsa	11	UGUUGAAUUAAUGUCCAUGGA	12
AM07351-AS	usGfsusugAfauuAfaUfgUfcCfauggsa	243	UGUUGAAUUAAUGUCCAUGGA	12
AM07352-AS	usGfsusUfgAfaUfuAfaUfgUfccauggsa	244	UGUUGAAUUAAUGUCCAUGGA	12
AM07356-AS	usGfsusugAfauuaaugUfcCfauggsa	245	UGUUGAAUUAAUGUCCAUGGA	12
AM07357-AS	usGfsusugaauuaaUfgUfcCfaUfgGfsg	246	UGUUGAAUUAAUGUCCAUGGG	12
AM07454-AS	asAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc	247	AACUGAUCAAAUAUGUUGAGC	382
AM07456-AS	D2usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc	248	UACUGAUCAAAUAUGUUGAGC	3
AM07457-AS	pD2usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc	249	UACUGAUCAAAUAUGUUGAGC	3
AM07458-AS	pusAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc	250	UACUGAUCAAAUAUGUUGAGC	3
AM07461-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsg	251	UACUGAUCAAAUAUGUUGAGG	385
AM07463-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfgGfsc	252	UACUGAUCAAAUAUGUUGGGC	386
AM07465-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfcGfsc	253	UACUGAUCAAAUAUGUUGCGC	387
AM07467-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfgGfsu	254	UACUGAUCAAAUAUGUUGGGU	388
AM07469-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfcGfsu	255	UACUGAUCAAAUAUGUUGCGU	389
AM07505-AS	usUfsTMsGfaAfuUfaAfuGfuCfcAfuGfgGfsu	256	UUTGAAUUAAUGUCCAUGGGU	390
AM07506-AS	usUfsusGfAMAfuUfaAfuGfuCfcAfuGfgGfsu	257	UUUGAAUUAAUGUCCAUGGGU	10
AM07507-AS	usUfsusGfaAfTMUfaAfuGfuCfcAfuGfgGfsu	258	UUUGAATUAAUGUCCAUGGGU	392

Antisense Strand ID:	Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07508-AS	usUfsTMsGfAMAfuUfaAfuGfuCfcAfuGfgGfsu	259	UUTGAAUUAAUGUCCAUGGGU	390
AM07581-AS	usAfscsugaucaaaUfaUfgUfuGfaGfsc	260	UACUGAUCAAAUAUGUUGAGC	3
AM07583-AS	usascsugaucaaaUfaUfgUfuGfaGfsc	261	UACUGAUCAAAUAUGUUGAGC	3
AM07588-AS	usAfscsugaucaaaUfgUfuGfaGfsc	262	UACUGAUCAAAUAUGUUGAGC	3
AM07589-AS	usAfscsUfgAfuCfaAfiUfaUfgUfuGfaGfsc	263	UACUGAUCAAIUAUGUUGAGC	393
AM07623-AS	usAfscsUfgAfuCfaAfaUfaUfgUfuGfasGfsc	264	UACUGAUCAAAUAUGUUGAGC	3
AM07624-AS	usAfscUfgAfuCfaAfaUfaUfgUfuGfasGfsc	265	UACUGAUCAAAUAUGUUGAGC	3
AM07634-AS	usUfsusgaauuaaauGfuCfcAfuGfgGfsu	266	UUUGAAUUAAUGUCCAUGGGU	10
AM07660-AS	asAfsgsUfcUfuuaagAfcCfaUfgUfcCfsc	267	AAGUCUUUAAGACCAUGUCCC	394
AM07662-AS	usAfsusUfgCfuucacUfaUfgGfaGfuAfsg	268	UAUUGCUUCACUAUGGAGUAG	395
AM07664-AS	usUfsusGfcUfuugugAfuCfcCfaAfgUfsc	269	UUUGCUUUGUGAUCCCAAGUC	396
AM07681-AS	asCfsasUfcGfucuaaCfaUfaGfcAfaCfsc	14	ACAUCGUCUAACAUAGCAACC	15
AM07683-AS	usCfsasCfuAfuggagUfaUfaUfcUfuCfsc	270	UCACUAUGGAGUAUAUCUUCC	397
AM07685-AS	usCfsgsUfaUfaguugGfuUfuCfgUfgAfsc	271	UCGUAUAGUUGGUUUCGUGAC	398
AM07687-AS	usCfsusUfuGfugaucCfcAfaGfuAfgAfsc	272	UCUUUGUGAUCCCAAGUAGAC	399
AM07911-AS	usCfsasCfuAfuGUNAgagUfaUfaUfcUfuCfsc	273	UCACUAUGGAGUAUAUCUUCC	397

Table 4. ANGPTL3 RNAi Agent Sense Strand Sequences

Sense Strand ID:	Modified Sense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM06992-SS	(NAG37)s(invAb)sagagcaccAfAfGfaacuacuccas(invAb)	274	AGAGCACCAAGAACUACUCCA	400
AM06994-SS	(NAG37)s(invAb)sggagcaccAfAfGfaacuacuucas(invAb)	275	GGAGCACCAAGAACUACUUCA	401
AM06996-SS	(NAG37)s(invAb)sgagcaccaAfGfAfacuacucucus(invAb)	276	GAGCACCAAGAACUACUCUCU	402
AM06998-SS	(NAG37)s(invAb)sguccauggAfCfAfuuaauucaaas(invAb)	277	GUCCAUGGACAUUAAUUCAAA	403
AM07000-SS	(NAG37)s(invAb)sgcccauggAfCfAfuuaauucaaas(invAb)	278	GCCCAUGGACAUUAAUUCAAA	26
AM07002-SS	(NAG37)s(invAb)succauggaCfAfUfuaauucaacas(invAb)	279	UCCAUGGACAUUAAUUCAACA	30
AM07004-SS	(NAG37)s(invAb)sgccauggaCfAfUfuaauucaacas(invAb)	280	GCCAUGGACAUUAAUUCAACA	406
AM07006-SS	(NAG37)s(invAb)saguguggaGfAfAfaacaaccuaas(invAb)	281	AGUGUGGAGAAAACAACCUAA	407
AM07008-SS	(NAG37)s(invAb)sgguguggaGfAfAfaacaaccuaas(invAb)	282	GGUGUGGAGAAAACAACCUAA	408
AM07010-SS	(NAG37)s(invAb)sgguggagaAfAfAfcaaccuaaaas(invAb)	283	GGUGGAGAAAACAACCUAAAA	409
AM07147-SS	(NAG37)s(invAb)scuccauggAfCfAfuuaauucaaas(invAb)	284	CUCCAUGGACAUUAAUUCAAA	410
AM07147-SS	(NAG37)s(invAb)scuccauggAfCfAfuuaauucaaas(invAb)	285	CUCCAUGGACAUUAAUUCAAA	410
AM07150-SS	(NAG37)s(invAb)scuccauggAfCfAfuUaauucaaas(invAb)	286	CUCCAUGGACAUUAAUUCAAA	410
AM07151-SS	(NAG37)s(invAb)scuccauggAfCfAfuuaauucaa_2Nas(invAb)	287	CUCCAUGGACAUUAAUUCA(A^{2N})A	411
AM07152-SS	(NAG37)s(invAb)scuccauggAfCfAfuuaauuca_2Naas(invAb)	288	CUCCAUGGACAUUAAUUC(A^{2N})AA	412
AM07153-SS	(NAG37)s(invAb)scuccauggAfCfAfuua_2Nauucaaas(invAb)	289	CUCCAUGGACAUU(A^{2N})AUUCAAA	413
AM07154-SS	(NAG37)s(invAb)scgccauggAfCfAfuuaauucaaas(invAb)	290	CGCCAUGGACAUUAAUUCAAA	414
AM07156-SS	(NAG37)s(invAb)sccccauggAfCfAfuuaauucaaas(invAb)	291	CCCCAUGGACAUUAAUUCAAA	415
AM07158-SS	(NAG37)s(invAb)sacccauggAfCfAfuuaauucaaas(invAb)	292	ACCCAUGGACAUUAAUUCAAA	28

Sense Strand ID:	Modified Sense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07160-SS	(NAG37)s(invAb)sccauggaCfAfUfuaauucaacas(invAb)	293	CCCAUGGACAUUAAUUCAACA	417
AM07162-SS	(NAG37)s(invAb)saccauggaCfAfUfuaauucaacas(invAb)	294	ACCAUGGACAUUAAUUCAACA	418
AM07165-SS	(NAG37)s(invAb)saccauggaCfAfUfuAauucaacas(invAb)	295	ACCAUGGACAUUAAUUCAACA	418
AM07166-SS	(NAG37)s(invAb)saccauggaCfAfUfuaa_2Nuucaacas(invAb)	296	ACCAUGGACAUUA(A^{2N})UUCAACA	419
AM07167-SS	(NAG37)s(invAb)sacca_2NuggaCfAfUfuaauucaacas(invAb)	297	ACC(A^{2N})UGGACAUUAAUUCAACA	420
AM07168-SS	(NAG37)s(invAb)sa_2NccauggaCfAfUfuaauucaacas(invAb)	298	(A^{2N})CCAUGGACAUUAAUUCAACA	421
AM07232-SS	(NAG37)s(invAb)sacucaacaUfAfUfuugaucaguas(invAb)	299	ACUCAACAUAUUUGAUCAGUA	24
AM07234-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuugaucaguas(invAb)	300	GCUCAACAUAUUUGAUCAGUA	17
AM07236-SS	(NAG37)s(invAb)succagagcCfAfAfaaucaagauus(invAb)	301	UCCAGAGCCAAAAUCAAGAUU	424
AM07238-SS	(NAG37)s(invAb)saccagagcCfAfAfaaucaagauus(invAb)	302	ACCAGAGCCAAAAUCAAGAUU	425
AM07240-SS	(NAG37)s(invAb)sgugauggaGfAfAfuuuugguugas(invAb)	303	GUGAUGGAGAAUUUUGGUUGA	426
AM07242-SS	(NAG37)s(invAb)sgcgauggaGfAfAfuuuugguugas(invAb)	304	GCGAUGGAGAAUUUUGGUUGA	427
AM07244-SS	(NAG37)s(invAb)sugauggagAfAfUfuuugguuggas(invAb)	305	UGAUGGAGAAUUUUGGUUGGA	428
AM07246-SS	(NAG37)usccauggaCfAfUfuaauucaacas(invAb)	306	UCCAUGGACAUUAAUUCAACA	30
AM07247-SS	(NAG37)asccauggaCfAfUfuaauucaacas(invAb)	307	ACCAUGGACAUUAAUUCAACA	418
AM07345-SS	(NAG37)s(invAb)succauggaCfAfUfuaauucaa_2Ncas(invAb)	308	UCCAUGGACAUUAAUUCA(A^{2N})CA	429
AM07346-SS	(NAG37)s(invAb)succauggaCfAfUfuaauuca_2Nacas(invAb)	309	UCCAUGGACAUUAAUUC(A^{2N})ACA	430
AM07353-SS	(NAG37)s(invAb)succauggaCfaUfuAfauucaacas(invAb)	310	UCCAUGGACAUUAAUUCAACA	30
AM07354-SS	(NAG37)s(invAb)succauggaCfaUfUfaauucaacas(invAb)	311	UCCAUGGACAUUAAUUCAACA	30
AM07355-SS	(NAG37)s(invAb)succaugGfaCfaUfuaauucaacas(invAb)	312	UCCAUGGACAUUAAUUCAACA	30
AM07358-SS	(NAG37)s(invAb)scccauggaCfaUfuAfauucaacas(invAb)	313	CCCAUGGACAUUAAUUCAACA	417

Sense Strand ID:	Modified Sense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07359-SS	(NAG37)ascccauggAfCfAfuuaauucaaas(invAb)	314	ACCCAUGGACAUUAAUUCAAA	28
AM07453-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuugaucaguus(invAb)	315	GCUCAACACAUAUUUGAUCAGUU	431
AM07455-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuugaucaiuas(invAb)	316	GCUCAACACAUAUUUGAUCAIUA	432
AM07459-SS	(NAG37)gscucaacaUfAfUfuugaucaguas(invAb)	317	GCUCAACACAUAUUUGAUCAGUA	17
AM07460-SS	(NAG37)s(invAb)sccucaacaUfAfUfuugaucaguas(invAb)	318	CCUCAACACAUAUUUGAUCAGUA	433
AM07462-SS	(NAG37)s(invAb)sgcccaacaUfAfUfuugaucaguas(invAb)	319	GCCCAACACAUAUUUGAUCAGUA	434
AM07464-SS	(NAG37)s(invAb)sgcgcaacaUfAfUfuugaucaguas(invAb)	320	GCGCAACACAUAUUUGAUCAGUA	435
AM07466-SS	(NAG37)s(invAb)sacccaacaUfAfUfuugaucaguas(invAb)	321	ACCCAACACAUAUUUGAUCAGUA	436
AM07468-SS	(NAG37)s(invAb)sacgcaacaUfAfUfuugaucaguas(invAb)	322	ACGCAACACAUAUUUGAUCAGUA	437
AM07502-SS	(NAG37)s(invAb)sacCfcAfuGfgAfCfAfuuaauucaaas(invAb)	323	ACCCAUGGACAUUAAUUCAAA	28
AM07503-SS	(NAG37)s(invAb)sacccauggAfCfAfuuaauucaAMas(invAb)	324	ACCCAUGGACAUUAAUUCAAA	28
AM07504-SS	(NAG37)s(invAb)sacccauggAfCfAfuuaaTMumCMaAMas(invAb)	325	ACCCAUGGACAUUAAUCAAA	438
AM07579-SS	(NAG37)s(invAb)sgcUfcAfaCfaUfAfUfuugaucaguas(invAb)	326	GCUCAACACAUAUUUGAUCAGUA	17
AM07580-SS	(NAG37)s(invAb)sgcUfcAfaCfaUfaUfuugaucaguas(invAb)	327	GCUCAACACAUAUUUGAUCAGUA	17
AM07582-SS	(NAG37)s(invAb)sgcucaacaUfaUfuUfgaucaguas(invAb)	328	GCUCAACACAUAUUUGAUCAGUA	17
AM07584-SS	(NAG37)s(invAb)sgcucaacaUfaUfuugaucaguas(invAb)	329	GCUCAACACAUAUUUGAUCAGUA	17
AM07585-SS	(NAG37)s(invAb)sgcucaacaUfauuugaucaguas(invAb)	330	GCUCAACACAUAUUUGAUCAGUA	17
AM07586-SS	(NAG37)s(invAb)sgcucaacauaUfuugaucaguas(invAb)	331	GCUCAACACAUAUUUGAUCAGUA	17
AM07587-SS	(NAG37)s(invAb)sgcUfcAfaCfaUfauuugaucaguas(invAb)	332	GCUCAACACAUAUUUGAUCAGUA	17
AM07607-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuuga_2Nucaguas(invAb)	333	GCUCAACACAUAUUUG(A^{2N})UCAGUA	439
AM07608-SS	(NAG37)s(invAb)sgcucaacaUfa_2NUfuugaucaguas(invAb)	334	GCUCAACAU(A^{2N})UUUGAUCAGUA	19

Sense Strand ID:	Modified Sense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07609-SS	(NAG37)s(invAb)sgcucaaca_2NUfAfUfuugaucaguas(invAb)	335	GCUCAAC(A^{2N})UAUUUGAUCAGUA	441
AM07610-SS	(NAG37)s(invAb)sgcucaaca_2NUfa_2NUfuugaucaguas(invAb)	336	GCUCAAC(A^{2N})U(A^{2N})UUUGAUCAGUA	21
AM07625-SS	(NAG25)s(invAb)sgcucaacaUfAfUfuugaucaguas(invAb)	337	GCUCAACAUAUUUGAUCAGUA	17
AM07626-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuUfgaucaguas(invAb)	338	GCUCAACAUAUUUGAUCAGUA	17
AM07627-SS	(NAG37)s(invAb)sgcucaacaUfAfUfUfugaucaguas(invAb)	339	GCUCAACAUAUUUGAUCAGUA	17
AM07628-SS	(NAG37)s(invAb)sgcucaacAfUfAfUfuugaucaguas(invAb)	340	GCUCAACAUAUUUGAUCAGUA	17
AM07629-SS	(NAG37)s(invAb)sgcUfcAfaCfaUfAfUfuUfgaucaguas(invAb)	341	GCUCAACAUAUUUGAUCAGUA	17
AM07630-SS	(NAG37)s(invAb)sgcUfcAfaCfaUfAfUfUfugaucaguas(invAb)	342	GCUCAACAUAUUUGAUCAGUA	17
AM07631-SS	(NAG37)s(invAb)sgcucaacAMUfAfUfuugaucaguas(invAb)	343	GCUCAACAUAUUUGAUCAGUA	17
AM07632-SS	(NAG37)s(invAb)sgcucaacaUfAfUfuugaucaGMuas(invAb)	344	GCUCAACAUAUUUGAUCAGUA	17
AM07633-SS	(NAG37)s(invAb)sgmCMumCMaAMcaUfAfUfuugaucaguas(invAb)	345	GCUCAACAUAUUUGAUCAGUA	17
AM07635-SS	(NAG37)s(invAb)saccauggAfCfAfuUfaAfuucaaas(invAb)	346	ACCCAUGGACAUUAAUUCAAA	28
AM07636-SS	(NAG37)s(invAb)saccauggAfCfAfuUfAfAfAfuucaaas(invAb)	347	ACCCAUGGACAUUAAUUCAAA	28
AM07637-SS	(NAG37)s(invAb)saccauggAfCfAfuUfaAfuUfcaaas(invAb)	348	ACCCAUGGACAUUAAUUCAAA	28
AM07638-SS	(NAG37)s(invAb)saccauggAfCfAfuuAMauucaaas(invAb)	349	ACCCAUGGACAUUAAUUCAAA	28
AM07639-SS	(NAG37)s(invAb)saccauggAfCfAfuuAMaTMucaaas(invAb)	350	ACCCAUGGACAUUAATUCAAA	438
AM07640-SS	(NAG37)s(invAb)saccauggAfCfAfuUfAMAfuucaaas(invAb)	351	ACCCAUGGACAUUAAUUCAAA	28
AM07641-SS	(NAG37)s(invAb)saccauggAfCfAfuuAMaTMucaAMas(invAb)	352	ACCCAUGGACAUUAATUCAAA	438
AM07642-SS	(NAG25)s(invAb)saccauggAfCfAfuuaauucaaas(invAb)	353	ACCCAUGGACAUUAAUUCAAA	28
AM07659-SS	(NAG37)s(invAb)sgggacaugGfUfCfuuaaagacuus(invAb)	354	GGGACAUGGUCUUAAAGACUU	443
AM07661-SS	(NAG37)s(invAb)scuacuccaUfAfGfugaagcaauas(invAb)	355	CUACUCCAUAGUGAAGCAAUA	444

Sense Strand ID:	Modified Sense Strand (5′ → 3′)	SEQ ID NO.	Underlying Base Sequence (5′ → 3′) (Shown as an Unmodified Nucleotide Sequence)	SEQ ID NO.
AM07663-SS	(NAG37)s(invAb)sgacuugggAfUfCfacaaagcaaas(invAb)	356	GACUUGGGAUCACAAAGCAAA	445
AM07680-SS	(NAG37)s(invAb)sgguugcuaUfGfUfuagacgaugus(invAb)	357	GGUUGCUAUGUUAGACGAUGU	32
AM07682-SS	(NAG37)s(invAb)sggaagauaUfAfCfuccauagugas(invAb)	358	GGAAGAUAUACUCCAUAGUGA	447
AM07684-SS	(NAG37)s(invAb)sgucacgaaAfCfCfaacuauacgas(invAb)	359	GUCACGAAACCAACUAUACGA	448
AM07686-SS	(NAG37)s(invAb)sgucuacuuGfGfGfaucacaaagas(invAb)	360	GUCUACUUGGGAUCACAAAGA	449
AM07910-SS	(NAG37)s(invAb)sggaagauaUfAfCfucCUNAauagugas(invAb)	361	GGAAGAUAUACUCCCAUAGUGA	447
AM07912-SS	(NAG37)s(invAb)sggaagauaUfAfCfuccauaiugas(invAb)	362	GGAAGAUAUACUCCCAUAIUGA	450

(A^{2N}) = 2-aminoadenine nucleotide

The ANGPTL3 RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2 or Table 4 can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

In some embodiments, the antisense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 3. In some embodiments, the sense strand of an ANGPTL3 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 4.

In some embodiments, an ANGPTL3 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2 or Table 3. In some embodiments, an ANGPTL3 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) 1-17, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, or 2-24 of any of the sequences in Table 2 or Table 3. In certain embodiments, an ANGPTL3 RNAi agent antisense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 3.

In some embodiments, an ANGPTL3 RNAi agent sense strand comprises the nucleotide sequence of any of the sequences in Table 2 or Table 4. In some embodiments, an ANGPTL3 RNAi agent sense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) 1-17, 2-17, 3-17, 4-17, 1-18, 2-18, 3-18, 4-18, 1-19, 2-19, 3-19, 4-19, 1-20, 2-20, 3-20, 4-20, 1-21, 2-21, 3-21, 4-21, 1-22, 2-22, 3-22, 4-22, 1-23, 2-23, 3-23, 4-23, 1-24, 2-24, 3-24, or 4-24 of any of the sequences in Table 2 or Table 4. In certain embodiments, an ANGPTL3 RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 4.

For the ANGPTL3 RNAi agents disclosed herein, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) can be perfectly complementary to an ANGPTL3 gene, or can be non-complementary to an ANGPTL3 gene. In some embodiments, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) is a U, A, or dT (or a

modified version thereof). In some embodiments, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) forms an A:U or U:A base pair with the sense strand.

In some embodiments, an ANGPTL3 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3. In some embodiments, an ANGPTL3 RNAi sense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) 1-17 or 1-18 of any of the sense strand sequences in Table 2 or Table 4.

In some embodiments, an ANGPTL3 RNAi agent includes (i) an antisense strand comprising the sequence of nucleotides (from 5′ end → 3′ end) 2-18 or 2-19 of any of the antisense strand sequences in Table 2 or Table 3, and (ii) a sense strand comprising the sequence of nucleotides (from 5′ end → 3′ end) 1-17 or 1-18 of any of the sense strand sequences in Table 2 or Table 4.

A sense strand containing a sequence listed in Table 2 or Table 4 can be hybridized to any antisense strand containing a sequence listed in Table 2 or Table 3, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence. In some embodiments, the ANGPTL3 RNAi agent has a sense strand consisting of the modified sequence of any of the modified sequences in Table 4, and an antisense strand consisting of the modified sequence of any of the modified sequences in Table 3. Certain representative sequence pairings are exemplified by the Duplex ID Nos. shown in Table 5.

In some embodiments, an ANGPTL3 RNAi agent comprises, consists of, or consists essentially of a duplex represented by any one of the Duplex ID Nos. presented herein. In some embodiments, an ANGPTL3 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the duplexes represented by any of the Duplex ID Nos. presented herein. In some embodiments, an ANGPTL3 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the duplexes represented by any of the Duplex ID Nos. presented herein and a targeting group and/or linking group wherein the targeting group and/or linking group is covalently linked (i.e., conjugated) to the sense strand or the antisense strand. In some embodiments, an ANGPTL3 RNAi agent

includes the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein. In some embodiments, an ANGPTL3 RNAi agent comprises the sense strand and antisense strand modified nucleotide sequences of any of the Duplex ID Nos. presented herein and a targeting group and/or linking group, wherein the targeting group and/or linking group is covalently linked to the sense strand or the antisense strand.

In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Table 2 or Table 5, and further comprises a targeting group. In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Table 2 or Table 5, and further comprises an asialoglycoprotein receptor ligand targeting group.

In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Table 2 or Table 5, and further comprises a targeting group selected from the group consisting of (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, each as defined in Table 6. In some embodiments, the targeting group is (NAG25) or (NAG25)s as defined in Table 6. In other embodiments, the targeting group is (NAG37) or (NAG37)s as defined in Table 6.

In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand and a sense strand having the modified nucleotide sequence of any of the antisense strand and/or sense strand nucleotide sequences in Table 3 or Table 4.

In some embodiments, an ANGPTL3 RNAi agent comprises an antisense strand and a sense strand having a modified nucleotide sequence of any of the antisense strand and/or sense strand nucleotide sequences of any of the duplexes Table 5, and further comprises an asialoglycoprotein receptor ligand targeting group.

In some embodiments, an ANGPTL3 RNAi agent comprises, consists of, or consists essentially of any of the duplexes of Table 5.

Table 5. ANGPTL3 RNAi Agents Duplexes with Corresponding Sense and Antisense Strand ID Numbers

Duplex ID	Antisense Strand ID	Sense Strand ID
AD05306	AM06999-AS	AM06998-SS
AD05307	AM07001-AS	AM07000-SS
AD05308	AM07003-AS	AM07002-SS
AD05309	AM07005-AS	AM07004-SS
AD05310	AM07007-AS	AM07006-SS
AD05311	AM07009-AS	AM07008-SS
AD05312	AM07011-AS	AM07010-SS
AD05342	AM07061-AS	AM06992-SS
AD05343	AM07062-AS	AM06994-SS
AD05344	AM07063-AS	AM06996-SS
AD05410	AM07148-AS	AM07147-SS
AD05411	AM07149-AS	AM07147-SS
AD05412	AM07148-AS	AM07150-SS
AD05413	AM07148-AS	AM07151-SS
AD05414	AM07148-AS	AM07152-SS
AD05415	AM07148-AS	AM07153-SS
AD05416	AM07155-AS	AM07154-SS
AD05417	AM07157-AS	AM07156-SS
AD05418	AM07159-AS	AM07158-SS
AD05419	AM07161-AS	AM07160-SS
AD05420	AM07163-AS	AM07162-SS
AD05421	AM07164-AS	AM07162-SS
AD05422	AM07163-AS	AM07165-SS
AD05423	AM07163-AS	AM07166-SS
AD05424	AM07163-AS	AM07167-SS
AD05425	AM07163-AS	AM07168-SS
AD05487	AM07233-AS	AM07232-SS
AD05488	AM07235-AS	AM07234-SS
AD05489	AM07237-AS	AM07236-SS
AD05490	AM07239-AS	AM07238-SS
AD05491	AM07241-AS	AM07240-SS
AD05492	AM07243-AS	AM07242-SS
AD05493	AM07245-AS	AM07244-SS
AD05494	AM07003-AS	AM07246-SS
AD05495	AM07163-AS	AM07247-SS
AD05572	AM07003-AS	AM07345-SS
AD05573	AM07003-AS	AM07346-SS

Duplex ID	Antisense Strand ID	Sense Strand ID
AD05574	AM07347-AS	AM07002-SS
AD05575	AM07348-AS	AM07002-SS
AD05576	AM07349-AS	AM07002-SS
AD05577	AM07350-AS	AM07002-SS
AD05578	AM07351-AS	AM07002-SS
AD05579	AM07352-AS	AM07002-SS
AD05580	AM07347-AS	AM07353-SS
AD05581	AM07348-AS	AM07353-SS
AD05582	AM07350-AS	AM07353-SS
AD05583	AM07351-AS	AM07353-SS
AD05584	AM07347-AS	AM07354-SS
AD05585	AM07356-AS	AM07355-SS
AD05586	AM07357-AS	AM07160-SS
AD05587	AM07357-AS	AM07358-SS
AD05588	AM07159-AS	AM07359-SS
AD05652	AM07454-AS	AM07453-SS
AD05653	AM07235-AS	AM07455-SS
AD05654	AM07456-AS	AM07234-SS
AD05655	AM07457-AS	AM07234-SS
AD05656	AM07458-AS	AM07234-SS
AD05657	AM07235-AS	AM07459-SS
AD05658	AM07461-AS	AM07460-SS
AD05659	AM07463-AS	AM07462-SS
AD05660	AM07465-AS	AM07464-SS
AD05661	AM07467-AS	AM07466-SS
AD05662	AM07469-AS	AM07468-SS
AD05693	AM07159-AS	AM07502-SS
AD05694	AM07159-AS	AM07503-SS
AD05695	AM07159-AS	AM07504-SS
AD05696	AM07505-AS	AM07158-SS
AD05697	AM07506-AS	AM07158-SS
AD05698	AM07507-AS	AM07158-SS
AD05699	AM07508-AS	AM07158-SS
AD05743	AM07235-AS	AM07579-SS
AD05744	AM07235-AS	AM07580-SS
AD05745	AM07581-AS	AM07234-SS
AD05746	AM07581-AS	AM07582-SS

Duplex ID	Antisense Strand ID	Sense Strand ID
AD05747	AM07583-AS	AM07582-SS
AD05748	AM07581-AS	AM07584-SS
AD05749	AM07581-AS	AM07585-SS
AD05750	AM07581-AS	AM07586-SS
AD05751	AM07581-AS	AM07580-SS
AD05752	AM07588-AS	AM07587-SS
AD05753	AM07589-AS	AM07234-SS
AD05756	AM07593-AS	AM07234-SS
AD05774	AM07235-AS	AM07607-SS
AD05775	AM07235-AS	AM07608-SS
AD05776	AM07235-AS	AM07609-SS
AD05777	AM07235-AS	AM07610-SS
AD05790	AM07623-AS	AM07234-SS
AD05791	AM07624-AS	AM07234-SS
AD05792	AM07235-AS	AM07625-SS
AD05793	AM07235-AS	AM07626-SS
AD05794	AM07235-AS	AM07627-SS
AD05795	AM07235-AS	AM07628-SS
AD05796	AM07235-AS	AM07629-SS
AD05797	AM07235-AS	AM07630-SS
AD05798	AM07235-AS	AM07631-SS

Duplex ID	Antisense Strand ID	Sense Strand ID
AD05799	AM07235-AS	AM07632-SS
AD05800	AM07235-AS	AM07633-SS
AD05801	AM07634-AS	AM07158-SS
AD05802	AM07634-AS	AM07635-SS
AD05803	AM07634-AS	AM07636-SS
AD05804	AM07634-AS	AM07637-SS
AD05805	AM07634-AS	AM07638-SS
AD05806	AM07634-AS	AM07639-SS
AD05807	AM07634-AS	AM07640-SS
AD05808	AM07634-AS	AM07641-SS
AD05809	AM07159-AS	AM07642-SS
AD05826	AM07660-AS	AM07659-SS
AD05827	AM07662-AS	AM07661-SS
AD05828	AM07664-AS	AM07663-SS
AD05840	AM07681-AS	AM07680-SS
AD05841	AM07683-AS	AM07682-SS
AD05842	AM07685-AS	AM07684-SS
AD05843	AM07687-AS	AM07686-SS
AD05991	AM07683-AS	AM07910-SS
AD05992	AM07911-AS	AM07682-SS
AD05993	AM07683-AS	AM07912-SS

In some embodiments, an ANGPTL3 RNAi agent is prepared or provided as a salt, mixed salt, or a free-acid. The RNAi agents described herein, upon delivery to a cell expressing an ANGPTL3 gene, inhibit or knockdown expression of one or more ANGPTL3 genes *in vivo* and/or *in vitro*.

Targeting Groups, Linking Groups, and Delivery Vehicles

In some embodiments, an ANGPTL3 RNAi agent is conjugated to one or more non-nucleotide groups including, but not limited to, a targeting group, a linking group, a delivery polymer, or a delivery vehicle. The non-nucleotide group can enhance targeting, delivery or attachment of the RNAi agent. Examples of targeting groups and linking groups are provided in Table 6. The non-nucleotide group can be covalently linked to the 3′ and/or 5′ end of either the sense strand and/or the antisense strand. In some embodiments, an ANGPTL3 RNAi agent contains a non-nucleotide group linked to the 3′ and/or 5′ end of the sense strand. In some embodiments, a non-nucleotide group is linked to the 5′ end of an ANGPTL3 RNAi agent sense strand. A non-nucleotide group may be linked directly or indirectly to the RNAi agent via a linker/linking group. In some embodiments, a non-nucleotide group is linked to the RNAi agent via a labile, cleavable, or reversible bond or linker.

In some embodiments, a non-nucleotide group enhances the pharmacokinetic or biodistribution properties of an RNAi agent or conjugate to which it is attached to improve cell- or tissue-specific distribution and cell-specific uptake of the RNAi agent or conjugate. In some embodiments, a non-nucleotide group enhances endocytosis of the RNAi agent.

Targeting groups or targeting moieties enhance the pharmacokinetic or biodistribution properties of a conjugate or RNAi agent to which they are attached to improve cell-specific (including, in some cases, organ specific) distribution and cell-specific (or organ specific) uptake of the conjugate or RNAi agent. A targeting group can be monovalent, divalent, trivalent, tetravalent, or have higher valency for the target to which it is directed. Representative targeting groups include, without limitation, compounds with affinity to cell surface molecules, cell receptor ligands, haptens, antibodies, monoclonal antibodies, antibody fragments, and antibody mimics with affinity to cell surface molecules. In some embodiments, a targeting group is linked to an RNAi agent using a linker, such as a PEG

linker or one, two, or three abasic and/or ribitol (abasic ribose) residues, which can in some instances serve as linkers. In some embodiments, a targeting group comprises a galactose-derivative cluster.

The ANGPTL3 RNAi agents described herein can be synthesized having a reactive group, such as an amino group (also referred to herein as an amine), at the 5′-terminus and/or the 3′-terminus. The reactive group can be used subsequently to attach a targeting moiety using methods typical in the art.

In some embodiments, a targeting group comprises an asialoglycoprotein receptor ligand. As used herein, an asialoglycoprotein receptor ligand is a ligand that contains a compound having affinity for the asialoglycoprotein receptor. As noted herein, the asialoglycoprotein receptor is highly expressed on hepatocytes. In some embodiments, an asialoglycoprotein receptor ligand includes or consists of one or more galactose derivatives. As used herein, the term galactose derivative includes both galactose and derivatives of galactose having affinity for the asialoglycoprotein receptor that is equal to or greater than that of galactose. Galactose derivatives include, but are not limited to: galactose, galactosamine, N-formylgalactosamine, N-acetyl-galactosamine, N-propionyl-galactosamine, N-n-butanoyl-galactosamine, and N-iso-butanoylgalactos-amine (see for example: S.T. Iobst and K. Drickamer, J.B.C., 1996, 271, 6686). Galactose derivatives, and clusters of galactose derivatives, that are useful for *in vivo* targeting of oligonucleotides and other molecules to the liver are known in the art (see, for example, Baenziger and Fiete, 1980, Cell, 22, 611-620; Connolly et al., 1982, J. Biol. Chem., 257, 939-945).

Galactose derivatives 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 hepatocytes and endocytosis of the molecule into hepatocytes. Asialoglycoprotein receptor ligands can be monomeric (e.g., having a single galactose derivative) or multimeric (e.g., having multiple galactose derivatives). The galactose derivative or galactose derivative cluster can be attached to the 3′ or 5′ end of the sense or antisense strand of the RNAi agent using methods known in the art. The preparation of targeting groups, such as galactose derivative clusters, is described in, for example,

International Patent Application Publication No. WO 2018/044350 to Arrowhead Pharmaceuticals, Inc., and International Patent Application Publication No. WO 2017/156012 to Arrowhead Pharmaceuticals, Inc., the contents of both of which are incorporated by reference herein in their entirety.

As used herein, a galactose derivative cluster comprises a molecule having two to four terminal galactose derivatives. A terminal galactose derivative is attached to a molecule through its C-1 carbon. In some embodiments, the galactose derivative cluster is a galactose derivative trimer (also referred to as tri-antennary galactose derivative or tri-valent galactose derivative). In some embodiments, the galactose derivative cluster comprises N-acetyl-galactosamines. In some embodiments, the galactose derivative cluster comprises three N-acetyl-galactosamines. In some embodiments, the galactose derivative cluster is a galactose derivative tetramer (also referred to as tetra-antennary galactose derivative or tetra-valent galactose derivative). In some embodiments, the galactose derivative cluster comprises four N-acetyl-galactosamines.

As used herein, a galactose derivative trimer contains three galactose derivatives, each linked to a central branch point. As used herein, a galactose derivative tetramer contains four galactose derivatives, each linked to a central branch point. The galactose derivatives can be attached to the central branch point through the C-1 carbons of the saccharides. In some embodiments, the galactose derivatives are linked to the branch point via linkers or spacers. In some embodiments, the linker or spacer is a flexible hydrophilic spacer, such as a PEG group (see, for example, U.S. Patent No. 5,885,968; Biessen et al. J. Med. Chem. 1995 Vol. 39 p. 1538-1546). In some embodiments, the PEG spacer is a PEG_3 spacer. The branch point can be any small molecule which permits attachment of three galactose derivatives and further permits attachment of the branch point to the RNAi agent. An example of branch point group is a di-lysine or di-glutamate. Attachment of the branch point to the RNAi agent can occur through a linker or spacer. In some embodiments, the linker or spacer comprises a flexible hydrophilic spacer, such as, but not limited to, a PEG spacer. In some embodiments, the linker comprises a rigid linker, such as a cyclic group. In some embodiments, a galactose derivative comprises or consists of N-acetyl-galactosamine. In some embodiments, the galactose derivative cluster is comprised of a galactose derivative tetramer, which can be, for example, an N-acetyl-galactosamine tetramer.

Embodiments of the present disclosure include pharmaceutical compositions for delivering an ANGPTL3 RNAi agent to a liver cell *in vivo*. Such pharmaceutical compositions can include, for example, an ANGPTL3 RNAi agent conjugated to a galactose derivative cluster. In some embodiments, the galactose derivative cluster is comprised of a galactose derivative trimer, which can be, for example, an N-acetyl-galactosamine trimer, or galactose derivative tetramer, which can be, for example, an N-acetyl-galactosamine tetramer.

Targeting groups include, but are not limited to, (PAZ), (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), and (NAG39)s as defined in Table 6. Other targeting groups, including galactose cluster targeting ligands, are known in the art.

In some embodiments, a linking group is conjugated to the RNAi agent. The linking group facilitates covalent linkage of the agent to a targeting group, delivery polymer, or delivery vehicle. The linking group can be linked to the 3′ and/or the 5′ end of the RNAi agent sense strand or antisense strand. In some embodiments, the linking group is linked to the RNAi agent sense strand. In some embodiments, the linking group is conjugated to the 5′ or 3′ end of an RNAi agent sense strand. In some embodiments, a linking group is conjugated to the 5′ end of an RNAi agent sense strand. Examples of linking groups, 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.

A linker or linking group is a connection between two atoms that links one chemical group (such as an RNAi agent) or segment of interest to another chemical group (such as a targeting group or delivery polymer) or segment of interest via one or more covalent bonds. A labile linkage contains a labile bond. A linkage can optionally include a spacer that increases the distance between the two joined atoms. A spacer can further add flexibility and/or length to the linkage. Spacers include, but are not be limited to, alkyl groups, alkenyl groups, alkynyl groups, aryl groups, aralkyl groups, aralkenyl groups, and aralkynyl groups;

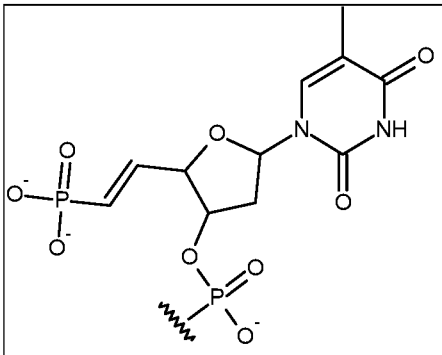
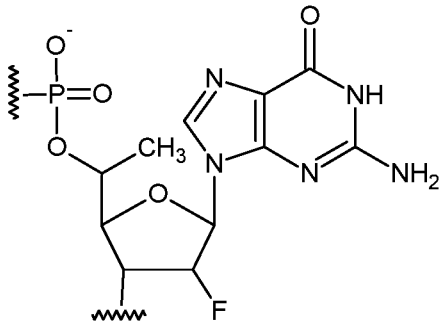
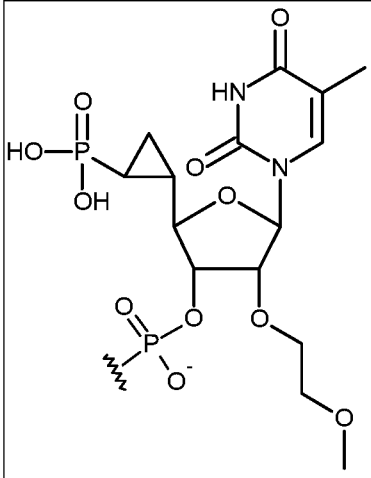
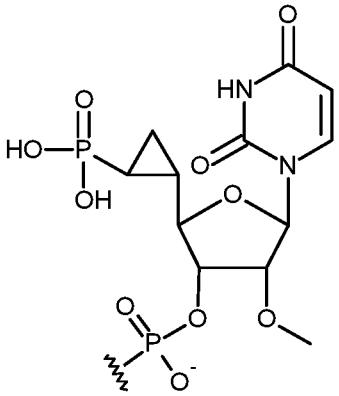
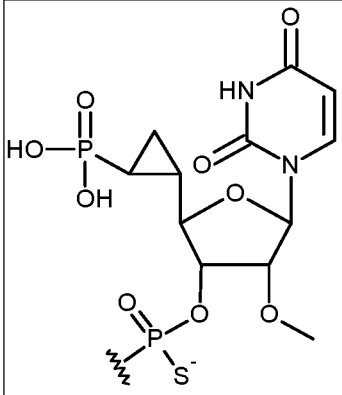
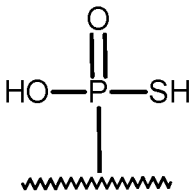
each of which can contain one or more heteroatoms, heterocycles, amino acids, nucleotides, and saccharides. Spacer groups are well known in the art and the preceding list is not meant to limit the scope of the description.

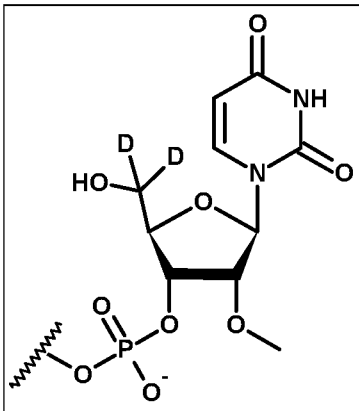
In some embodiments, when two or more RNAi agents are included in a single composition, each of the RNAi agents may be linked to the same targeting group or two a different targeting groups (i.e., targeting groups having different chemical structure). In some embodiments, targeting groups are linked to the ANGPTL3 RNAi agents disclosed herein without the use of an additional linker. In some embodiments, the targeting group itself is designed having a linker or other site to facilitate conjugation readily present. In some embodiments, when two or more ANGPTL3 RNAi agents are included in a single, each of the RNAi agents may utilize the same linker or different linkers (i.e., linkers having different chemical structures).

Any of the ANGPTL3 RNAi agent nucleotide sequences listed in Tables 2, 3, or 4, whether modified or unmodified, can contain 3′ and/or 5′ targeting group(s) or linking group(s). Any of the ANGPTL3 RNAi agent sequences listed in Table 3 or 4, or are otherwise described herein, which contain a 3′ or 5′ targeting group or linking group, can alternatively contain no 3′ or 5′ targeting group or linking group, or can contain a different 3′ or 5′ targeting group or linking group including, but not limited to, those depicted in Table 6. Any of the ANGPTL3 RNAi agent duplexes listed in Table 5, whether modified or unmodified, can further comprise a targeting group or linking group, including, but not limited to, those depicted in Table 6, and the targeting group or linking group can be attached to the 3′ or 5′ terminus of either the sense strand or the antisense strand of the ANGPTL3 RNAi agent duplex.

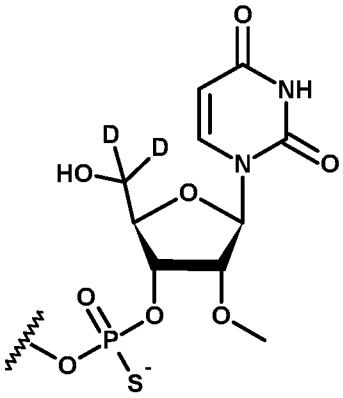
Examples of targeting groups and linking groups are provided in Table 6. Table 4 provides several embodiments of ANGPTL3 RNAi agent sense strands having a targeting group or linking group linked to the 5′ or 3′ end.

Table 6. Structures Representing Various Modified Nucleotides, Targeting Groups, and Linking Groups

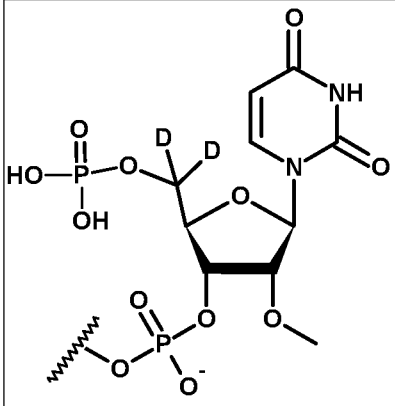
 vpdT	 5Me-Gf
 cPrpTM	 cPrpu
 cPrpus	 sp



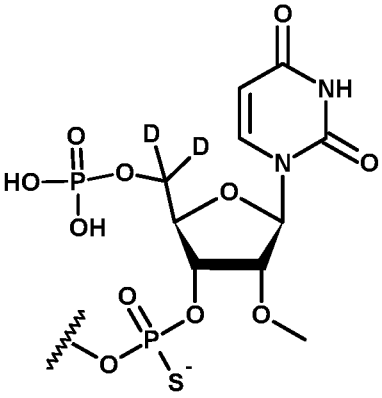
D2u



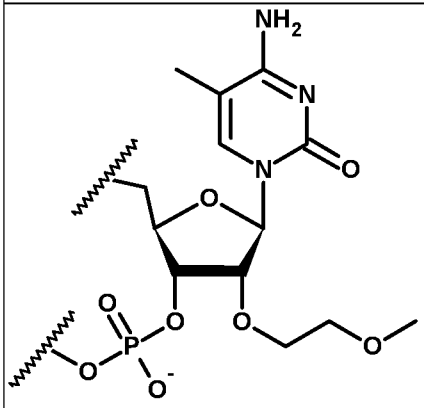
D2us



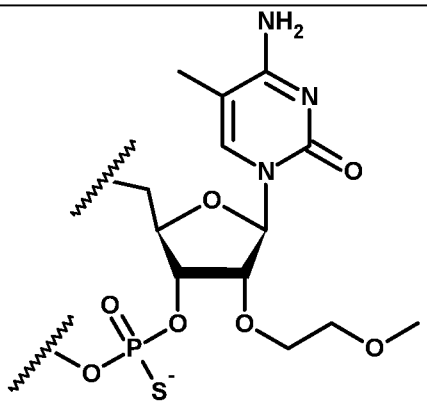
pD2u



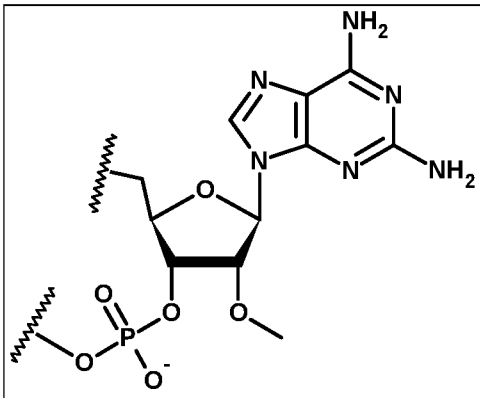
pD2us



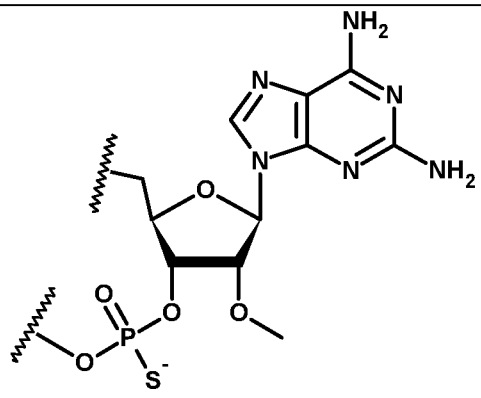
mCM



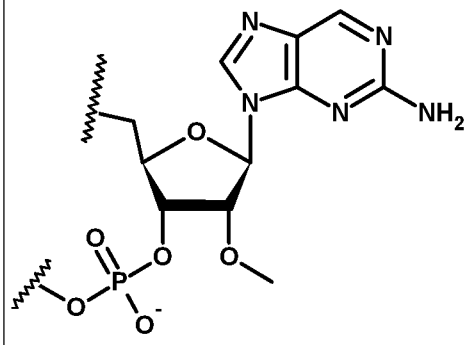
mCMs



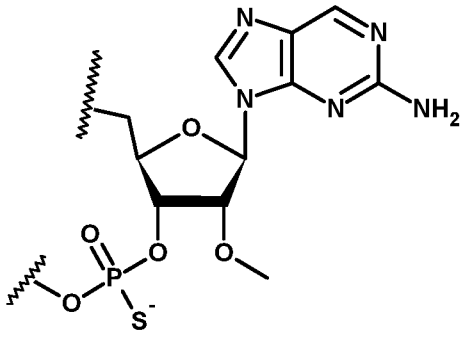
a_2N



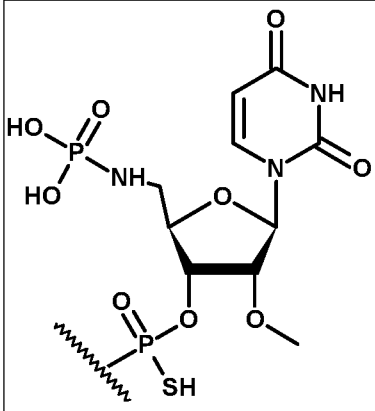
a_2Ns



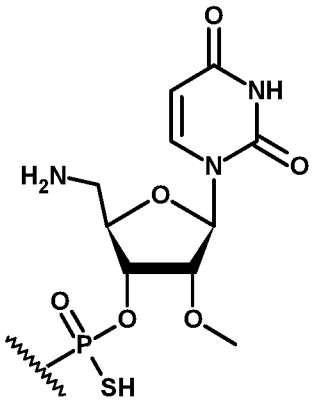
pu_2N



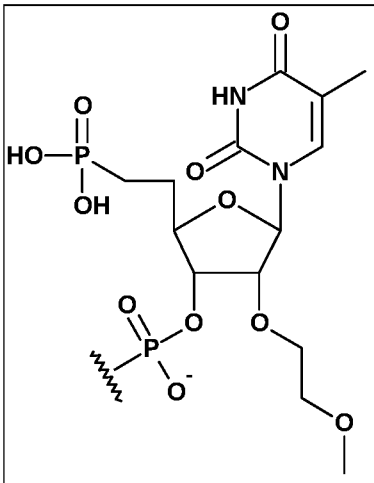
pu_2Ns



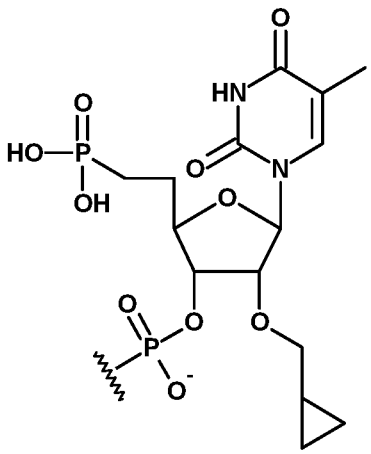
Npus



Nus

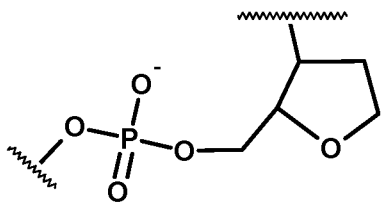


epTM



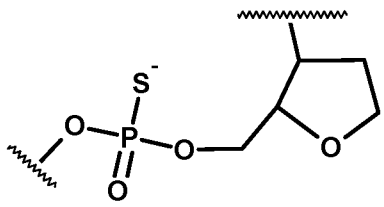
epTcPr

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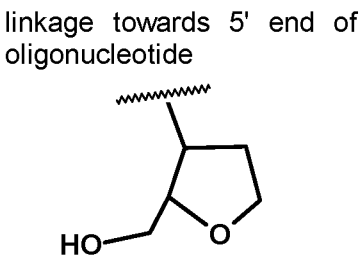
(invAb)

When positioned internally in oligonucleotide:

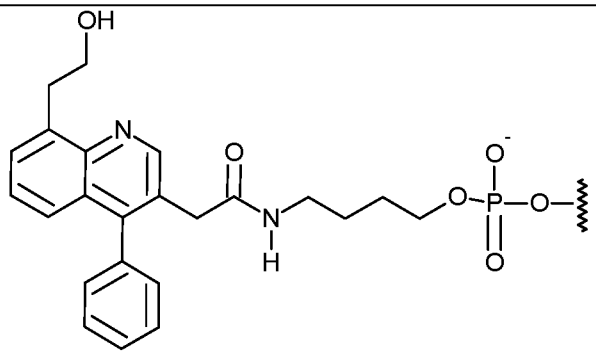


(invAb)s

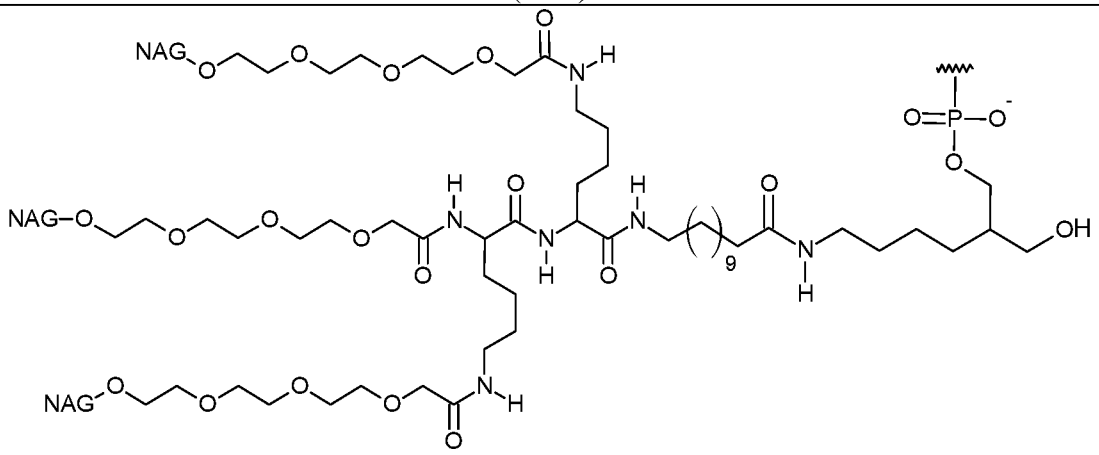
When positioned at the 3′ terminal end of oligonucleotide:



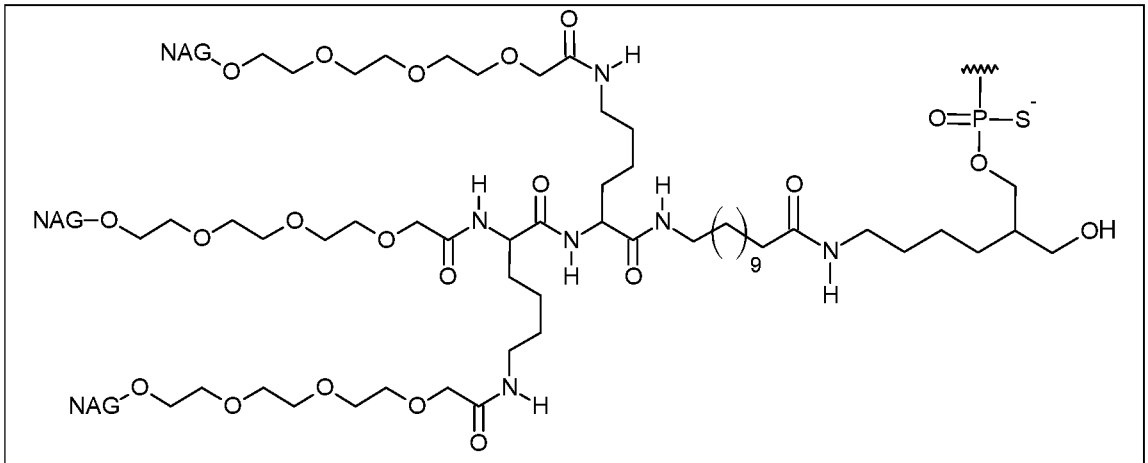
(invAb)



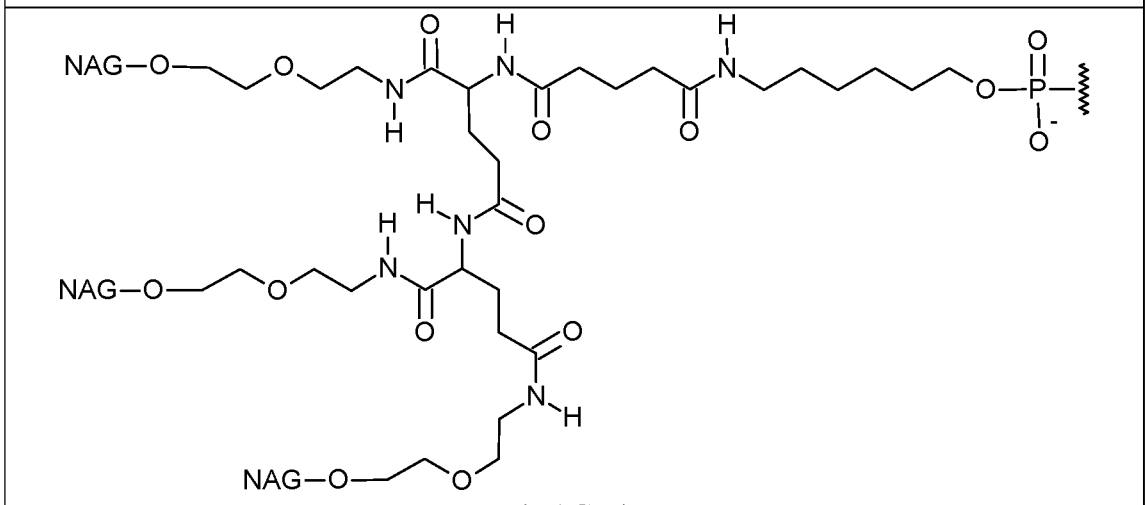
(PAZ)



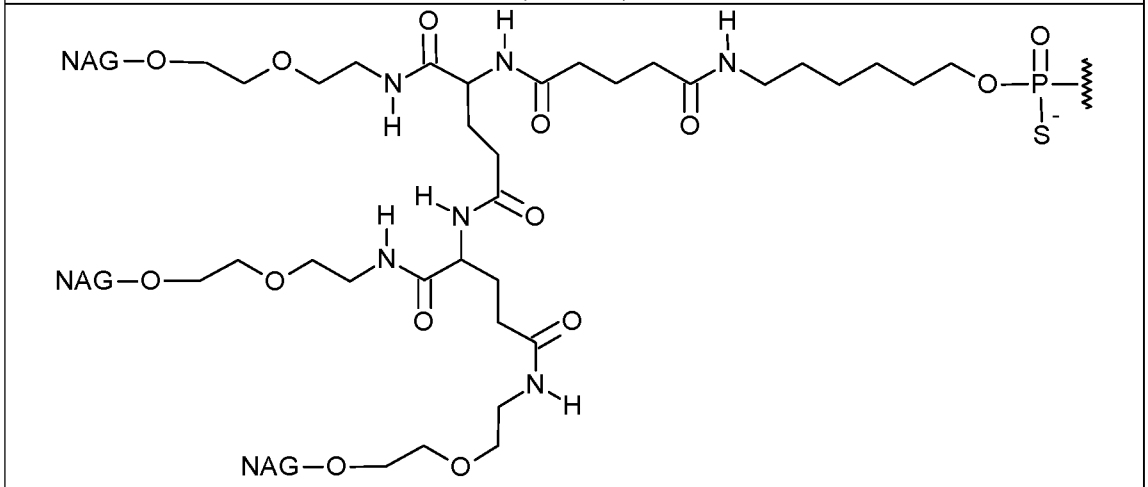
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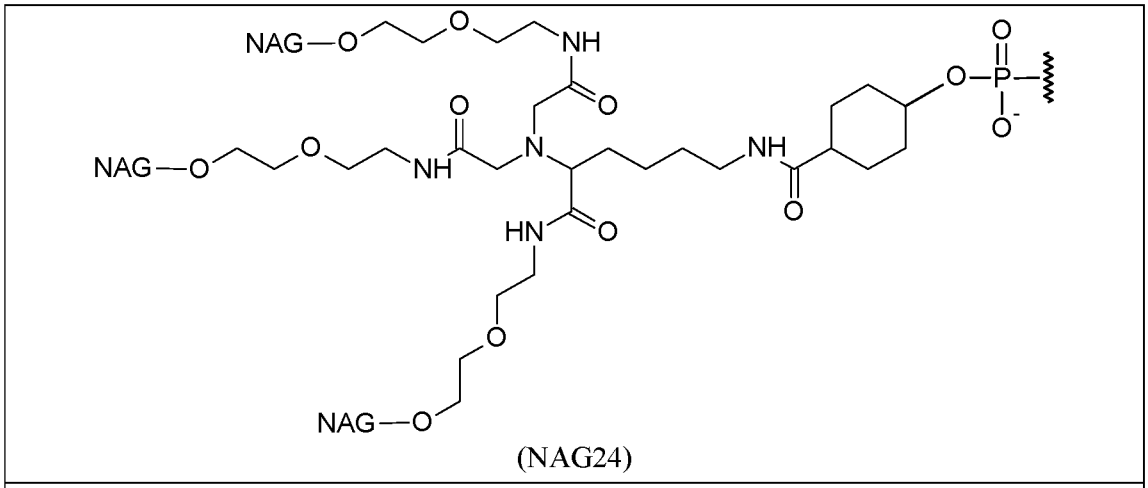
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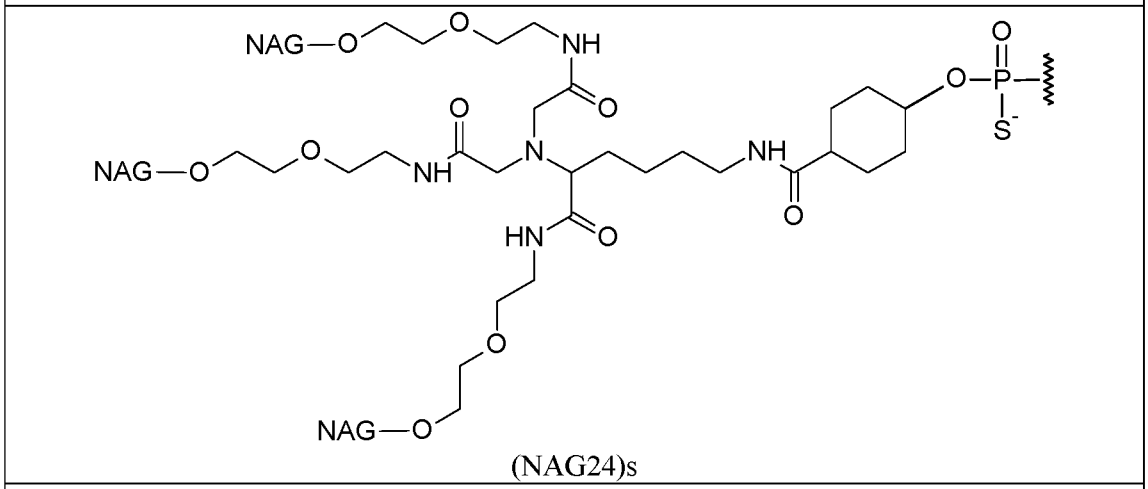
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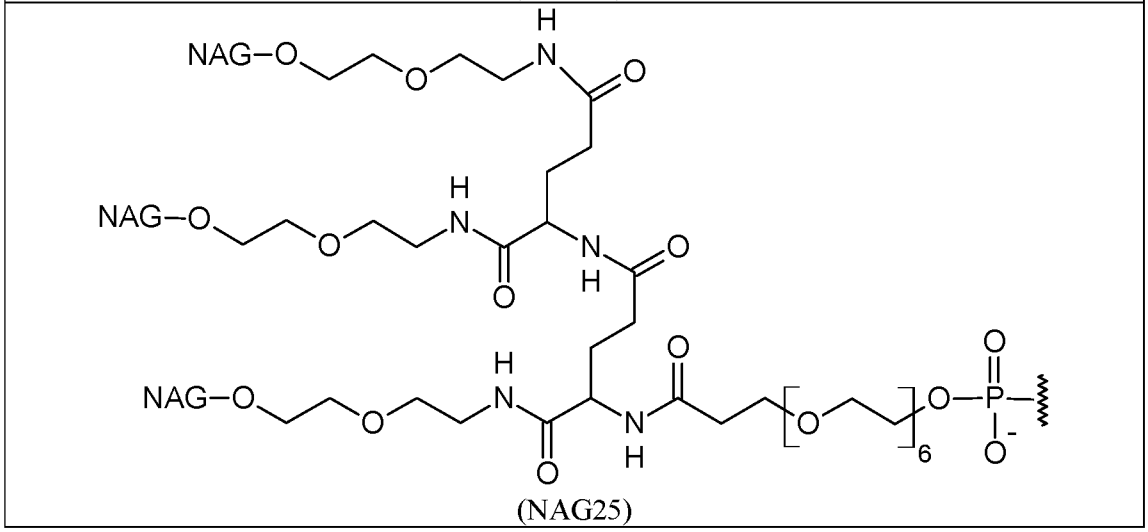
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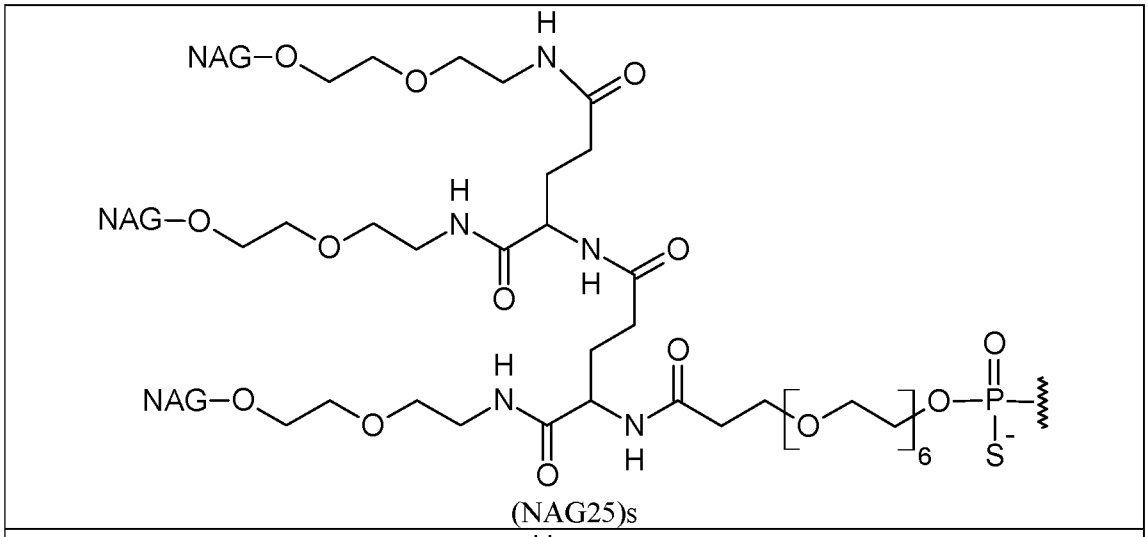
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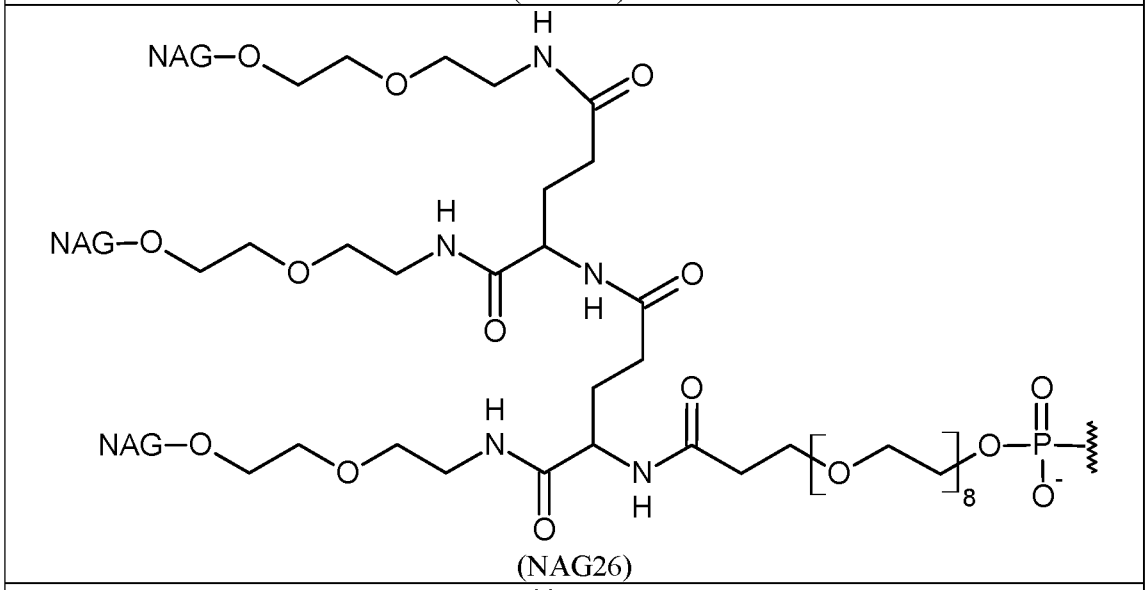
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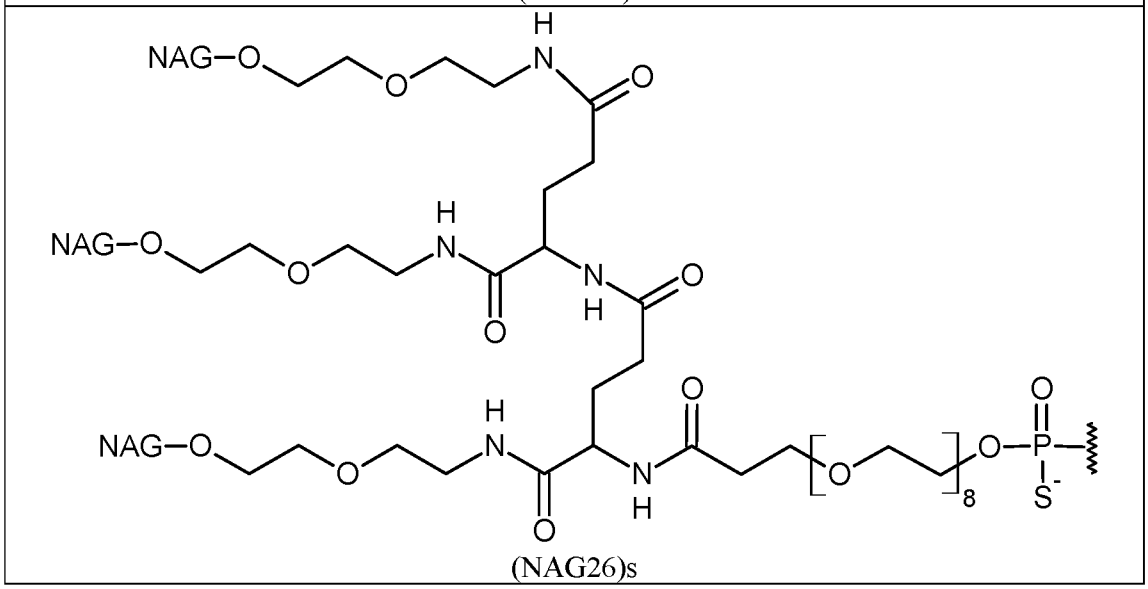
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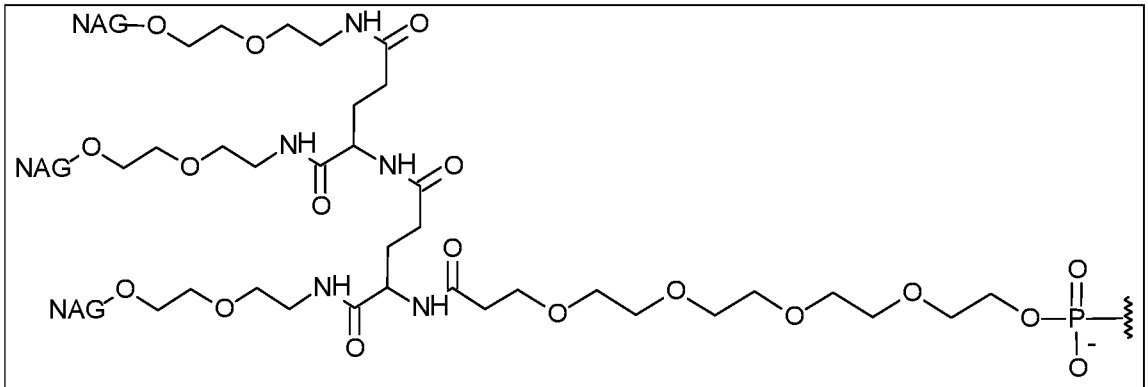
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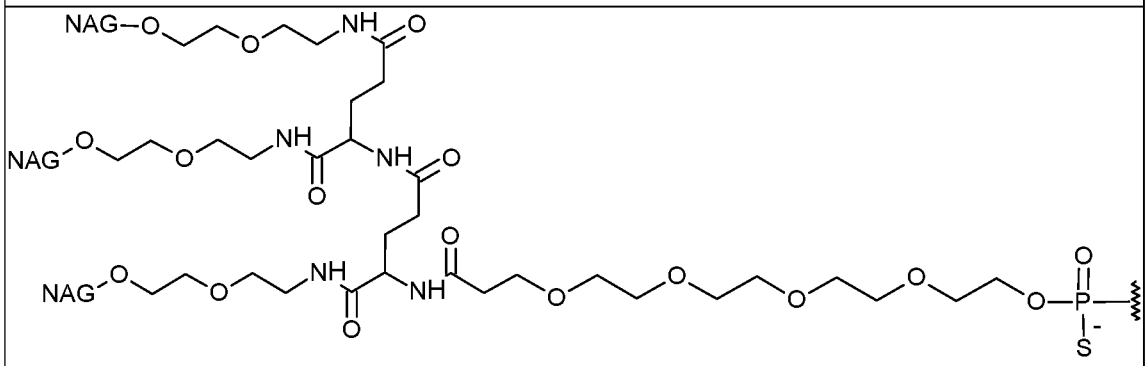
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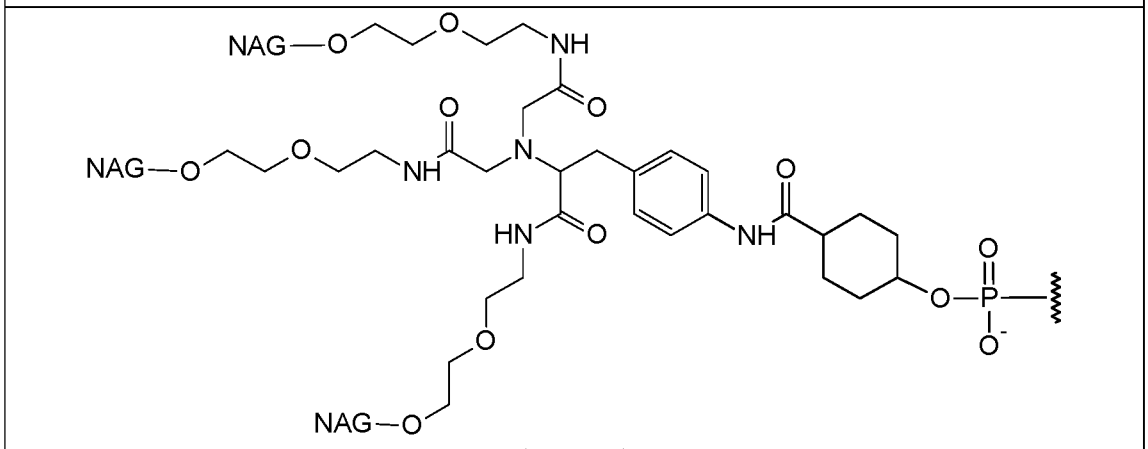
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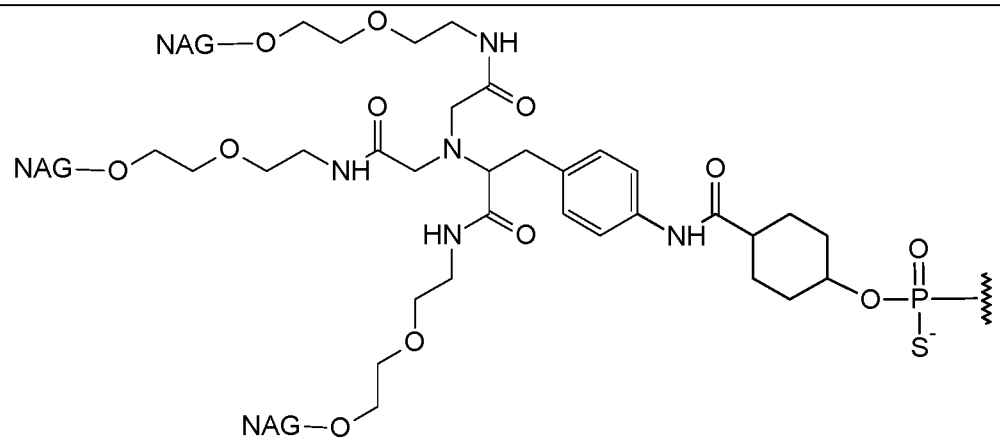
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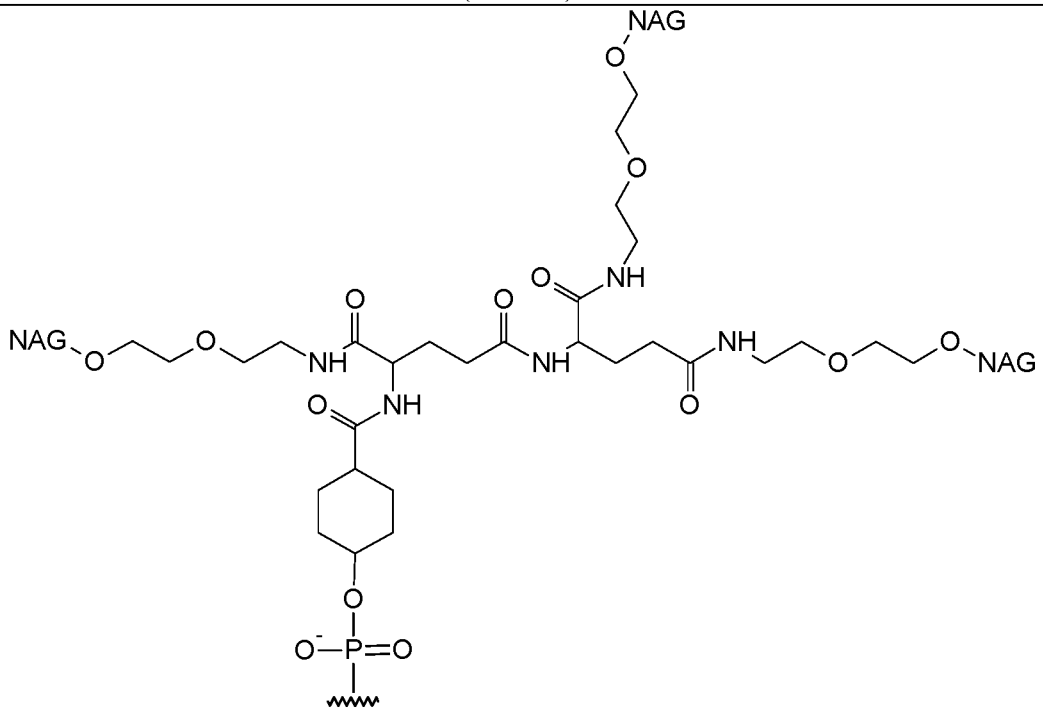
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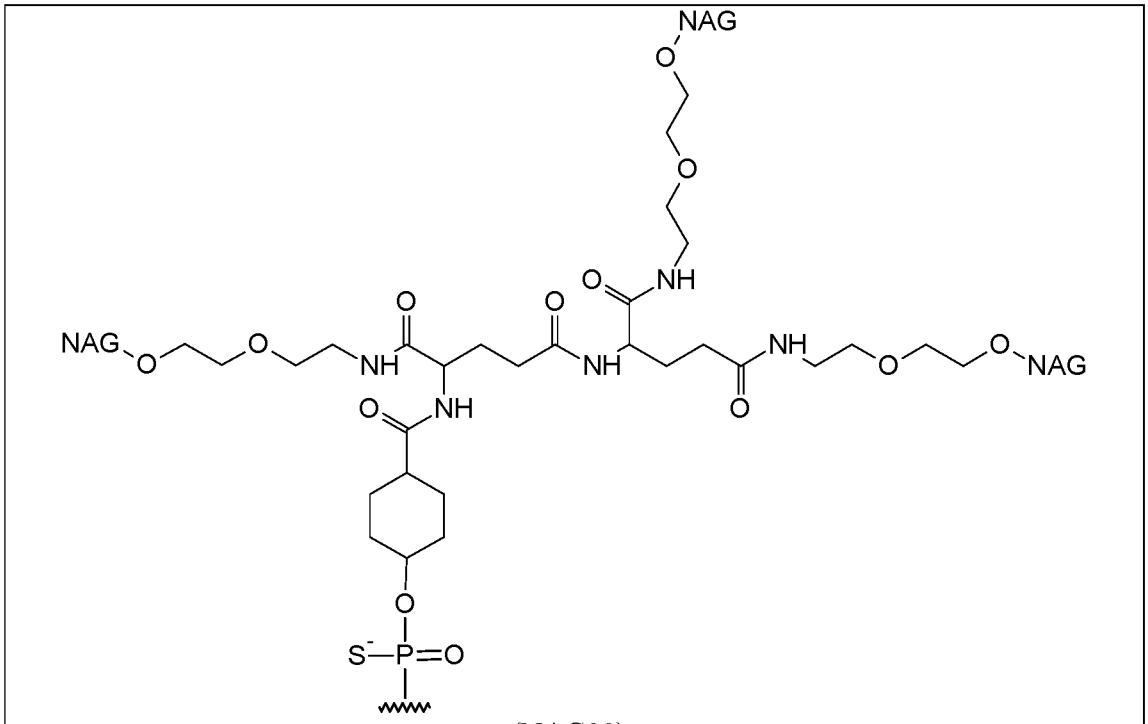
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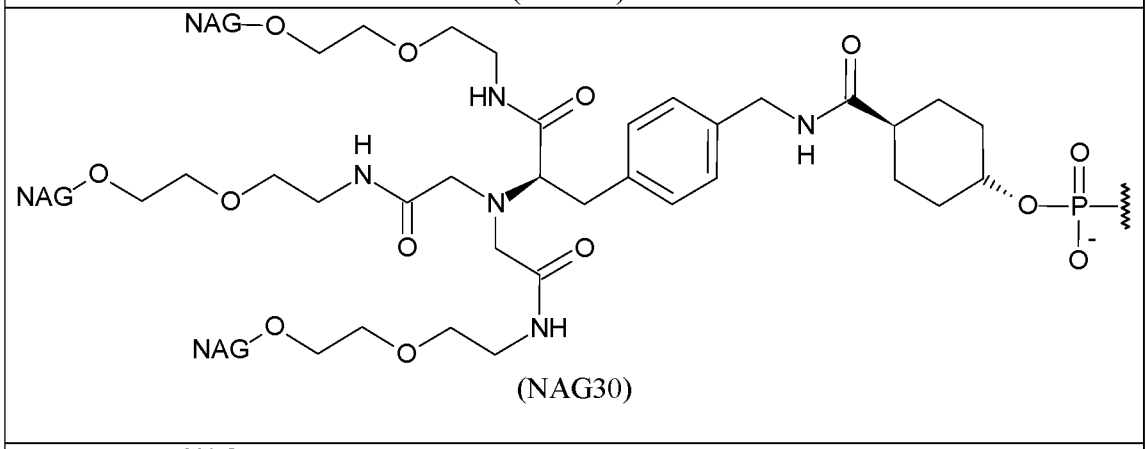
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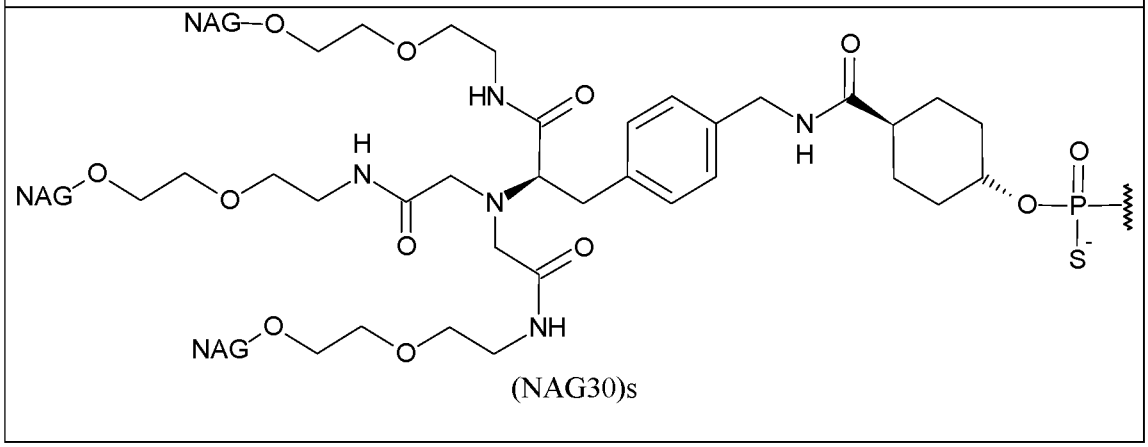
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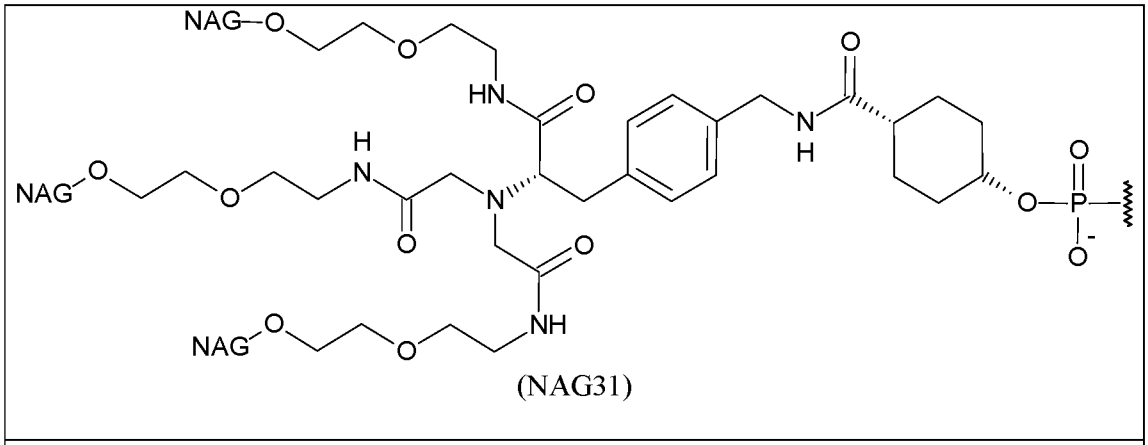
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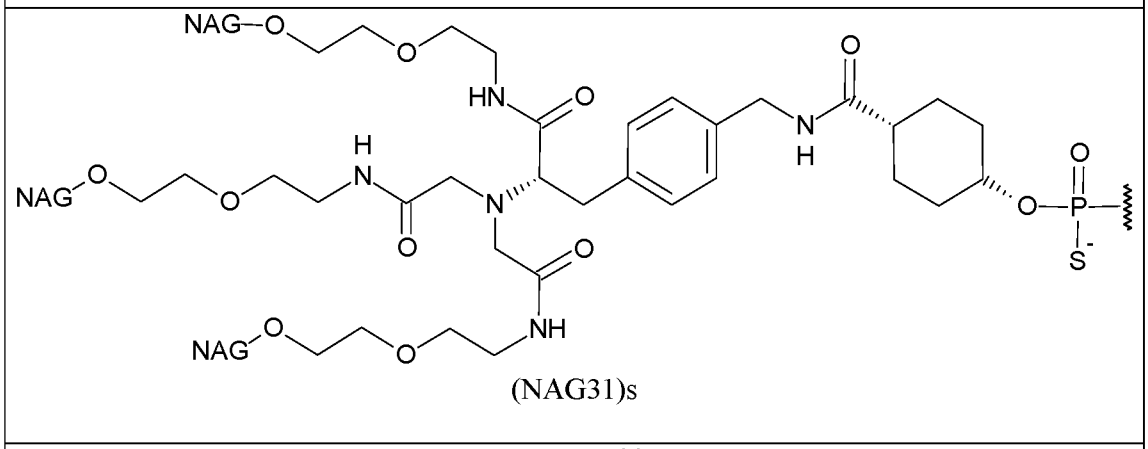
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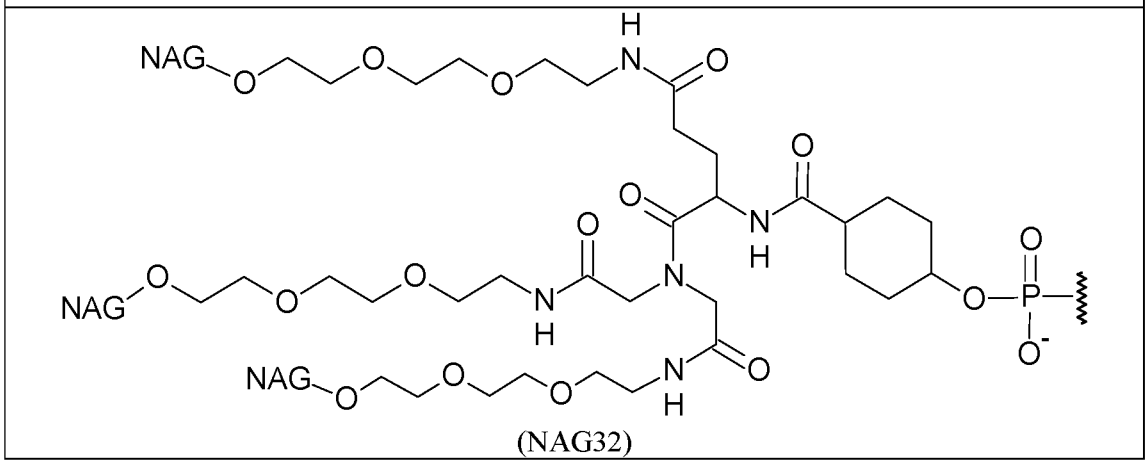
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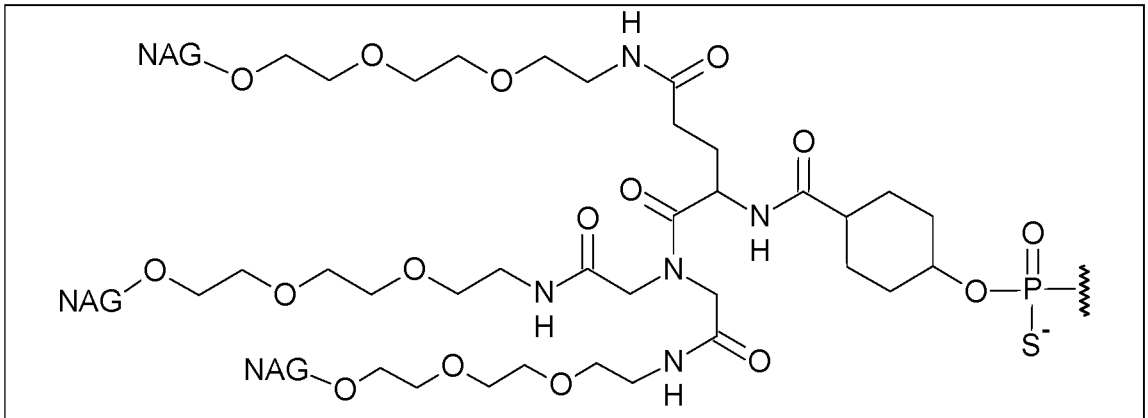
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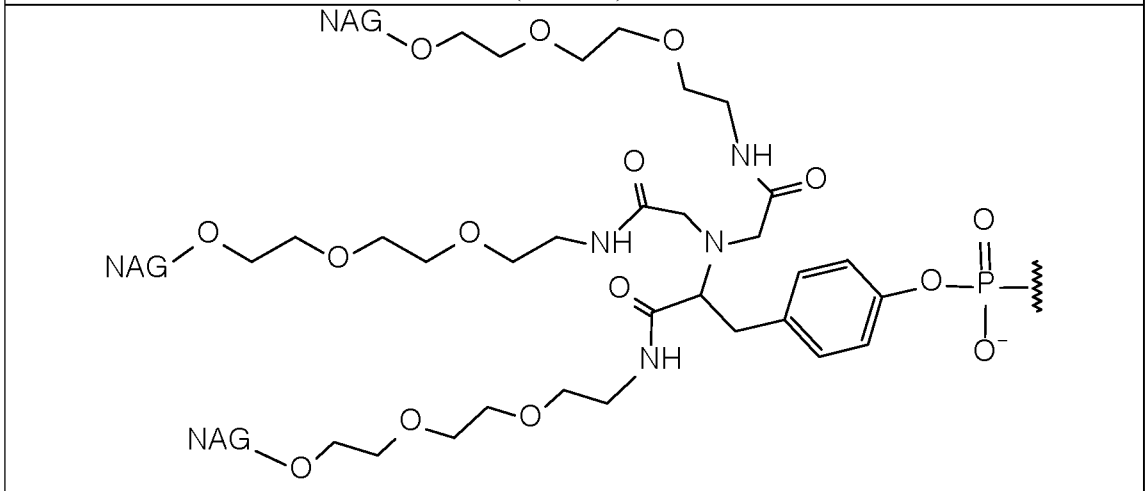
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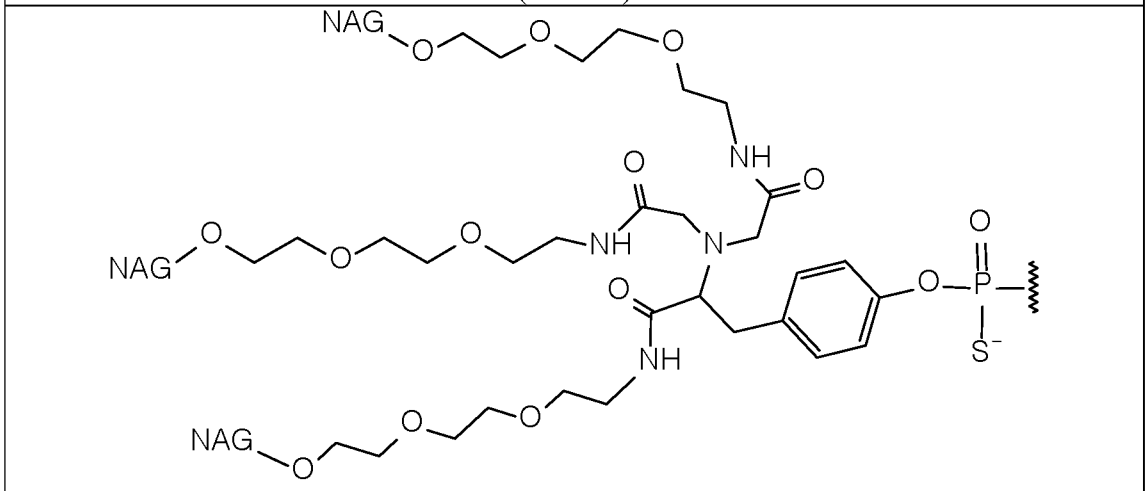
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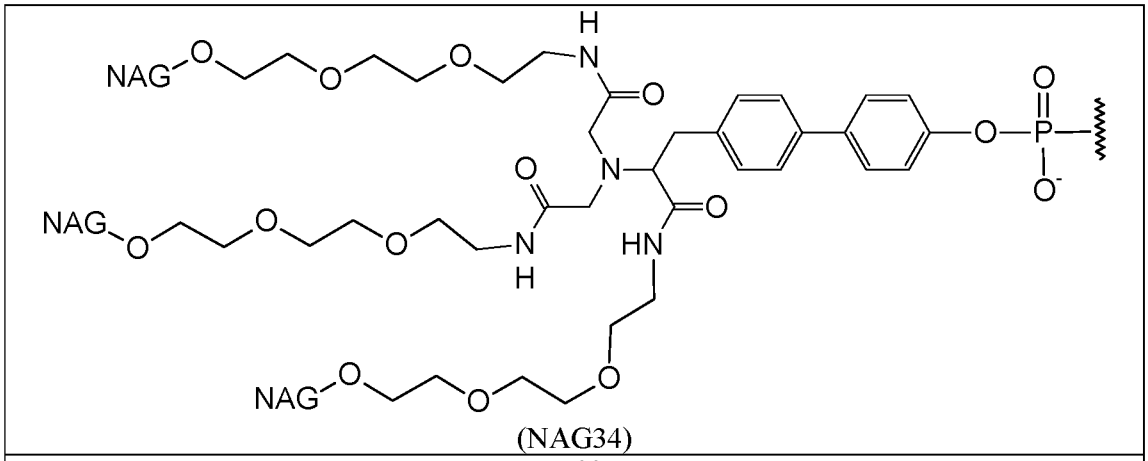
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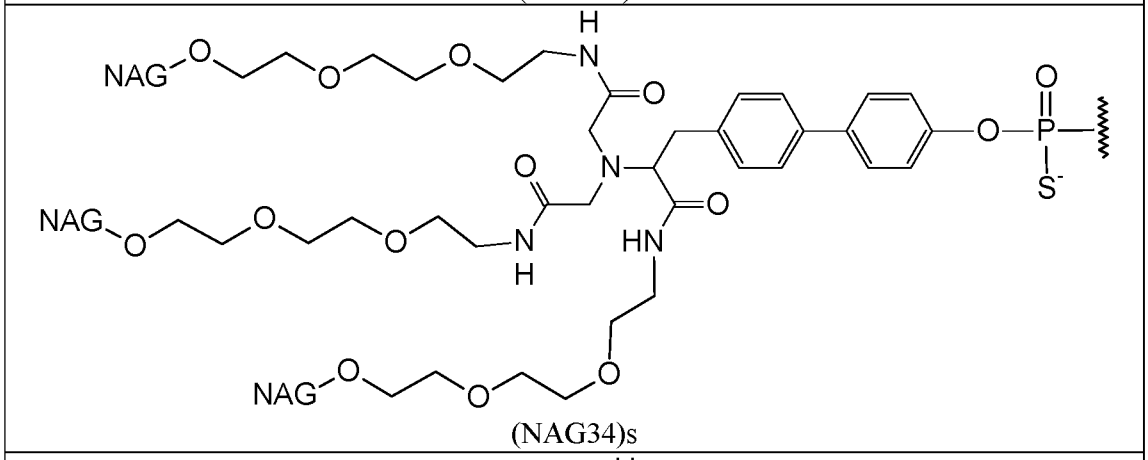
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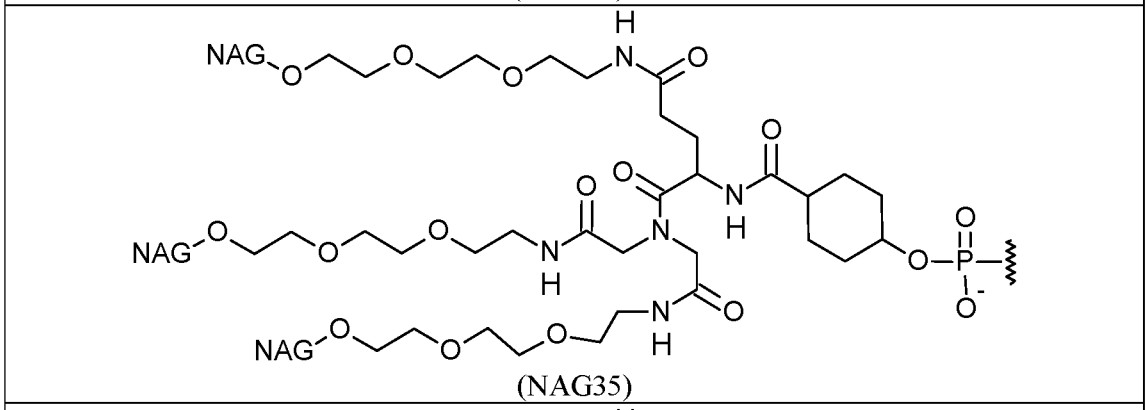
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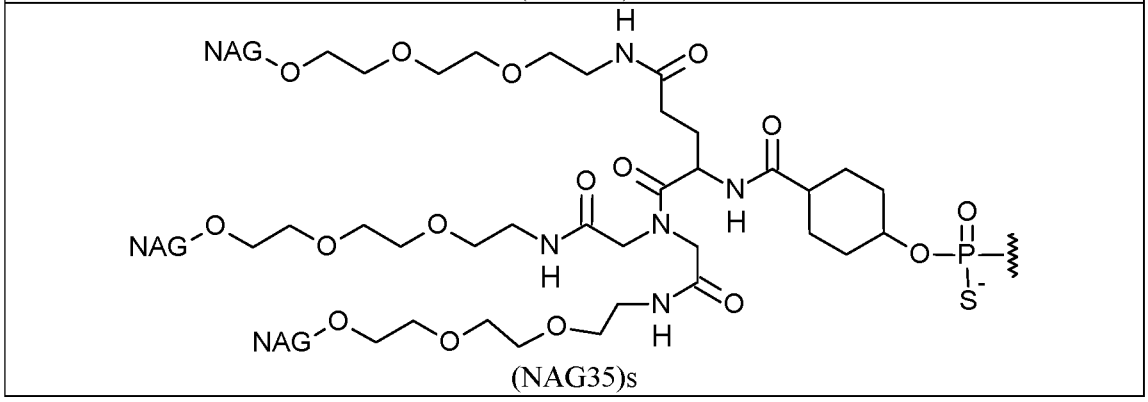
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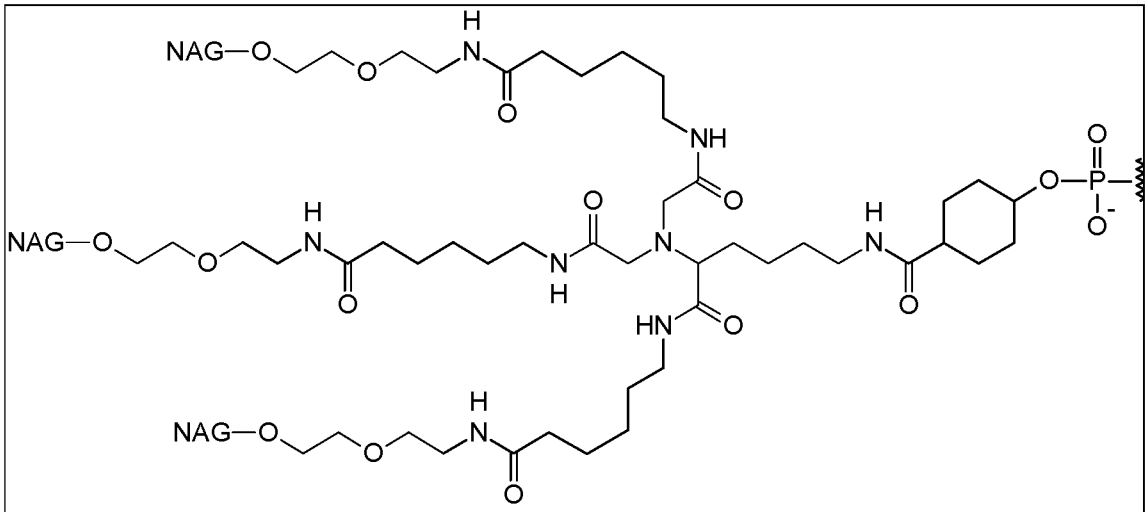
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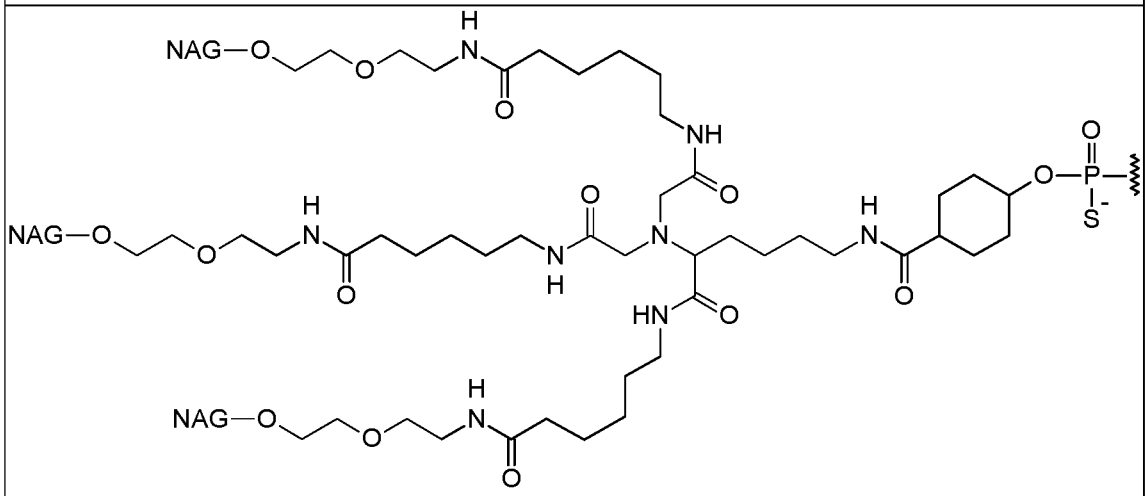
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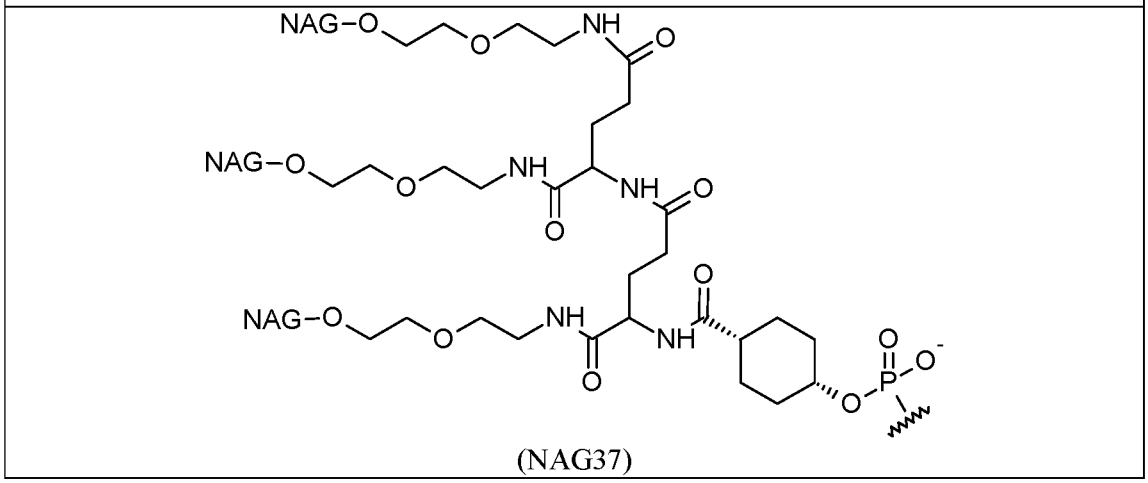
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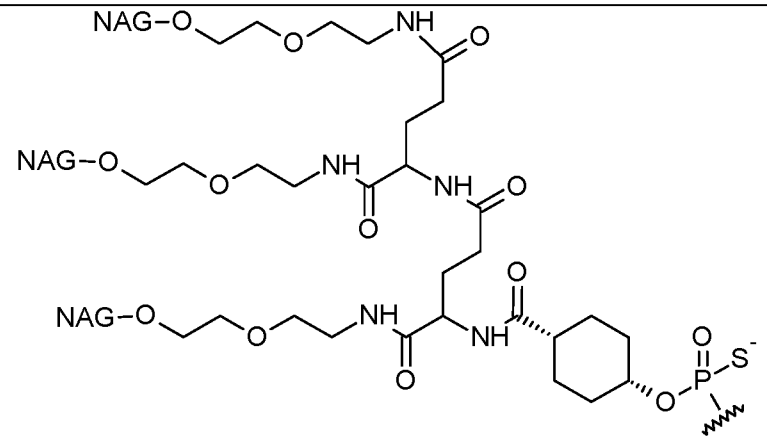
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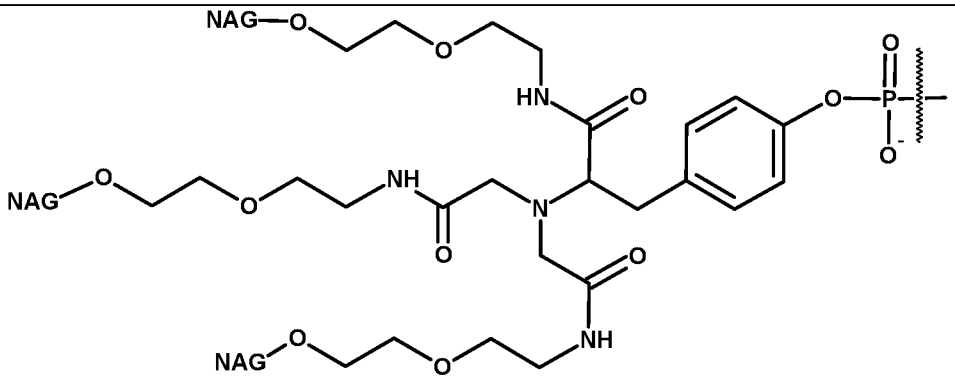
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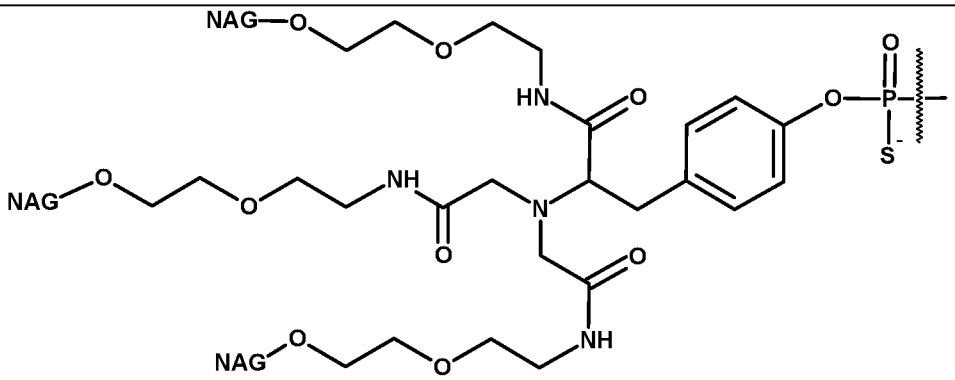
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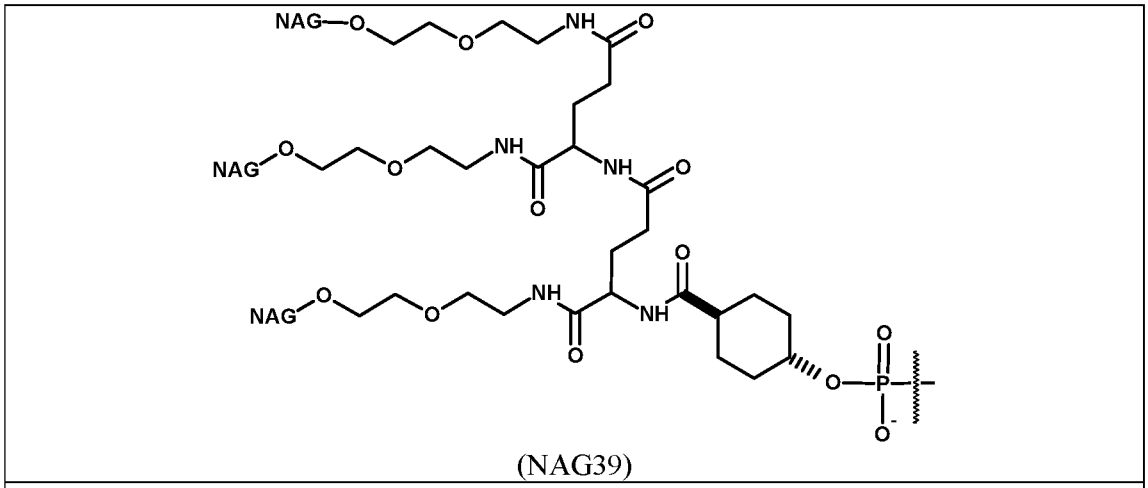
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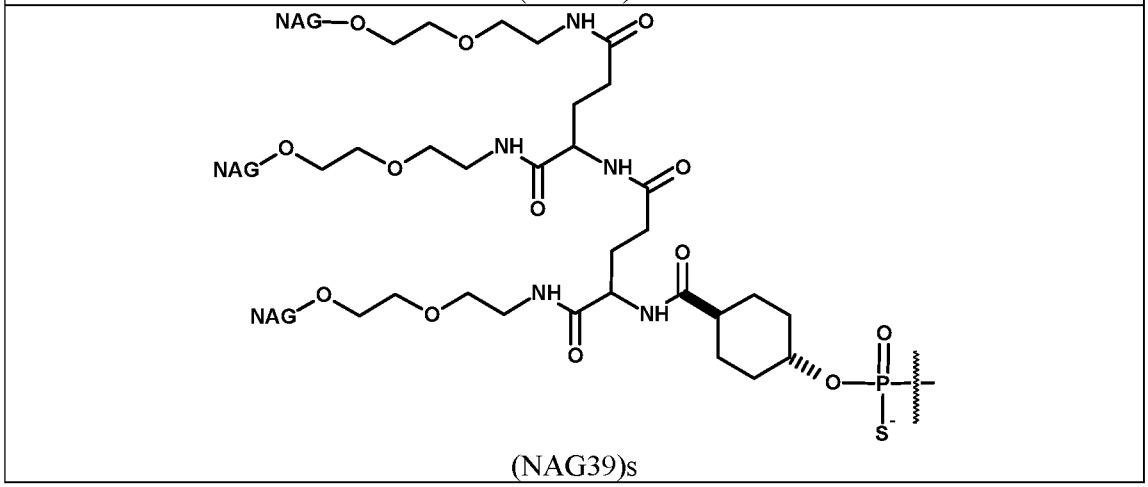
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(NAG 38)s

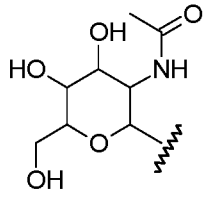


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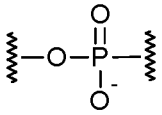
(NAG39)s

In each of the above structures in Table 6, NAG comprises an N-acetyl-galactosamine or another galactose derivative, as would be understood by a person of ordinary skill in the art to be attached in view of the structures above and description provided herein. For example, in some embodiments, NAG in the structures provided in Table 6 is represented by the following structure:

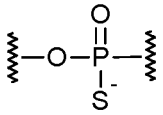


(N-acetyl-galactosamine).

Each (NAGx) may be attached to an ANGPTL3 RNAi agent via a phosphate group (as in (NAG25), (NAG30), and (NAG31)), or a phosphorothioate group, (as is (NAG25)s, (NAG29)s, (NAG30)s, (NAG31)s, or (NAG37)s), or another linking group.



Phosphate group



Phosphorothioate group

Other linking groups known in the art may be used.

In some embodiments, a delivery vehicle can be used to deliver an RNAi agent to a cell or tissue. A delivery vehicle is a compound that improves delivery of the 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, the RNAi agents can be combined with lipids, nanoparticles, polymers, liposomes, micelles, DPCs or other delivery systems available in the art. The RNAi agents can also be chemically conjugated to targeting groups, lipids (including, but not limited to cholesterol and cholesteryl derivatives), nanoparticles, polymers, liposomes, micelles, DPCs (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, WO 2013/032829, WO 2013/158141, each of which is incorporated herein by reference), or other delivery systems available in the art.

Pharmaceutical Compositions and Formulations

The ANGPTL3 RNAi agents disclosed herein can be prepared as pharmaceutical compositions or formulations (also referred to herein as "medicaments"). In some embodiments, pharmaceutical compositions include at least one ANGPTL3 RNAi agent. These pharmaceutical compositions are particularly useful in the inhibition of the expression of the target mRNA in a target cell, a group of cells, a tissue, or an organism. The pharmaceutical compositions can be used to treat a subject having a disease, disorder, or

condition that would benefit from reduction in the level of the target mRNA, or inhibition in expression of the target gene. The pharmaceutical compositions can be used to treat a subject at risk of developing a disease, disorder, or condition that would benefit from reduction of the level of the target mRNA or an inhibition in expression the target gene. In one embodiment, the method includes administering an ANGPTL3 RNAi agent linked to a targeting ligand as described herein, to a subject to be treated. In some embodiments, one or more pharmaceutically acceptable excipients (including vehicles, carriers, diluents, and/or delivery polymers) are added to the pharmaceutical compositions that include an ANGPTL3 RNAi agent, thereby forming a pharmaceutical formulation or medicament suitable for *in vivo* delivery to a subject, including a human.

The pharmaceutical compositions that include an ANGPTL3 RNAi agent and methods disclosed herein decrease the level of the target mRNA in a cell, group of cells, group of cells, tissue, organ, or subject, including by administering to the subject a therapeutically effective amount of a herein described ANGPTL3 RNAi agent, thereby inhibiting the expression of ANGPTL3 mRNA in the subject. In some embodiments, the subject has been previously identified or diagnosed as having a pathogenic upregulation of the target gene in the targeted cell or tissue. In some embodiments, the subject has been previously identified or diagnosed as having elevated triglyceride (TG) and/or elevated cholesterol levels or some other dyslipidemia. In some embodiments, the subject has been previously diagnosed with having one or more cardiometabolic diseases such as hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic-related disorders and diseases. In some embodiments, the subject has been suffering from symptoms associated with one or more cardiometabolic diseases that is associated with or caused by elevated or increased TG levels, elevated or increased cholesterol levels, or hepatic steatosis.

In some embodiments, the described pharmaceutical compositions including an ANGPTL3 RNAi agent are used for treating or managing clinical presentations associated with elevated TG levels, elevated cholesterol levels, hepatic steatosis, and/or over-expression of

ANGPTL3 mRNA in a subject. In some embodiments, a therapeutically (including prophylactically) effective amount of one or more of pharmaceutical compositions is administered to a subject in need of such treatment. In some embodiments, administration of any of the disclosed ANGPTL3 RNAi agents can be used to decrease the number, severity, and/or frequency of symptoms of a disease in a subject.

The described pharmaceutical compositions that include an ANGPTL3 RNAi agent can be used to treat at least one symptom in a subject having a disease or disorder that would benefit from reduction or inhibition in expression of ANGPTL3 mRNA. In some embodiments, the subject is administered a therapeutically effective amount of one or more pharmaceutical compositions that include an ANGPTL3 RNAi agent thereby treating the symptom. In other embodiments, the subject is administered a prophylactically effective amount of one or more ANGPTL3 RNAi agents, thereby preventing or inhibiting the at least one symptom.

The route of administration is the path by which an ANGPTL3 RNAi agent is brought into contact with the body. In general, methods of administering drugs and oligonucleotides and nucleic acids for treatment of a mammal are well known in the art and can be applied to administration of the compositions described herein. The ANGPTL3 RNAi agents disclosed herein can be administered via any suitable route in a preparation appropriately tailored to the particular route. Thus, herein described pharmaceutical compositions can be administered by injection, for example, intravenously, intramuscularly, intracutaneously, subcutaneously, intraarticularly, or intraperitoneally. In some embodiments, the herein described pharmaceutical compositions are administered via subcutaneous injection.

The pharmaceutical compositions including an ANGPTL3 RNAi agent described herein can be delivered to a cell, group of cells, tissue, or subject using oligonucleotide delivery technologies known in the art. In general, any suitable method recognized in the art for delivering a nucleic acid molecule (in vitro or in vivo) can be adapted for use with the compositions described herein. For example, delivery can be by local administration, (e.g., direct injection, implantation, or topical administering), systemic administration, or subcutaneous, intravenous, intraperitoneal, or parenteral routes, including intracranial (e.g., intraventricular, intraparenchymal and intrathecal), intramuscular, transdermal, airway (aerosol), nasal, oral, rectal, or topical (including buccal and sublingual) administration. In

certain embodiments, the compositions are administered by subcutaneous or intravenous infusion or injection.

In some embodiments, the pharmaceutical compositions described herein comprise one or more pharmaceutically acceptable excipients. The pharmaceutical compositions described herein are formulated for administration to a subject.

As used herein, a pharmaceutical composition or medicament includes a pharmacologically effective amount of at least one of the described therapeutic compounds and one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients (excipients) are substances other than the Active Pharmaceutical Ingredient (API, therapeutic product, e.g., ANGPTL3 RNAi agent) that are intentionally included in the drug delivery system. Excipients do not exert or are not intended to exert a therapeutic effect at the intended dosage. Excipients can act to a) aid in processing of the drug delivery system during manufacture, b) protect, support or enhance stability, bioavailability or patient acceptability of the API, c) assist in product identification, and/or d) enhance any other attribute of the overall safety, effectiveness, of delivery of the API during storage or use. A pharmaceutically acceptable excipient may or may not be an inert substance.

Excipients include, but are not limited to: absorption enhancers, anti-adherents, anti-foaming agents, anti-oxidants, binders, buffering agents, carriers, coating agents, colors, delivery enhancers, delivery polymers, detergents, dextran, dextrose, diluents, disintegrants, emulsifiers, extenders, fillers, flavors, glidants, humectants, lubricants, oils, polymers, preservatives, saline, salts, solvents, sugars, surfactants, suspending agents, sustained release matrices, sweeteners, thickening agents, tonicity agents, vehicles, water-repelling agents, and wetting agents.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water-soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor® ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). Suitable carriers should be stable under the conditions of manufacture and storage and should be preserved against the

contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Formulations suitable for intra-articular administration can be in the form of a sterile aqueous preparation of the drug that can be in microcrystalline form, for example, in the form of an aqueous microcrystalline suspension. Liposomal formulations or biodegradable polymer systems can also be used to present the drug for both intra-articular and ophthalmic administration.

The active compounds can be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as

pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

The ANGPTL3 RNAi agents can be formulated in compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

A pharmaceutical composition can contain other additional components commonly found in pharmaceutical compositions. Such additional components include, but are not limited to: anti-pruritics, astringents, local anesthetics, analgesics, antihistamines, or anti-inflammatory agents (e.g., acetaminophen, NSAIDs, diphenhydramine, etc.). It is also envisioned that cells, tissues, or isolated organs that express or comprise the herein defined RNAi agents may be used as "pharmaceutical compositions." As used herein, "pharmacologically effective amount," "therapeutically effective amount," or simply "effective amount" refers to that amount of an RNAi agent to produce a pharmacological, therapeutic, or preventive result.

In some embodiments, the methods disclosed herein further comprise the step of administering a second therapeutic or treatment in addition to administering an RNAi agent disclosed herein. In some embodiments, the second therapeutic is another ANGPTL3 RNAi agent (e.g., an ANGPTL3 RNAi agent that targets a different sequence within the ANGPTL3 target). In other embodiments, the second therapeutic can be a small molecule drug, an antibody, an antibody fragment, or an aptamer.

Generally, an effective amount of an active compound will be in the range of from about 0.1 to about 100 mg/kg of body weight/day, e.g., from about 1.0 to about 50 mg/kg of body weight/day. In some embodiments, an effective amount of an active compound will be in

the range of from about 0.25 to about 5 mg/kg of body weight per dose. In some embodiments, an effective amount of an active ingredient will be in the range of from about 0.5 to about 4 mg/kg of body weight per dose. The amount administered will also 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 be increased beyond the above upper level to rapidly achieve the desired blood-level or tissue level, or the initial dosage can be smaller than the optimum.

For treatment of disease or for formation of a medicament or composition for treatment of a disease, the pharmaceutical compositions described herein including an ANGPTL3 RNAi agent can be combined with an excipient or with a second therapeutic agent or treatment including, but not limited to: a second or other RNAi agent, a small molecule drug, an antibody, an antibody fragment, peptide and/or an aptamer.

The described ANGPTL3 RNAi agents, when added to pharmaceutically acceptable excipients or adjuvants, can be packaged into kits, containers, packs, or dispensers. The pharmaceutical compositions described herein may be packaged in pre-filled syringes or vials.

Methods of Treatment and Inhibition of Expression

The ANGPTL3 RNAi agents disclosed herein can be used to treat a subject (e.g., a human or other mammal) having a disease or disorder that would benefit from administration of the RNAi agent. In some embodiments, the RNAi agents disclosed herein can be used to treat a subject (e.g., a human) that would benefit from reduction and/or inhibition in expression of ANGPTL3 mRNA and/or ANGPTL3 protein levels, for example, a subject that has been diagnosed with hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, type II diabetes mellitus, cardiovascular disease, coronary artery disease, non-alcoholic steatohepatitis, non-alcoholic fatty liver

disease, homozygous and heterozygous familial hypercholesterolemia, statin resistant hypercholesterolemia and other metabolic-related disorders and diseases.

In some embodiments, the subject is administered a therapeutically effective amount of any one or more ANGPTL3 RNAi agents. Treatment of a subject can include therapeutic and/or prophylactic treatment. The subject is administered a therapeutically effective amount of any one or more ANGPTL3 RNAi agents described herein. The subject can be a human, patient, or human patient. The subject may be an adult, adolescent, child, or infant. Administration of a pharmaceutical composition described herein can be to a human being or animal.

The ANGPTL3 RNAi agents described herein can be used to treat at least one symptom in a subject having an ANGPTL3-related disease or disorder, or having a disease or disorder that is mediated at least in part by ANGPTL3 gene expression. In some embodiments, the ANGPTL3 RNAi agents are used to treat or manage a clinical presentation of a subject with an ANGPTL3-related disease or disorder. The subject is administered a therapeutically effective amount of one or more of the ANGPTL3 RNAi agents or ANGPTL3 RNAi agent-containing compositions described herein. In some embodiments, the methods disclosed herein comprise administering a composition comprising an ANGPTL3 RNAi agent described herein to a subject to be treated. In some embodiments, the subject is administered a prophylactically effective amount of any one or more of the described ANGPTL3 RNAi agents, thereby treating the subject by preventing or inhibiting the at least one symptom.

In certain embodiments, the present disclosure provides methods for treatment of diseases, disorders, conditions, or pathological states mediated at least in part by ANGPTL3 gene expression, in a patient in need thereof, wherein the methods include administering to the patient any of the ANGPTL3 RNAi agents described herein.

In some embodiments, the gene expression level and/or mRNA level of an ANGPTL3 gene in a subject to whom a described ANGPTL3 RNAi agent is administered is reduced by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 95%, 96%, 97%, 98%, 99%, or greater than 99% relative to the subject prior to being administered the ANGPTL3 RNAi agent or to a subject not receiving the ANGPTL3 RNAi agent. The gene

expression level and/or mRNA level in the subject may be reduced in a cell, group of cells, and/or tissue of the subject.

In some embodiments, the ANGPTL3 protein level in a subject to whom a described ANGPTL3 RNAi agent has been administered is reduced by at least about 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99% relative to the subject prior to being administered the ANGPTL3 RNAi agent or to a subject not receiving the ANGPTL3 RNAi agent. The protein level in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject.

In some embodiments, the triglyceride (TG) levels in a subject to whom a described ANGPTL3 RNAi agent has been administered is reduced by at least about 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99% relative to the subject prior to being administered the ANGPTL3 RNAi agent or to a subject not receiving the ANGPTL3 RNAi agent. The TG level in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject.

In some embodiments, the total cholesterol levels in a subject to whom a described ANGPTL3 RNAi agent has been administered is reduced by at least about 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99% relative to the subject prior to being administered the ANGPTL3 RNAi agent or to a subject not receiving the ANGPTL3 RNAi agent. In some embodiments, the low-density lipoprotein (LDL) cholesterol levels in a subject to whom a described ANGPTL3 RNAi agent has been administered is reduced by at least about 10%, 20%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or greater than 99% relative to the subject prior to being administered the ANGPTL3 RNAi agent or to a subject not receiving the ANGPTL3 RNAi agent. The total cholesterol levels and/or LDL cholesterol levels in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject.

A reduction in ANGPTL3 mRNA levels, ANGPTL3 protein levels, TG levels, cholesterol levels, and LDL cholesterol levels can be assessed by any methods known in the art. As used herein, a reduction or decrease in ANGPTL3 mRNA level and/or protein level are collectively referred to herein as a reduction or decrease in ANGPTL3 or inhibiting or reducing the expression of ANGPTL3. The Examples set forth herein illustrate known methods for assessing inhibition of ANGPTL3 gene expression.

Cells, Tissues, Organs, and Non-Human Organisms

Cells, tissues, organs, and non-human organisms that include at least one of the ANGPTL3 RNAi agents described herein are contemplated. The cell, tissue, organ, or non-human organism is made by delivering the RNAi agent to the cell, tissue, organ or non-human organism.

The above provided embodiments and items are now illustrated with the following, non-limiting examples.

EXAMPLES

Example 1. Synthesis of ANGPTL3 RNAi Agents.

ANGPTL3 RNAi agent duplexes shown in Table 5, above, were synthesized in accordance with the following general procedures:

A. *Synthesis*. The sense and antisense strands of the RNAi agents were synthesized according to phosphoramidite technology on solid phase used in oligonucleotide synthesis. Depending on the scale, either a MerMade96E® (Bioautomation), a MerMade12® (Bioautomation), or an OP Pilot 100 (GE Healthcare) was used. Syntheses were performed on a solid support made of controlled pore glass (CPG, 500 Å or 600Å, obtained from Prime Synthesis, Aston, PA, USA) All RNA and 2′-modified RNA phosphoramidites were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA) or Hongene Biotech (Shanghai, PRC). The 2′-O-methyl phosphoramidites included the following: (5′-O-dimethoxytrityl-N^6-(benzoyl)-2′-O-methyl-adenosine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, 5′-O-dimethoxy-trityl-N^4-(acetyl)-2′-O-methyl-cytidine-3′-O-(2-cyanoethyl-N,N-diisopropyl-amino) phosphoramidite, (5′-O-

dimethoxytrityl-N^2-(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 amidites. 5′-(4,4′-Dimethoxytrityl)-2′,3′-seco-uridine, 2′-benzoyl-3′-[(2- cyanoethyl)-(N,N- diisopropyl)]-phosphoramidite was also purchased from Thermo Fisher Scientific or Hongene Biotech. 5′-dimethoxytrityl-2′-O-methyl-inosine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from Glen Research (Virginia) or Hongene Biotech. The abasic (3′-O-dimethoxytrityl-2′-deoxyribose-5′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from ChemGenes (Wilmington, MA, USA) or SAFC (St Louis, MO, USA) The 5'-O-dimethoxytrityl-N^2,N^6-(phenoxyacetate)-2'-O-methyl-diaminopurine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite was obtained from ChemGenes or Hongene Biotech.

Targeting ligand containing phosphoramidites were dissolved in anhydrous dichloromethane or anhydrous acetonitrile (50 mM), while all other amidites were dissolved in anhydrous acetonitrile (50 mM), or anhydrous dimethylformamide and molecular sieves (3Å) were added. 5-Benzylthio-1H-tetrazole (BTT, 250 mM in acetonitrile) or 5-Ethylthio-1H-tetrazole (ETT, 250 mM in acetonitrile) was used as activator solution. Coupling times were 12 min (RNA), 15 min (targeting ligand), 90 sec (2′OMe), and 60 sec (2′F). In order to introduce phosphorothioate linkages, a 100 mM solution of 3-phenyl 1,2,4-dithiazoline-5-one (POS, obtained from PolyOrg, Inc., Leominster, MA, USA) in anhydrous Acetonitrile was employed. Unless specifically identified as a "naked" RNAi agent having no targeting ligand present, each of the ANGPTL3 RNAi agent duplexes synthesized and tested in the following Examples utilized N-acetyl-galactosamine as "NAG" in the targeting ligand chemical structures represented in Table 6.

B. Cleavage and deprotection of support bound oligomer. After finalization of the solid phase synthesis, the dried solid support was treated with a 1:1 volume solution of 40 wt. % methylamine in water and 28% ammonium hydroxide solution (Aldrich) for 1.5 hours at 30°C. The solution was evaporated and the solid residue was reconstituted in water (see below).

C. Purification. Crude oligomers were purified by anionic exchange HPLC using a TSKgel SuperQ-5PW 13µm column and Shimadzu LC-8 system. Buffer A was 20 mM Tris, 5 mM EDTA, pH 9.0 and contained 20% Acetonitrile and buffer B was the same as buffer A with the addition of 1.5 M sodium chloride. UV traces at 260 nm were recorded. Appropriate fractions were pooled then run on size exclusion HPLC using a GE Healthcare XK 26/40 column packed with Sephadex G-25 fine with a running buffer of filtered DI water or 100mM ammonium bicarbonate, pH 6.7 and 20% Acetonitrile.

D. Annealing. Complementary strands were mixed by combining equimolar RNA solutions (sense and antisense) in 1×Phosphate-Buffered Saline (Corning, Cellgro) to form the RNAi agents. Some RNAi agents were lyophilized and stored at −15 to −25°C. Duplex concentration was determined by measuring the solution absorbance on a UV-Vis spectrometer in 1× Phosphate-Buffered Saline. The solution absorbance at 260 nm was then multiplied by a conversion factor and the dilution factor to determine the duplex concentration. The conversion factor used was either 0.037 mg/(mL·cm) or was calculated from an experimentally determined extinction coefficient.

Example 2. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the *in vivo* activity of ANGPTL3 RNAi agents that are designed to target different positions on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing either 3 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 7.

Table 7. Dosing Groups of Example 2

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	3.0 mg/kg AD05342	Single injection on day 1
3	3.0 mg/kg AD05343	Single injection on day 1
4	3.0 mg/kg AD05344	Single injection on day 1
5	3.0 mg/kg AD05306	Single injection on day 1

6	3.0 mg/kg AD05307	Single injection on day 1
7	3.0 mg/kg AD05308	Single injection on day 1
8	3.0 mg/kg AD05309	Single injection on day 1
9	3.0 mg/kg AD05310	Single injection on day 1
10	3.0 mg/kg AD05311	Single injection on day 1
11	3.0 mg/kg AD05312	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 8, 13, 22, 29, and day 36 (for Groups 1 and 5-11 only). Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, high-density lipoprotein (HDL), and total cholesterol in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, HDL levels, and total cholesterol levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 8 through 11:

Table 8. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 2

	Day 8		Day 13		Day 22		Day 29		Day 36	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.338	1.000	0.234	1.000	0.341	1.000	0.217	1.000	0.226
Group 2 (3.0 mg/kg AD05342)	0.640	0.072	0.720	0.122	0.811	0.041	0.907	0.020		
Group 3 (3.0 mg/kg AD05343)	0.939	0.238	1.024	0.371	1.146	0.116	0.914	0.120		
Group 4 (3.0 mg/kg AD05344)	0.521	0.040	0.584	0.164	0.731	0.150	0.829	0.139		
Group 5 (3.0 mg/kg AD05306)	0.113	0.019	0.135	0.045	0.154	0.022	0.240	0.054	0.292	0.079
Group 6 (3.0 mg/kg AD05307)	0.117	0.072	0.120	0.069	0.134	0.054	0.206	0.119	0.216	0.086
Group 7 (3.0 mg/kg AD05308)	0.106	0.030	0.076	0.043	0.084	0.048	0.088	0.030	0.167	0.075
Group 8 (3.0 mg/kg AD05309)	0.197	0.055	0.240	0.028	0.206	0.066	0.274	0.112	0.321	0.092
Group 9 (3.0 mg/kg AD05310)	0.196	0.058	0.331	0.118	0.343	0.171	0.589	0.289	0.637	0.273
Group 10 (3.0 mg/kg AD05311)	0.143	0.025	0.206	0.055	0.183	0.019	0.288	0.050	0.389	0.020
Group 11 (3.0 mg/kg AD05312)	0.162	0.073	0.216	0.069	0.229	0.077	0.326	0.079	0.386	0.072

Table 9. Average Triglycerides Normalized to Pre-Treatment and Control from Example 2

	Day 8		Day 13		Day 22		Day 29		Day 36	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.047	1.000	0.016	1.000	0.068	1.000	0.144	1.000	0.206
Group 2 (3.0 mg/kg AD05342)	0.726	0.028	0.870	0.101	0.832	0.119	0.771	0.159	N/A	N/A
Group 3 (3.0 mg/kg AD05343)	0.636	0.186	0.878	0.301	0.591	0.228	0.810	0.146	N/A	N/A
Group 4 (3.0 mg/kg AD05344)	0.709	0.131	0.821	0.094	0.617	0.160	0.783	0.105	N/A	N/A
Group 5 (3.0 mg/kg AD05306)	0.571	0.083	0.752	0.088	0.712	0.167	0.742	0.019	0.768	0.143
Group 6 (3.0 mg/kg AD05307)	0.504	0.084	0.655	0.047	0.459	0.050	0.629	0.122	0.602	0.076
Group 7 (3.0 mg/kg AD05308)	0.375	0.026	0.554	0.040	0.399	0.028	0.473	0.099	0.430	0.045
Group 8 (3.0 mg/kg AD05309)	0.390	0.060	0.604	0.092	0.406	0.047	0.587	0.129	0.581	0.161
Group 9 (3.0 mg/kg AD05310)	0.494	0.142	0.538	0.051	0.443	0.076	0.482	0.029	0.535	0.090
Group 10 (3.0 mg/kg AD05311)	0.402	0.077	0.640	0.095	0.518	0.052	0.614	0.136	0.567	0.092
Group 11 (3.0 mg/kg AD05312)	0.379	0.117	0.539	0.119	0.417	0.044	0.558	0.087	0.483	0.081

Table 10. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 2

	Day 8		Day 13		Day 22		Day 29		Day 36	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.067	1.000	0.067	1.000	0.132	1.000	0.047	1.000	0.019
Group 2 (3.0 mg/kg AD05342)	1.051	0.105	1.111	0.052	0.988	0.117	0.974	0.169	N/A	N/A
Group 3 (3.0 mg/kg AD05343)	1.234	0.104	1.290	0.107	1.222	0.043	1.105	0.106	N/A	N/A
Group 4 (3.0 mg/kg AD05344)	1.062	0.162	1.033	0.115	1.101	0.035	1.017	0.050	N/A	N/A
Group 5 (3.0 mg/kg AD05306)	0.686	0.124	0.733	0..089	0.800	0.081	0.810	0.076	0.938	0.078
Group 6 (3.0 mg/kg AD05307)	0.649	0.160	0.570	0.057	0.628	0.015	0.753	0.099	0.755	0.134
Group 7 (3.0 mg/kg AD05308)	0.671	0.037	0.645	0.129	0.621	0.160	0.669	0.088	0.905	0.171
Group 8 (3.0 mg/kg AD05309)	0.677	0.093	0.817	0.054	0.703	0.011	0.842	0.052	0.951	0.153
Group 9 (3.0 mg/kg AD05310)	0.844	0.149	0.913	0.135	0.911	0.162	0.932	0.102	1.114	0.183
Group 10 (3.0 mg/kg AD05311)	0.659	0.068	0.757	0.124	0.666	0.063	0.778	0.093	0.998	0.160
Group 11 (3.0 mg/kg AD05312)	0.730	0.150	0.826	0.153	0.704	0.062	0.876	0.080	0.978	0.068

Table 11. Average HDL Normalized to Pre-Treatment and Control from Example 2

	Day 8		Day 13		Day 22		Day 29		Day 36	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.069	1.000	0.068	1.000	0.143	1.000	0.056	1.000	0.007
Group 2 (3.0 mg/kg AD05342)	1.063	0.088	1.085	0.045	0.974	0.111	1.004	0.199	N/A	N/A
Group 3 (3.0 mg/kg AD05343)	1.253	0.116	1.250	0.108	1.202	0.039	1.069	0.103	N/A	N/A
Group 4 (3.0 mg/kg AD05344)	1.089	0.141	1.023	0.127	1.101	0.064	1.023	0.032	N/A	N/A
Group 5 (3.0 mg/kg AD05306)	0.654	0.119	0.643	0.063	0.721	0.059	0.756	0.052	0.856	0.091
Group 6 (3.0 mg/kg AD05307)	0.633	0.151	0.524	0.048	0.588	0.023	0.724	0.082	0.702	0.128
Group 7 (3.0 mg/kg AD05308)	0.634	0.035	0.583	0.138	0.572	0.161	0.606	0.086	0.854	0.177
Group 8 (3.0 mg/kg AD05309)	0.665	0.094	0.791	0.059	0.688	0.021	0.826	0.007	0.901	0.115
Group 9 (3.0 mg/kg AD05310)	0.792	0.131	0.867	0.122	0.867	0.161	0.914	0.098	1.045	0.158
Group 10 (3.0 mg/kg AD05311)	0.648	0.063	0.708	0.120	0.621	0.065	0.755	0.086	0.953	0.178
Group 11 (3.0 mg/kg AD05312)	0.671	0.158	0.761	0.159	0.652	0.059	0.849	0.089	0.958	0.048

The ANGPTL3 RNAi agents AD05342 and AD05343 (Groups 2 and 3) included nucleotide sequences that were designed to inhibit expression of an ANGPTL3 gene at position 743 of the gene; ANGPTL3 RNAi agent AD05344 (Group 4) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 744 of the gene; ANGPTL3 RNAi agents AD05306 and AD05307 (Groups 5 and 6) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 921 of the gene; ANGPTL3 RNAi agents AD05308 and AD05309 (Groups 7 and 8) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 922 of the gene; ANGPTL3 RNAi agents AD05310 and AD05311 (Groups 9 and 10) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 1302 of the gene; and ANGPTL3 RNAi agents AD05312 (Group 11) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 1304 of the gene. (*See* SEQ ID NO:1 for the ANGPTL3 gene sequence referenced).

As shown in Table 8, above, each of the RNAi agents in at least Groups 5, 6, 7, 8, 9, 10, and 11 showed ANGPTL3 inhibition. For example, on day 29, Group 7 (ANGPTL3 RNAi agent AD05308), which included nucleotide sequences designed to target position 922 of the ANGPTL3 gene, showed an approximately 91% percent reduction (0.088) in ANGPTL3 protein compared to control. Similarly, both Group 5 (ANGPTL3 RNAi agent AD05306) and Group 6 (ANGPTL3 RNAi agent AD05307), which were designed to target position 921 of the ANGPTL3 gene, showed greater than 75% reduction in ANGPTL3 protein at day 29 (i.e., 0.240 and 0.206).

Example 3. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the *in vivo* activity of ANGPTL3 RNAi agents that are designed to target positions 921 and 922 on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing either 1 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 12.

Table 12. Dosing Groups of Example 3

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	1.0 mg/kg AD05307	Single injection on day 1
3	1.0 mg/kg AD05410	Single injection on day 1
4	1.0 mg/kg AD05411	Single injection on day 1
5	1.0 mg/kg AD05412	Single injection on day 1
6	1.0 mg/kg AD05413	Single injection on day 1
7	1.0 mg/kg AD05414	Single injection on day 1
8	1.0 mg/kg AD05415	Single injection on day 1
9	1.0 mg/kg AD05416	Single injection on day 1
10	1.0 mg/kg AD05417	Single injection on day 1
11	1.0 mg/kg AD05418	Single injection on day 1
12	1.0 mg/kg AD05308	Single injection on day 1
13	1.0 mg/kg AD05419	Single injection on day 1
14	1.0 mg/kg AD05420	Single injection on day 1
15	1.0 mg/kg AD05421	Single injection on day 1
16	1.0 mg/kg AD05422	Single injection on day 1
17	1.0 mg/kg AD05423	Single injection on day 1
18	1.0 mg/kg AD05424	Single injection on day 1
19	1.0 mg/kg AD05425	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 9, 15, 22, and 29. Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, high-density lipoprotein (HDL), and total cholesterol in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, HDL levels, and total cholesterol levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 13 through 16:

Table 13. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 3

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.294	1.000	0.154	1.000	0.167	1.000	0.191
Group 2 (1.0 mg/kg AD05307)	0.323	0.117	0.311	0.098	0.346	0.142	0.486	0.096
Group 3 (1.0 mg/kg AD05410)	0.376	0.042	0.406	0.140	0.515	0.089	0.571	0.085
Group 4 (1.0 mg/kg AD05411)	0.750	0.066	0.822	0.176	0.670	0.091	0.821	0.206
Group 5 (1.0 mg/kg AD05412)	0.289	0.066	0.348	0.073	0.387	0.075	0.564	0.111
Group 6 (1.0 mg/kg AD05413)	0.274	0.054	0.348	0.025	0.373	0.051	0.500	0.102
Group 7 (1.0 mg/kg AD05414)	0.559	0.292	0.608	0.209	0.753	0.275	0.706	0.171
Group 8 (1.0 mg/kg AD05415)	0.291	0.079	0.340	0.065	0.415	0.015	0.498	0.100
Group 9 (1.0 mg/kg AD05416)	0.325	0.141	0.382	0.146	0.417	0.189	0.607	0.128
Group 10 (1.0 mg/kg AD05417)	0.352	0.103	0.287	0.057	0.371	0.053	0.416	0.111
Group 11 (1.0 mg/kg AD05418)	0.236	0.049	0.290	0.084	0.394	0.058	0.517	0.113

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 12 (1.0 mg/kg AD05308)	0.196	0.008	0.202	0.040	0.209	0.027	0.309	0.026
Group 13 (1.0 mg/kg AD05419)	0.268	0.020	0.305	0.101	0.404	0.037	0.361	0.024
Group 14 (1.0 mg/kg AD05420)	0.391	0.106	0.548	0.233	0.564	0.240	0.572	0.167
Group 15 (1.0 mg/kg AD05421)	0.308	0.089	0.407	0.115	0.383	0.058	0.570	0.117
Group 16 (1.0 mg/kg AD05422)	0.463	0.130	0.758	0.205	0.717	0.144	0.921	0.184
Group 17 (1.0 mg/kg AD05423)	0.302	0.055	0.353	0.079	0.331	0.067	0.454	0.137
Group 18 (1.0 mg/kg AD05424)	0.260	0.031	0.308	0.077	0.310	0.053	0.375	0.006
Group 19 (1.0 mg/kg AD05425)	0.319	0.056	0.383	0.071	0.423	0.108	0.518	0.190

Table 14. Average Triglycerides Normalized to Pre-Treatment and Control from Example 3

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.117	1.000	0.122	1.000	0.184	1.000	0.186

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 2 (1.0 mg/kg AD05307)	0.786	0.027	0.692	0.041	0.903	0.153	0.852	0.054
Group 3 (1.0 mg/kg AD05410)	0.873	0.152	1.005	0.133	1.259	0.288	1.046	0.167
Group 4 (1.0 mg/kg AD05411)	1.302	0.214	1.311	0.181	1.687	0.390	1.498	0.174
Group 5 (1.0 mg/kg AD05412)	0.642	0.019	0.617	0.052	1.139	0.601	0.780	0.216
Group 6 (1.0 mg/kg AD05413)	0.720	0.028	0.728	0.097	0.889	0.058	0.925	0.087
Group 7 (1.0 mg/kg AD05414)	0.765	0.160	0.660	0.169	0.905	0.281	0.626	0.096
Group 8 (1.0 mg/kg AD05415)	0.675	0.173	0.780	0.204	0.877	0.196	0.647	0.048
Group 9 (1.0 mg/kg AD05416)	0.785	0.136	0.821	0.100	0.944	0.104	0.787	0.116
Group 10 (1.0 mg/kg AD05417)	0.898	0.054	0.906	0.194	1.121	0.227	0.898	0.259
Group 11 (1.0 mg/kg AD05418)	0.718	0.035	0.791	0.124	0.855	0.090	0.881	0.123
Group 12 (1.0 mg/kg AD05308)	0.952	0.319	0.749	0.262	0.854	0.239	1.009	0.136

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 13 (1.0 mg/kg AD05419)	0.652	0.024	0.786	0.074	0.794	0.092	0.901	0.045
Group 14 (1.0 mg/kg AD05420)	0.644	0.234	0.861	0.244	0.978	0.313	0.836	0.260
Group 15 (1.0 mg/kg AD05421)	0.597	0.253	0.586	0.085	0.796	0.048	0.700	0.088
Group 16 (1.0 mg/kg AD05422)	0.698	0.137	0.594	0.066	0.972	0.129	0.837	0.073
Group 17 (1.0 mg/kg AD05423)	0.811	0.140	0.593	0.076	1.157	0.429	0.848	0.031
Group 18 (1.0 mg/kg AD05424)	0.847	0.219	0.667	0.173	1.171	0.297	0.898	0.264
Group 19 (1.0 mg/kg AD05425)	0.710	0.063	0.517	0.018	0.721	0.149	0.682	0.077

Table 15. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 3

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.060	1.000	0.047	1.000	0.079	1.000	0.115
Group 2 (1.0 mg/kg AD05307)	0.940	0.181	0.840	0.031	0.948	0.086	1.088	0.051

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 3 (1.0 mg/kg AD05410)	0.929	0.071	0.881	0.087	1.022	0.124	0.945	0.134
Group 4 (1.0 mg/kg AD05411)	1.043	0.013	0.905	0.060	1.017	0.056	1.071	0.060
Group 5 (1.0 mg/kg AD05412)	0.886	0.117	0.857	0.022	0.972	0.104	1.063	0.155
Group 6 (1.0 mg/kg AD05413)	0.940	0.095	0.893	0.027	0.982	0.106	0.854	0.065
Group 7 (1.0 mg/kg AD05414)	1.076	0.197	0.919	0.103	1.064	0.093	0.969	0.088
Group 8 (1.0 mg/kg AD05415)	0.897	0.063	0.817	0.059	0.927	0.026	0.986	0.109
Group 9 (1.0 mg/kg AD05416)	0.893	0.104	0.841	0.080	0.950	0.118	0.925	0.116
Group 10 (1.0 mg/kg AD05417)	1.045	0.074	0.838	0.093	1.054	0.082	1.045	0.076
Group 11 (1.0 mg/kg AD05418)	0.783	0.032	0.842	0.096	0.916	0.049	0.953	0.090
Group 12 (1.0 mg/kg AD05308)	0.872	0.071	0.768	0.034	1.048	0.424	0.978	0.088
Group 13 (1.0 mg/kg AD05419)	0.876	0.016	0.848	0.060	1.106	0.102	0.985	0.051

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 14 (1.0 mg/kg AD05420)	1.036	0.183	0.950	0.188	1.014	0.122	1.033	0.125
Group 15 (1.0 mg/kg AD05421)	0.893	0.059	0.914	0.045	1.011	0.019	1.049	0.020
Group 16 (1.0 mg/kg AD05422)	1.034	0.143	1.042	0.096	0.982	0.063	1.130	0.153
Group 17 (1.0 mg/kg AD05423)	1.004	0.055	0.997	0.129	0.973	0.076	0.947	0.087
Group 18 (1.0 mg/kg AD05424)	0.762	0.048	0.776	0.118	0.824	0.080	0.847	0.111
Group 19 (1.0 mg/kg AD05425)	1.032	0.127	0.835	0.061	0.954	0.138	1.045	0.161

Table 16. Average HDL Normalized to Pre-Treatment and Control from Example 3

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.060	1.000	0.043	1.000	0.067	1.000	0.120
Group 2 (1.0 mg/kg AD05307)	0.916	0.181	0.856	0.009	0.913	0.083	1.053	0.026
Group 3 (1.0 mg/kg AD05410)	0.904	0.062	0.878	0.065	1.003	0.121	0.916	0.103

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 4 (1.0 mg/kg AD05411)	1.025	0.011	0.910	0.059	0.997	0.065	1.007	0.025
Group 5 (1.0 mg/kg AD05412)	0.869	0.190	0.875	0.065	0.954	0.128	1.044	0.159
Group 6 (1.0 mg/kg AD05413)	0.935	0.072	0.916	0.023	0.964	0.091	0.867	0.066
Group 7 (1.0 mg/kg AD05414)	1.059	0.163	0.960	0.095	1.079	0.086	0.993	0.083
Group 8 (1.0 mg/kg AD05415)	0.889	0.048	0.832	0.047	0.942	0.053	1.013	0.071
Group 9 (1.0 mg/kg AD05416)	0.848	0.112	0.838	0.077	0.921	0.112	0.928	0.098
Group 10 (1.0 mg/kg AD05417)	0.986	0.074	0.820	0.076	0.996	0.095	1.026	0.089
Group 11 (1.0 mg/kg AD05418)	0.766	0.049	0.823	0.094	0.910	0.064	0.916	0.098
Group 12 (1.0 mg/kg AD05308)	0.838	0.053	0.754	0.019	0.739	0.052	0.953	0.090
Group 13 (1.0 mg/kg AD05419)	0.865	0.022	0.851	0.072	1.093	0.097	0.991	0.061
Group 14 (1.0 mg/kg AD05420)	1.021	0.155	0.967	0.164	1.020	0.111	1.039	0.123

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 15 (1.0 mg/kg AD05421)	0.865	0.052	0.920	0.068	0.972	0.037	1.043	0.054
Group 16 (1.0 mg/kg AD05422)	0.987	0.115	1.032	0.080	0.953	0.052	1.101	0.124
Group 17 (1.0 mg/kg AD05423)	0.968	0.069	0.999	0.126	0.972	0.056	0.945	0.072
Group 18 (1.0 mg/kg AD05424)	0.760	0.077	0.790	0.099	0.831	0.105	0.854	0.101
Group 19 (1.0 mg/kg AD05425)	0.985	0.115	0.840	0.080	0.929	0.145	1.022	0.150

While having different sequences and modification patterns, the ANGPTL3 RNAi agents in Groups 2 through 11 included nucleotide sequences that were each designed to inhibit expression of an ANGPTL3 gene at position 921 of the gene; and the ANGPTL3 RNAi agents in Groups 12 through 19 included nucleotide sequences that were each designed to inhibit expression of an ANGPTL3 gene at position 922 of the gene. (*See* SEQ ID NO:1 for the ANGPTL3 gene sequence referenced). As shown in Table 12 above, each of the RNAi agents achieved inhibition of ANGPTL3 as compared to control. For example, Group 12 (ANGPTL3 RNAi agent AD05308) achieved nearly an 80% reduction in ANGPTL3 protein levels compared in control (0.209) on day 22.

Example 4. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the *in vivo* activity of ANGPTL3 RNAi agents that are designed to target additional positions on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing either 1 mg/kg (mpk) or 0.5 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 17.

Table 17. Dosing Groups of Example 4

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	1.0 mg/kg AD05487	Single injection on day 1
3	1.0 mg/kg AD05488	Single injection on day 1
4	1.0 mg/kg AD05489	Single injection on day 1
5	1.0 mg/kg AD05490	Single injection on day 1
6	1.0 mg/kg AD05491	Single injection on day 1
7	1.0 mg/kg AD05492	Single injection on day 1
8	1.0 mg/kg AD05493	Single injection on day 1
9	1.0 mg/kg AD05494	Single injection on day 1
10	1.0 mg/kg AD05495	Single injection on day 1
11	1.0 mg/kg AD05308	Single injection on day 1

12	0.5 mg/kg AD05308	Single injection on day 1
13	1.0 mg/kg AD05418	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 8, 15, 22, 29, and day 43 (Groups 1-3, 9, and 11-13 only). Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, high-density lipoprotein (HDL), and total cholesterol in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, HDL levels, and total cholesterol levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 18 through 22:

Table 18. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 4

	Day 8		Day 15		Day 22		Day 29		Day 43	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.355	1.000	0.428	1.000	0.431	1.000	0.443	1.000	0.256
Group 2 (1.0 mg/kg AD05487)	0.173	0.022	0.170	0.056	0.138	0.030	0.226	0.034	0.477	0.052
Group 3 (1.0 mg/kg AD05488)	0.090	0.011	0.070	0.023	0.092	0.011	0.124	0.024	0.254	0.074
Group 4 (1.0 mg/kg AD05489)	0.875	0.229	0.933	0.181	0.911	0.288	1.009	0.331		
Group 5 (1.0 mg/kg AD05490)	0.821	0.064	0.826	0.091	0.773	0.216	0.837	0.173		
Group 6 (1.0 mg/kg AD05491)	0.822	0.030	0.739	0.180	0.851	0.128	1.034	0.212		
Group 7 (1.0 mg/kg AD05492)	0.498	0.016	0.575	0.018	0.561	0.025	0.716	0.031		
Group 8 (1.0 mg/kg AD05493)	0.667	0.078	0.774	0.084	0.765	0.129	0.802	0.250		
Group 9 (1.0 mg/kg AD05494)	0.174	0.023	0.192	0.027	0.238	0.044	0.230	0.053	0.467	0.056
Group 10 (1.0 mg/kg AD05495)	0.448	0.263	0.680	0.526	0.721	0.609	0.745	0.319		
Group 11 (1.0 mg/kg AD05308)	0.299	0.103	0.298	0.116	0.252	0.100	0.322	0.097	0.643	0.277

Group 12 (0.5 mg/kg AD05308)	0.340	0.038	0.310	0.030	0.319	0.021	0.345	0.040	0.582	0.020
Group 13 (1.0 mg/kg AD05418)	0.258	0.033	0.270	0.027	0.387	0.064	0.407	0.010	0.847	0.054

Table 19. Average Triglycerides Normalized to Pre-Treatment and Control from Example 4

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 43**	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.085	1.000	0.013	1.000	0.291	1.000	0.131	1.000	0.193
Group 2 (1.0 mg/kg AD05487)	0.776	0.051	0.912	0.117	0.761	0.070	0.556	0.228	0.757	0.119
Group 3 (1.0 mg/kg AD05488)	0.624	0.040	0.795	0.106	0.837	0.047	0.721	0.077	0.901	0.316
Group 4 (1.0 mg/kg AD05489)	0.877	0.065	1.424	0.231	1.189	0.155	1.216	0.030		
Group 5 (1.0 mg/kg AD05490)	0.930	0.196	1.341	0.359	1.385	0.215	0.820	0.424		
Group 6 (1.0 mg/kg AD05491)	1.240	0.334	1.896	0.376	1.590	0.311	1.238	0.071		
Group 7 (1.0 mg/kg AD05492)	0.775	0.086	0.991	0.154	1.085	0.128	1.188	0.129		
Group 8 (1.0 mg/kg AD05493)	1.255	0.320	1.459	0.436	1.326	0.494	1.190	0.281		

Group 9 (1.0 mg/kg AD05494)	0.667	0.054	1.118	0.249	1.341	0.198	0.909	0.183	0.995	0.256
Group 10 (1.0 mg/kg AD05495)	0.864	0.061	1.343	0.211	1.294	0.097	1.116	0.444		
Group 11 (1.0 mg/kg AD05308)	0.749	0.074	1.164	0.037	1.152	0.121	1.021	0.057	0.994	0.072
Group 12 (0.5 mg/kg AD05308)	1.330	0.203	1.598	0.419	1.732	0.342	1.446	0.515	1.547	0.174
Group 13 (1.0 mg/kg AD05418)	0.858	0.068	1.065	0.029	1.079	0.072	0.980	0.037	0.892	0.136

Table 20. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 4

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 43**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.110	1.000	0.159	1.000	0.074	1.000	0.153	1.000	0.122
Group 2 (1.0 mg/kg AD05487)	0.728	0.055	0.944	0.110	0.697	0.050	0.560	0.305	0.923	0.086
Group 3 (1.0 mg/kg AD05488)	0.827	0.096	0.667	0.086	0.759	0.057	0.683	0.077	0.837	0.072
Group 4 (1.0 mg/kg AD05489)	1.139	0.062	1.058	0.110	1.040	0.175	1.068	0.153		
Group 5 (1.0 mg/kg AD05490)	0.986	0.043	1.064	0.025	1.021	0.140	0.779	0.427		

Group 6 (1.0 mg/kg AD05491)	1.047	0.069	1.029	0.072	0.934	0.048	0.960	0.072	
Group 7 (1.0 mg/kg AD05492)	0.957	0.048	0.883	0.059	0.907	0.059	1.001	0.040	
Group 8 (1.0 mg/kg AD05493)	1.060	0.006	0.879	0.066	0.952	0.068	1.011	0.110	
Group 9 (1.0 mg/kg AD05494)	0.823	0.045	0.829	0.075	0.980	0.054	0.793	0.100	0.947	0.051
Group 10 (1.0 mg/kg AD05495)	0.930	0.128	0.919	0.082	1.025	0.167	0.758	0.240		
Group 11 (1.0 mg/kg AD05308)	0.868	0.018	0.793	0.026	0.770	0.081	0.819	0.073	1.073	0.149
Group 12 (0.5 mg/kg AD05308)	0.992	0.061	0.838	0.028	1.000	0.046	0.958	0.059	1.157	0.088
Group 13 (1.0 mg/kg AD05418)	0.818	0.071	0.761	0.087	0.915	0.105	0.853	0.039	1.002	0.076

Table 21. Average HDL Normalized to Pre-Treatment and Control from Example 4

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 43**	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.090	1.000	0.158	1.000	0.087	1.000	0.158	1.000	0.141
Group 2 (1.0 mg/kg AD05487)	0.716	0.074	0.918	0.120	0.658	0.050	0.745	0.065	0.934	0.094

Group 3 (1.0 mg/kg AD05488)	0.839	0.094	0.644	0.061	0.727	0.035	0.771	0.104	0.833	0.079
Group 4 (1.0 mg/kg AD05489)	1.203	0.071	1.068	0.116	1.056	0.195	1.111	0.177		
Group 5 (1.0 mg/kg AD05490)	1.022	0.020	1.043	0.027	0.981	0.109	1.021	0.153		
Group 6 (1.0 mg/kg AD05491)	1.071	0.052	1.015	0.065	0.905	0.058	0.979	0.093		
Group 7 (1.0 mg/kg AD05492)	1.003	0.076	0.920	0.088	0.920	0.092	1.047	0.055		
Group 8 (1.0 mg/kg AD05493)	1.096	0.006	0.882	0.053	0.966	0.061	1.046	0.110		
Group 9 (1.0 mg/kg AD05494)	0.874	0.059	0.782	0.065	0.911	0.045	0.802	0.083	0.978	0.073
Group 10 (1.0 mg/kg AD05495)	0.931	0.124	0.863	0.096	0.953	0.157	0.978	0.091		
Group 11 (1.0 mg/kg AD05308)	0.904	0.026	0.801	0.039	0.728	0.078	0.824	0.082	1.096	0.196
Group 12 (0.5 mg/kg AD05308)	1.007	0.085	0.770	0.021	0.921	0.037	0.934	0.064	1.101	0.106
Group 13 (1.0 mg/kg AD05418)	0.828	0.103	0.730	0.115	0.846	0.104	0.845	0.050	1.018	0.082

Table 22. Average LDL Normalized to Pre-Treatment and Control from Example 4

	Day 8		Day 15		Day 22		Day 29		Day 43	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.166	1.000	0.257	1.000	0.244	1.000	0.122	1.000	0.247
Group 2 (1.0 mg/kg AD05487)	0.916	0.207	1.125	0.079	0.845	0.020	0.744	0.095	0.989	0.031
Group 3 (1.0 mg/kg AD05488)	0.936	0.201	0.714	0.189	0.811	0.105	0.801	0.084	0.902	0.189
Group 4 (1.0 mg/kg AD05489)	1.197	0.191	0.874	0.068	0.985	0.226	0.943	0.132	0.000	0.000
Group 5 (1.0 mg/kg AD05490)	0.937	0.059	1.045	0.183	1.116	0.235	1.135	0.138	0.000	0.000
Group 6 (1.0 mg/kg AD05491)	0.966	0.199	0.867	0.147	0.912	0.202	0.839	0.197	0.000	0.000
Group 7 (1.0 mg/kg AD05492)	0.857	0.135	0.777	0.118	0.747	0.116	0.851	0.157	0.000	0.000
Group 8 (1.0 mg/kg AD05493)	0.946	0.014	0.776	0.112	0.782	0.053	0.927	0.329	0.000	0.000
Group 9 (1.0 mg/kg AD05494)	0.846	0.030	1.129	0.203	1.086	0.054	0.922	0.132	0.845	0.081
Group 10 (1.0 mg/kg AD05495)	1.192	0.150	1.084	0.087	1.232	0.263	0.898	0.114	0.000	0.000
Group 11 (1.0 mg/kg AD05308)	0.842	0.103	0.688	0.072	0.829	0.144	0.897	0.143	1.033	0.164

Group 12 (0.5 mg/kg AD05308)	0.965	0.172	0.830	0.055	1.209	0.146	1.251	0.139	1.375	0.102
Group 13 (1.0 mg/kg AD05418)	1.044	0.035	0.841	0.033	1.142	0.231	0.966	0.080	0.921	0.135

The ANGPTL3 RNAi agents AD05487 and AD05488 (Groups 2 and 3) included nucleotide sequences that were designed to inhibit expression of an ANGPTL3 gene at position 304 of the gene; ANGPTL3 RNAi agent AD05489 and AD05490 (Groups 4 and 5) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 172 of the gene; ANGPTL3 RNAi agents AD05491 and AD05492 (Groups 6 and 7) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 1008 of the gene; ANGPTL3 RNAi agent AD0593 (Group 8) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 1009 of the gene; ANGPTL3 RNAi agents AD05494, AD05495, and AD05308 (Groups 9, 10, 11, and 12) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 1302 of the gene; and ANGPTL3 RNAi agents AD05418 (Group 13) included nucleotide sequences designed to inhibit expression of an ANGPTL3 gene at position 921 of the gene. (*See* SEQ ID NO:1 for the ANGPTL3 gene sequence referenced).

As shown in Table 18 above, while most of the RNAi agents achieved a reduction in ANGPTL3 protein levels at nearly every time point measured, the ANGPTL3 RNAi agents in Group 2 (AD05487) and Group 3 (AD05488), which each included nucleotide sequences designed to inhibit ANGPTL3 gene expression at position 304 of the gene, outperformed the other Groups in this study. For example, at days 15 and 22, ANGPTL3 RNAi agent AD05488 (Group 3) achieved greater than 90% knockdown of ANGPTL3 protein (*i.e.*, 0.070 on day 15 and 0.092 on day 22). Similarly, ANGPTL3 RNAi agent AD05487 (Group 2) achieved nearly 75% knockdown on days 15 and 22 (*i.e.*, 0.170 on day 15 and 0.138 on day 22). Moreover, the same trends were seen across the additional measured parameters including TG, total cholesterol, and LDL, as both Group 2 (AD05487) and Group 3 (AD05488) generally outperformed the other RNAi agents tested (*see* Tables 19-22). For example, For Groups 2 and 3, on day 29, triglyceride levels were reduced by at least 28% (i.e., 0.556 or 0.721), total cholesterol was reduced by at least 31% (i.e., 0.560 or 0.683), and LDL levels were reduced by nearly 20% (0.744 or 0.801).

Example 5. ***In Vivo Testing of ANGPTL3 RNAi Agents in Cynomolgus Monkeys.***

ANGPTL3 RNAi agents were evaluated in cynomolgus monkeys. On day 1, cynomolgus macaque (Macaca fascicularis) primates (also referred to herein as "cynos") were administered a single subcutaneous injection of 0.3 mL/kg (approximately 2-3 mL volume,

depending on animal mass) containing 3.0 mg/kg of ANGPTL3 RNAi agent AD05308 or AD05418, formulated in saline. Each of the ANGPTL3 RNAi agents contained modified nucleotides and included N-acetyl-galactosamine targeting ligands conjugated to the 5′-terminal end of the sense strand, as shown in Tables 3, 4, and 5.

Two (2) cynos in each group were tested (n=2). Blood samples were drawn and serum samples were analyzed on days -37 (pre-dose), -15 (pre-dose), and -1 (pre-dose), 8, 16, 23, 30, and 37. Cynos were fasted overnight prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. The ANGPTL3 protein levels were normalized. For normalization, the level of ANGPTL3 protein for each animal at a time point, was divided by the average pre-treatment level of expression in that animal (in this case at days -37, -15, and -1) to determine the ratio of expression "normalized to pre-treatment."

Data from the study set forth in this Example are shown in the following Tables 23 and 24:

Table 23. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 5 By Group

	Day 8		Day 16		Day 23		Day 30		Day 37	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05308)	0.416	0.183	0.447	0.034	0.649	0.260	0.647	0.106	0.565	0.204
Group 2 (3.0 mg/kg AD05418)	0.544	0.058	0.334	0.035	0.300	0.057	0.389	0.110	0.270	0.082

Table 24. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 5 By Individual Animal

	Day 8		Day 16		Day 23		Day 30		Day 37	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
AD05308 (3.0 mg/kg) (Cyno A)	0.545	0.021	0.423	0.011	0.832	0.014	0.722	0.025	0.709	0.020
AD05308 (3.0 mg/kg) (Cyno B)	0.287	0.004	0.471	0.010	0.465	0.017	0.572	0.025	0.421	0.002
AD05418 (3.0 mg/kg) (Cyno A)	0.585	0.006	0.358	0.008	0.260	0.012	0.311	0.010	0.212	0.001
AD05418 (3.0 mg/kg) (Cyno B)	0.503	0.013	0.309	0.008	0.340	0.021	0.467	0.022	0.328	0.010

Each of the cynomolgus monkeys dosed with either AD05308 or AD05418 showed a reduction in ANGPTL3 protein compared to pre-treatment measurements across all measured time points. For example, for individual animals, on Day 16, the cynos dosed with AD05418 showed a reduction of either approximately 64% (0.358 normalized protein level) or 69% (0.309 normalized protein level) in ANGPTL3 protein. Further, even on day 37, the cynos of Group 2 (AD05418) showed an average reduction of approximately 73% (0.270) in ANGPTL protein levels.

Example 6. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the *in vivo* activity of additional ANGPTL3 RNAi agents that are designed to target position 304 on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing 0.5 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 25.

Table 25. Dosing Groups of Example 6

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	0.5 mg/kg AD05488	Single injection on day 1
3	0.5 mg/kg AD05652	Single injection on day 1
4	0.5 mg/kg AD05653	Single injection on day 1
5	0.5 mg/kg AD05654	Single injection on day 1
6	0.5 mg/kg AD05655	Single injection on day 1
7	0.5 mg/kg AD05656	Single injection on day 1
8	0.5 mg/kg AD05657	Single injection on day 1
9	0.5 mg/kg AD05658	Single injection on day 1
10	0.5 mg/kg AD05660	Single injection on day 1
11	0.5 mg/kg AD05661	Single injection on day 1
12	0.5 mg/kg AD05662	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables

3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 8, 15, 22, 30, and on day 43 for certain groups (i.e., Groups 1, 2, 5, and 10 only). Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables:

Table 26. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 6

	Day 8		Day 15		Day 22		Day 30		Day 43	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.117	1.000	0.134	1.000	0.136	1.000	0.013	1.000	0.100
Group 2 (0.5 mg/kg AD05488)	0.221	0.031	0.272	0.046	0.254	0.022	0.381	0.043	0.475	0.054
Group 3 (0.5 mg/kg AD05652)	0.394	0.130	0.346	0.089	0.333	0.102	0.478	0.047		
Group 4 (0.5 mg/kg AD05653)	0.388	0.056	0.416	0.087	0.378	0.055	0.548	0.055		
Group 5 (0.5 mg/kg AD05654)	0.261	0.061	0.305	0.014	0.308	0.048	0.340	0.012	0.413	0.051
Group 6 (0.5 mg/kg AD05655)	0.301	0.037	0.338	0.027	0.304	0.017	0.403	0.052		
Group 7 (0.5 mg/kg AD05656)	0.402	0.138	0.347	0.074	0.328	0.039	0.445	0.078		
Group 8 (0.5 mg/kg AD05657)	0.341	0.137	0.385	0.052	0.371	0.011	0.425	0.121		
Group 9 (0.5 mg/kg AD05658)	0.427	0.077	0.484	0.093	0.492	0.032	0.477	0.045		
Group 10 (0.5 mg/kg AD05660)	0.342	0.042	0.372	0.047	0.368	0.085	0.404	0.032	0.485	0.081
Group 11 (0.5 mg/kg AD05661)	0.436	0.120	0.322	0.057	0.372	0.028	0.430	0.037		

Group 12 (0.5 mg/kg AD05662)	0.602	0.106	0.609	0.189	0.688	0.294	0.736	0.128	

Table 27. Average Triglycerides Normalized to Pre-Treatment and Control from Example 6

	Day 8		**Day 15**		**Day 22**		**Day 30**		**Day 43**	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.181	1.000	0.078	1.000	0.171	1.000	0.203	1.000	0.065
Group 2 (0.5 mg/kg AD05488)	0.568	0.095	0.637	0.035	0.484	0.052	0.652	0.086	0.689	0.089
Group 3 (0.5 mg/kg AD05652)	0.653	0.102	0.636	0.058	0.582	0.120	0.858	0.182		
Group 4 (0.5 mg/kg AD05653)	0.628	0.205	0.528	0.123	0.469	0.111	0.737	0.036		
Group 5 (0.5 mg/kg AD05654)	0.522	0.054	0.624	0.110	0.536	0.047	0.652	0.060	1.001	0.175
Group 6 (0.5 mg/kg AD05655)	0.512	0.142	0.672	0.162	0.491	0.095	0.785	0.132		
Group 7 (0.5 mg/kg AD05656)	0.633	0.109	0.631	0.044	0.442	0.021	0.657	0.031		
Group 8 (0.5 mg/kg AD05657)	0.579	0.075	0.589	0.024	0.416	0.061	0.670	0.214		
Group 9 (0.5 mg/kg AD05658)	0.529	0.037	0.555	0.074	0.490	0.087	0.720	0.108		
Group 10 (0.5 mg/kg AD05660)	0.567	0.032	0.713	0.083	0.480	0.153	0.644	0.074	1.040	0.228
Group 11 (0.5 mg/kg AD05661)	0.574	0.139	0.697	0.208	0.480	0.112	0.596	0.151		
Group 12 (0.5 mg/kg AD05662)	0.563	0.091	0.755	0.086	0.592	0.078	0.644	0.062		

Table 28. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 6

	Day 8	**Day 15**	**Day 22**	**Day 30**	**Day 43**

Group ID	Avg Total Chol	Std Dev (+/-)	Avg Total Chol	Std Dev (+/-)	Avg Total Chol	Std Dev (+/-)	Avg Total Chol	Std Dev (+/-)	Avg Total Chol	Std Dev (+/-)
Group 1 (D5W)	1.000	0.062	1.000	0.077	1.000	0.081	1.000	0.041	1.000	0.113
Group 2 (0.5 mg/kg AD05488)	0.782	0.086	0.768	0.011	0.801	0.070	0.961	0.114	1.037	0.123
Group 3 (0.5 mg/kg AD05652)	0.829	0.094	0.815	0.078	0.724	0.069	0.835	0.069		
Group 4 (0.5 mg/kg AD05653)	0.907	0.129	0.891	0.120	0.781	0.067	1.024	0.106		
Group 5 (0.5 mg/kg AD05654)	0.752	0.083	0.741	0.035	0.741	0.081	0.788	0.087	0.764	0.076
Group 6 (0.5 mg/kg AD05655)	0.751	0.043	0.815	0.089	0.708	0.048	0.845	0.126		
Group 7 (0.5 mg/kg AD05656)	0.779	0.078	0.696	0.068	0.717	0.097	0.842	0.038		
Group 8 (0.5 mg/kg AD05657)	0.772	0.032	0.736	0.053	0.690	0.021	0.797	0.059		
Group 9 (0.5 mg/kg AD05658)	0.760	0.068	0.784	0.075	0.778	0.037	0.784	0.062		
Group 10 (0.5 mg/kg AD05660)	0.774	0.060	0.824	0.106	0.898	0.108	0.820	0.019	0.928	0.121
Group 11 (0.5 mg/kg AD05661)	0.719	0.076	0.755	0.013	0.784	0.076	0.758	0.061		
Group 12 (0.5 mg/kg AD05662)	0.744	0.024	0.918	0.063	0.864	0.039	0.905	0.046		

Table 29. Average HDL Normalized to Pre-Treatment and Control from Example 6

	Day 8		Day 15		Day 22		Day 30		Day 43	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.072	1.000	0.069	1.000	0.083	1.000	0.040	1.000	0.098
Group 2 (0.5 mg/kg AD05488)	0.783	0.069	0.763	0.020	0.793	0.076	0.956	0.122	1.088	0.131
Group 3 (0.5 mg/kg AD05652)	0.811	0.106	0.778	0.087	0.690	0.098	0.806	0.054		
Group 4 (0.5 mg/kg AD05653)	0.915	0.154	0.898	0.155	0.773	0.087	1.027	0.113		
Group 5 (0.5 mg/kg AD05654)	0.708	0.120	0.725	0.040	0.717	0.105	0.776	0.097	0.731	0.056
Group 6 (0.5 mg/kg AD05655)	0.752	0.045	0.825	0.098	0.708	0.063	0.835	0.119		
Group 7 (0.5 mg/kg AD05656)	0.747	0.078	0.682	0.081	0.726	0.087	0.833	0.050		
Group 8 (0.5 mg/kg AD05657)	0.757	0.034	0.716	0.054	0.695	0.013	0.801	0.065		
Group 9 (0.5 mg/kg AD05658)	0.778	0.084	0.787	0.075	0.807	0.062	0.793	0.069		
Group 10 (0.5 mg/kg AD05660)	0.768	0.056	0.807	0.108	0.912	0.101	0.800	0.008	0.899	0.129
Group 11 (0.5 mg/kg AD05661)	0.739	0.082	0.752	0.014	0.796	0.086	0.782	0.034		
Group 12 (0.5 mg/kg AD05662)	0.740	0.020	0.919	0.096	0.887	0.086	0.955	0.045		

Table 30. Average LDL Normalized to Pre-Treatment and Control from Example 6

	Day 8		Day 15		Day 22		Day 30		Day 43	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.138	1.000	0.136	1.000	0.177	1.000	0.097	1.000	0.120
Group 2 (0.5 mg/kg AD05488)	0.805	0.162	0.880	0.075	0.991	0.139	1.093	0.171	0.970	0.114
Group 3 (0.5 mg/kg AD05652)	0.867	0.093	1.015	0.060	0.878	0.102	0.904	0.175		
Group 4 (0.5 mg/kg AD05653)	1.043	0.198	1.134	0.219	1.034	0.229	1.259	0.233		
Group 5 (0.5 mg/kg AD05654)	1.087	0.094	0.845	0.033	0.893	0.070	1.006	0.136	0.840	0.112
Group 6 (0.5 mg/kg AD05655)	0.877	0.048	0.911	0.081	0.752	0.111	0.976	0.194		
Group 7 (0.5 mg/kg AD05656)	0.818	0.120	0.837	0.052	0.782	0.216	0.963	0.117		
Group 8 (0.5 mg/kg AD05657)	0.888	0.051	0.853	0.049	0.791	0.073	0.903	0.136		
Group 9 (0.5 mg/kg AD05658)	0.715	0.065	0.818	0.070	0.741	0.041	0.764	0.076		
Group 10 (0.5 mg/kg AD05660)	0.795	0.078	0.909	0.094	1.041	0.040	0.998	0.187	0.941	0.078
Group 11 (0.5 mg/kg AD05661)	0.687	0.048	0.855	0.051	0.877	0.079	0.798	0.102		
Group 12 (0.5 mg/kg AD05662)	0.723	0.037	0.908	0.068	0.865	0.125	0.832	0.106		

Each of the ANGPTL3 RNAi agents tested (*i.e.*, AD05488, AD05652, AD05653, AD05654, AD05655, AD05656, AD05657, AD05658, AD05660, AD05661, and AD05662) each included nucleotide sequences designed to target the ANGPTL3 gene at position 304 (*see, e.g.*, SEQ ID NO:1). As shown above, each of the RNAi agents showed a substantial reduction in ANGPTL3 protein levels through at least day 22. Reductions in TG levels and total cholesterol were also observed.

Example 7. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To further assess the *in vivo* activity of additional ANGPTL3 RNAi agents that are designed to target position 304 on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing 0.5 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 31.

Table 31. Dosing Groups of Example 7

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	0.5 mg/kg AD05488	Single injection on day 1
3	0.5 mg/kg AD05774	Single injection on day 1
4	0.5 mg/kg AD05775	Single injection on day 1
5	0.5 mg/kg AD05776	Single injection on day 1
6	0.5 mg/kg AD05777	Single injection on day 1
7	0.5 mg/kg AD05308	Single injection on day 1
8	0.5 mg/kg AD05418	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 8, 15, 22, and 29. Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D

Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 32-36:

Table 32. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 7

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.139	1.000	0.060	1.000	0.414	1.000	0.227
Group 2 (0.5 mg/kg AD05488)	0.339	0.014	0.379	0.098	0.346	0.031	0.469	0.058
Group 3 (0.5 mg/kg AD05774)	0.343	0.075	0.302	0.062	0.287	0.018	0.425	0.060
Group 4 (0.5 mg/kg AD05775)	0.247	0.033	0.232	0.038	0.218	0.008	0.300	0.061
Group 5 (0.5 mg/kg AD05776)	0.327	0.121	0.297	0.099	0.300	0.096	0.378	0.043
Group 6 (0.5 mg/kg AD05777)	0.297	0.056	0.246	0.035	0.257	0.068	0.345	0.035
Group 7 (0.5 mg/kg AD05308)	0.447	0.101	0.388	0.139	0.440	0.092	0.523	0.171
Group 8 (0.5 mg/kg AD05418)	0.534	0.117	0.565	0.077	0.639	0.042	0.758	0.119

Table 33. Average Triglycerides Normalized to Pre-Treatment and Control from Example 7

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.074	1.000	0.116	1.000	0.151	1.000	0.089
Group 2 (0.5 mg/kg AD05488)	0.856	0.223	0.947	0.279	0.922	0.116	0.877	0.400
Group 3 (0.5 mg/kg AD05774)	0.867	0.165	0.641	0.037	0.832	0.177	0.723	0.027
Group 4 (0.5 mg/kg AD05775)	0.837	0.109	0.610	0.107	0.819	0.063	0.885	0.140
Group 5 (0.5 mg/kg AD05776)	0.738	0.130	0717	0.120	0.601	0.105	0.718	0.180

Group 6 (0.5 mg/kg AD05777)	0.755	0.099	0.702	0.001	0.700	0.120	0.648	0.093
Group 7 (0.5 mg/kg AD05308)	0.836	0.343	0.755	0.152	0.839	0.199	0.705	0.219
Group 8 (0.5 mg/kg AD05418)	0.830	0.316	0.655	0.049	0.815	0.184	0.586	0.243

Table 34. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 7

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.058	1.000	0.162	1.000	0.094	1.000	0.032
Group 2 (0.5 mg/kg AD05488)	0.802	0.033	0.810	0.012	0.810	0.043	0.906	0.064
Group 3 (0.5 mg/kg AD05774)	1.001	0.047	0.923	0.070	0.932	0.064	1.231	0.041
Group 4 (0.5 mg/kg AD05775)	0.794	0.041	0.777	0.049	0.812	0.037	1.108	0.216
Group 5 (0.5 mg/kg AD05776)	0.762	0.073	0.745	0.087	0.784	0.075	1.011	0.120
Group 6 (0.5 mg/kg AD05777)	0.797	0.078	0.800	0.019	0.794	0.122	0.995	0.127
Group 7 (0.5 mg/kg AD05308)	0.756	0.037	0.814	0.098	0.896	0.116	0.927	0.096
Group 8 (0.5 mg/kg AD05418)	0.950	0.082	0.878	0.044	0.926	0.012	1.023	0.056

Table 35. Average HDL Normalized to Pre-Treatment and Control from Example 7

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.055	1.000	0.152	1.000	0.088	1.000	0.029

Group 2 (0.5 mg/kg AD05488)	0.783	0.020	0.793	0.009	0.780	0.030	0.886	0.070
Group 3 (0.5 mg/kg AD05774)	0.982	0.038	0.930	0.013	0.901	0.085	1.263	0.021
Group 4 (0.5 mg/kg AD05775)	0.754	0.028	0.757	0.038	0.736	0.017	1.069	0.193
Group 5 (0.5 mg/kg AD05776)	0.760	0.059	0.743	0.062	0.773	0.055	1.022	0.093
Group 6 (0.5 mg/kg AD05777)	0.784	0.085	0.768	0.003	0.801	0.113	1.008	0.117
Group 7 (0.5 mg/kg AD05308)	0.750	0.033	0.791	0.079	0.846	0.115	0.905	0.107
Group 8 (0.5 mg/kg AD05418)	0.907	0.087	0.857	0.035	0.910	0.014	1.048	0.050

Table 36. Average LDL Normalized to Pre-Treatment and Control from Example 7

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.119	1.000	0.088	1.000	0.110	1.000	0.151
Group 2 (0.5 mg/kg AD05488)	0.953	0.186	0.825	0.074	0.860	0.187	1.055	0.095
Group 3 (0.5 mg/kg AD05774)	1.188	0.200	1.101	0.197	1.055	0.043	1.474	0.267
Group 4 (0.5 mg/kg AD05775)	0.975	0.188	0.918	0.135	1.095	0.180	1.534	0.417
Group 5 (0.5 mg/kg AD05776)	0.849	0.143	0.764	0.223	0.861	0.158	1.207	0.269
Group 6 (0.5 mg/kg AD05777)	0.886	0.116	0.869	0.165	0.966	0.383	1.224	0.407
Group 7 (0.5 mg/kg AD05308)	0.710	0.077	0.801	0.105	0.933	0.123	1.047	0.146
Group 8 (0.5 mg/kg AD05418)	1.160	0.143	0.928	0.128	0.950	0.085	1.131	0.150

Example 8. ***In Vivo Testing of ANGPTL3 RNAi Agents in Cynomolgus Monkeys.***

Additional ANGPTL3 RNAi agents were evaluated in cynomolgus monkeys. On day 1, cynomolgus macaque (Macaca fascicularis) primates (also referred to herein as "cynos") were administered a single subcutaneous injection of 0.3 mL/kg (approximately 1-2 mL volume, depending on animal mass) containing 3.0 mg/kg of one of ANGPTL3 RNAi agent AD05577, AD05307, AD05488, AD05654, or AD05659, each formulated in saline. Each of the ANGPTL3 RNAi agents contained modified nucleotides and included N-acetyl-galactosamine targeting ligands conjugated to the 5′-terminal end of the sense strand, as shown in Tables 3, 4, and 5.

Two (2) cynos in each group were tested (n=2). Blood samples were drawn and serum samples were analyzed on days -8 (pre-dose), 1 (pre-dose), 8, 15, 22, 29, and 36. Cynos were fasted overnight prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the average pre-treatment level of expression in that animal (in this case at days -8, and 1 (pre-dose)) to determine the ratio of expression "normalized to pre-treatment."

Data from the study set forth in this Example are shown in the following Tables 37-41:

Table 37. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 8

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05577)	0.503	0.015	0.544	0.037	0.754	0.147	0.586	0.026	0.479	0.054
Group 2 (3.0 mg/kg AD05307)	0.486	0.116	0.402	0.134	0.726	0.306	0.518	0.210	0.392	0.159
Group 3 (3.0 mg/kg AD05488)	0.423	0.071	0.334	0.067	0.343	0.110	0.276	0.069	0.229	0.123
Group 4 (3.0 mg/kg AD05654)	0.366	0.048	0.262	0.055	0.394	0.082	0.202	0.062	0.203	0.024
Group 5 (3.0 mg/kg AD05659)	0.406	0.040	0.434	0.095	0.610	0.033	0.522	0.014	0.349	0.030

Table 38. Average Triglycerides Normalized to Pre-Treatment from Example 8

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05577)	1.159	0.247	0.977	0.076	0.855	0.034	0.886	0.010	0.748	0.014
Group 2 (3.0 mg/kg AD05307)	1.157	0.127	1.058	0.389	0.895	0.118	0.969	0.264	0.806	0.008

Group 3 (3.0 mg/kg AD05488)	0.727	0.158	0.586	0.175	0.399	0.075	0.534	0.157	0.364	0.079
Group 4 (3.0 mg/kg AD05654)	0.949	0.282	0.645	0.293	0.534	0.292	0.542	0.281	0.429	0.231
Group 5 (3.0 mg/kg AD05659)	0.893	0.225	0.670	0.194	0.707	0.092	0.600	0.044	0.635	0.094

Table 39. Average Total Cholesterol Normalized to Pre-Treatment from Example 8

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 36**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05577)	0.885	0.110	0.931	0.123	0.844	0.181	0.839	0.188	0.936	0.195
Group 2 (3.0 mg/kg AD05307)	0.994	0.017	1.006	0.017	0.905	0.021	0.954	0.074	0.909	0.038
Group 3 (3.0 mg/kg AD05488)	0.840	0.020	0.779	0.067	0.743	0.033	0.674	0.004	0.722	0.021
Group 4 (3.0 mg/kg AD05654)	0.912	0.007	0.933	0.004	0.794	0.071	0.806	0.011	0.832	0.042
Group 5 (3.0 mg/kg AD05659)	0.928	0.053	0.841	0.004	0.748	0.020	0.796	0.048	0.797	0.028

Table 40. Average HDL Normalized to Pre-Treatment from Example 8

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05577)	0.855	0.101	0.788	0.096	0.818	0.192	0.857	0.124	0.852	0.119
Group 2 (3.0 mg/kg AD05307)	1.007	0.039	0.946	0.088	0.879	0.022	0.998	0.063	0.863	0.070
Group 3 (3.0 mg/kg AD05488)	0.832	0.006	0.695	0.075	0.688	0.097	0.657	0.032	0.622	0.099
Group 4 (3.0 mg/kg AD05654)	0.865	0.018	0.804	0.017	0.698	0.056	0.760	0.014	0.682	0.015
Group 5 (3.0 mg/kg AD05659)	0.910	0.008	0.865	0.084	0.765	0.073	0.857	0.129	0.761	0.104

Table 41. Average LDL Normalized to Pre-Treatment from Example 8

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (3.0 mg/kg AD05577)	0.951	0.136	1.091	0.150	0.860	0.220	0.991	0.288	0.925	0.258
Group 2 (3.0 mg/kg AD05307)	1.008	0.093	1.102	0.012	0.971	0.113	1.106	0.103	0.894	0.021
Group 3 (3.0 mg/kg AD05488)	0.934	0.051	0.926	0.020	0.891	0.092	0.872	0.085	0.805	0.081
Group 4 (3.0 mg/kg AD05654)	1.015	0.014	1.134	0.026	0.957	0.097	1.022	0.035	0.962	0.068
Group 5 (3.0 mg/kg AD05659)	1.188	0.065	1.085	0.099	0.928	0.045	1.105	0.208	0.952	0.024

Each of the cynomolgus monkeys dosed with any of AD05577, AD05307, AD05488, AD05654, or AD05659 showed a reduction in ANGPTL3 protein compared to pre-treatment measurements across all measured time points.

Example 9. ***In Vivo Testing of ANGPTL3 RNAi Agents in Cynomolgus Monkeys.***

Additional ANGPTL3 RNAi agents were evaluated in cynomolgus monkeys. On day 1, cynomolgus macaque (Macaca fascicularis) primates (also referred to herein as "cynos") were administered a single subcutaneous injection of 0.3 mL/kg (approximately 2-3 mL volume, depending on animal mass) containing 2.0 mg/kg of an ANGPTL3 RNAi agent, which included either AD05488, AD05743, AD05775, or AD05841, each formulated in saline. Each of the ANGPTL3 RNAi agents contained modified nucleotides and included N-acetyl-galactosamine targeting ligands conjugated to the 5′-terminal end of the sense strand, as shown in Tables 3, 4, and 5. ANGPTL3 RNAi agents AD05488, AD05743, and AD05775 included nucleotide sequences designed to target position 304 of the ANGPTL3 gene. ANGPTL3 RNAi agent AD05841 included nucleotide sequences designed to target position 1035 of the ANGPTL3 gene.

Three (3) cynos in each group were tested (n=3). Blood samples were drawn and serum samples were analyzed on days -14 (predose), -7 (predose), 1 (pre-dose), 8, 15, 22, 29, and 35. Cynos were fasted overnight prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the average pre-treatment level of expression in that animal (in this case at days -14, -7, and 1) to determine the ratio of expression "normalized to pre-treatment."

Data from the study set forth in this Example are shown in the following Tables 42-45:

Table 42. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 9

	Day 8		Day 15		Day 22		Day 29		Day 35	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (2.0 mg/kg AD05488)	0.232	0.083	0.240	0.114	0.239	0.087	0.258	0.090	0.332	0.133
Group 2 (2.0 mg/kg AD05743)	0.349	0.029	0.316	0.070	0.322	0.075	0.381	0.068	0.346	0.066
Group 3 (2.0 mg/kg AD05775)	0.463	0.089	0.352	0.053	0.330	0.053	0.365	0.106	0.379	0.111
Group 4 (2.0 mg/kg AD05841)	0.672	0.188	0.646	0.213	0.489	0.196	0.582	0.187	0.460	0.154

Table 43. Average Triglycerides Normalized to Pre-Treatment from Example 9

	Day 8		Day 15		Day 22		Day 29		Day 35	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (2.0 mg/kg AD05488)	0.413	0.081	0.403	0.131	0.288	0.184	0.344	0.254	0.350	0.083
Group 2 (2.0 mg/kg AD05743)	0.646	0.134	0.708	0.373	0.458	0.163	0.479	0.063	0.521	0.101
Group 3 (2.0 mg/kg AD05775)	0.466	0.209	0.427	0.065	0.552	0.254	0.391	0.056	0.431	0.150

Group 4 (2.0 mg/kg AD05841)	0.600	0.160	0.506	0.083	0.579	0.073	0.687	0.182	0.600	0.107

Table 44. Average Total Cholesterol Normalized to Pre-Treatment from Example 8

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 35**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (2.0 mg/kg AD05488)	0.823	0.065	0.744	0.014	0.709	0.037	0.687	0.029	0.659	0.041
Group 2 (2.0 mg/kg AD05743)	0.925	0.050	0.758	0.042	0.768	0.041	0.807	0.093	0.752	0.055
Group 3 (2.0 mg/kg AD05775)	0.965	0.067	0.811	0.058	0.811	0.075	0.813	0.015	0.770	0.022
Group 4 (2.0 mg/kg AD05841)	0.863	0.209	0.844	0.178	0.820	0.141	0.819	0.265	0.798	0.069

Table 45. Average HDL Normalized to Pre-Treatment from Example 9

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 35**	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (2.0 mg/kg AD05488)	0.985	0.354	0.911	0.261	0.849	0.353	0.856	0.333	0.810	0.296
Group 2 (2.0 mg/kg AD05743)	0.849	0.048	0.797	0.058	0.666	0.155	0.757	0.138	0.677	0.135
Group 3 (2.0 mg/kg AD05775)	0.904	0.078	0.871	0.161	0.737	0.085	0.781	0.055	0.723	0.042
Group 4 (2.0 mg/kg AD05841)	0.842	0.268	0.919	0.186	0.876	0.204	0.896	0.247	0.919	0.102

Table 46. Average LDL Normalized to Pre-Treatment from Example 9

Group ID	Day 8		Day 15		Day 22		Day 29		Day 35	
	Avg LDL	Std Dev (+/-)	Avg LDL	Std Dev (+/-)	Avg LDL	Std Dev (+/-)	Avg LDL	Std Dev (+/-)	Avg LDL	Std Dev (+/-)
Group 1 (2.0 mg/kg AD05488)	0.971	0.291	0.913	0.193	0.949	0.216	0.853	0.145	0.845	0.108
Group 2 (2.0 mg/kg AD05743)	1.055	0.061	0.825	0.054	0.986	0.013	0.941	0.124	0.911	0.069
Group 3 (2.0 mg/kg AD05775)	1.134	0.156	0.909	0.111	1.089	0.180	1.034	0.184	1.008	0.143
Group 4 (2.0 mg/kg AD05841)	0.918	0.135	0.953	0.193	0.980	0.069	0.894	0.270	1.002	0.048

Each of the cynomolgus monkeys dosed with any of AD05488, AD05743, AD05775, and AD05841, each at 2.0 mg/kg dosage levels, showed a reduction in ANGPTL3 protein compared to pre-treatment measurements across each of the measured time points.

Example 10. ***Additional In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the *in vivo* activity of further ANGPTL3 RNAi agents that are designed to target position 304 on the ANGPTL3 gene, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing 0.5 mg/kg (mpk) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 47.

Table 47. Dosing Groups of Example 10

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	0.5 mg/kg AD05488	Single injection on day 1
3	0.5 mg/kg AD05790	Single injection on day 1
4	0.5 mg/kg AD05791	Single injection on day 1
5	0.5 mg/kg AD05792	Single injection on day 1
6	0.5 mg/kg AD05793	Single injection on day 1
7	0.5 mg/kg AD05794	Single injection on day 1
8	0.5 mg/kg AD05795	Single injection on day 1
9	0.5 mg/kg AD05796	Single injection on day 1
10	0.5 mg/kg AD05797	Single injection on day 1
11	0.5 mg/kg AD05798	Single injection on day 1
12	0.5 mg/kg AD05799	Single injection on day 1
13	0.5 mg/kg AD05800	Single injection on day 1

Each of the RNAi agents included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). As noted above, each of the ANGPTL3 RNAi agents dosed in this study included nucleotide sequences designed to

target the ANGPTL3 gene at position 304. The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Three (3) mice in each group were tested (n=3). Serum was collected on days 8, 15, 22, 29, and for some groups on day 36 (*i.e.*, Groups 1, 2, and 9-13 only). Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 48-52:

Table 48. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 10

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.128	1.000	0.246	1.000	0.116	1.000	0.202	1.000	0.218
Group 2 (0.5 mg/kg AD05488)	0.179	0.019	0.197	0.010	0.196	0.014	0.258	0.042	0.297	0.039
Group 3 (0.5 mg/kg AD05790)	0.207	0.076	0.144	0.025	0.208	0.017	0.308	0.036		
Group 4 (0.5 mg/kg AD05791)	0.145	0.040	0.170	0.016	0.214	0.076	0.246	0.045		
Group 5 (0.5 mg/kg AD05792)	0.195	0.049	0.192	0.097	0.171	0.046	0.309	0.184		
Group 6 (0.5 mg/kg AD05793)	0.205	0.038	0.156	0.048	0.162	0.011	0.287	0.016		
Group 7 (0.5 mg/kg AD05794)	0.223	0.014	0.217	0.031	0.224	0.048	0.285	0.044		
Group 8 (0.5 mg/kg AD05795)	0.246	0.076	0.343	0.021	0.288	0.042	0.453	0.134		
Group 9 (0.5 mg/kg AD05796)	0.183	0.058	0.213	0.062	0.223	0.047	0.241	0.040	0.315	0.098
Group 10 (0.5 mg/kg AD05797)	0.250	0.098	0.201	0.051	0.238	0.097	0.269	0.027	0.371	0.042
Group 11 (0.5 mg/kg AD05798)	0.175	0.018	0.167	0.015	0.228	0.044	0.233	0.069	0.242	0.033

Group 12 (0.5 mg/kg AD05799)	0.167	0.047	0.150	0.026	0.227	0.032	0.221	0.024	0.231	0.015
Group 13 (0.5 mg/kg AD05800)	0.194	0.013	0.196	0.050	0.214	0.029	0.227	0.053	0.235	0.005

Table 49. Average Triglycerides Normalized to Pre-Treatment and Control from Example 10

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 36**	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.097	1.000	0.099	1.000	0.185	1.000	0.135	1.000	0.174
Group 2 (0.5 mg/kg AD05488)	0.646	0.083	0.669	0.209	0.723	0.227	0.739	0.136	0.843	0.239
Group 3 (0.5 mg/kg AD05790)	0.832	0.086	0.784	0.172	0.974	0.242	0.718	0.085		
Group 4 (0.5 mg/kg AD05791)	1.125	0.266	0.930	0.141	1.631	0.218	0.854	0.179		
Group 5 (0.5 mg/kg AD05792)	0.888	0.196	0.796	0.144	1.094	0.217	0.794	0.026		
Group 6 (0.5 mg/kg AD05793)	1.143	0.137	0.972	0.116	0.998	0.280	1.028	0.408		
Group 7 (0.5 mg/kg AD05794)	0.560	0.083	0.545	0.145	0.713	0.097	0.730	0.175		
Group 8 (0.5 mg/kg AD05795)	0.739	0.072	0.753	0.078	1.034	0.121	0.842	0.065		

Group 9 (0.5 mg/kg AD05796)	0.602	0.075	0.586	0.142	0.832	0.132	0.702	0.085	0.958	0.076
Group 10 (0.5 mg/kg AD05797)	0.851	0.159	0.651	0.075	0.969	0.065	0.713	0.030	0.929	0.186
Group 11 (0.5 mg/kg AD05798)	0.869	0.166	0.586	0.107	0.653	0.059	0.573	0.118	0.690	0.081
Group 12 (0.5 mg/kg AD05799)	0.683	0.092	0.593	0.166	0.751	0.061	0.546	0.075	0.725	0.179
Group 13 (0.5 mg/kg AD05800)	0.676	0.046	0.634	0.048	0.655	0.019	0.635	0.088	1.033	0.068

Table 50. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 10

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 36**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.060	1.000	0.018	1.000	0.104	1.000	0.038	1.000	0.056
Group 2 (0.5 mg/kg AD05488)	0.659	0.023	0.808	0.018	0.763	0.113	0.743	0.028	0.824	0.065
Group 3 (0.5 mg/kg AD05790)	0.698	0.104	0.730	0.026	0.711	0.031	0.757	0.083		
Group 4 (0.5 mg/kg AD05791)	0.664	0.035	0.694	0.062	0.631	0.041	0.677	0.046		
Group 5 (0.5 mg/kg AD05792)	0.716	0.055	0.725	0.081	0.568	0.074	0.727	0.133		

Group 6 (0.5 mg/kg AD05793)	0.813	0.102	0.805	0.091	0.689	0.026	0.769	0.128	
Group 7 (0.5 mg/kg AD05794)	0.715	0.055	0.861	0.031	0.673	0.080	0.768	0.110	
Group 8 (0.5 mg/kg AD05795)	0.852	0.124	0.973	0.187	0.745	0.087	0.866	0.067	
Group 9 (0.5 mg/kg AD05796)	0.666	0.113	0.793	0.047	0.595	0.054	0.735	0.082	0.795	0.125
Group 10 (0.5 mg/kg AD05797)	0.734	0.024	0.734	0.017	0.642	0.026	0.741	0.113	0.861	0.102
Group 11 (0.5 mg/kg AD05798)	0.719	0.031	0.784	0.065	0.711	0.077	0.721	0.086	0.649	0.019
Group 12 (0.5 mg/kg AD05799)	0.700	0.052	0.684	0.014	0.698	0.092	0.632	0.070	0.714	0.040
Group 13 (0.5 mg/kg AD05800)	0.842	0.079	0.794	0.048	0.691	0.071	0.750	0.100	0.853	0.186

Table 51. Average HDL Normalized to Pre-Treatment and Control from Example 10

	Day 8		**Day 15**		**Day 22**		**Day 29**		**Day 36**	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.090	1.000	0.014	1.000	0.123	1.000	0.058	1.000	0.036
Group 2 (0.5 mg/kg AD05488)	0.648	0.029	0.807	0.041	0.733	0.096	0.746	0.012	0.816	0.038

Group 3 (0.5 mg/kg AD05790)	0.674	0.110	0.705	0.040	0.649	0.030	0.748	0.095		
Group 4 (0.5 mg/kg AD05791)	0.632	0.029	0.674	0.063	0.563	0.036	0.679	0.041		
Group 5 (0.5 mg/kg AD05792)	0.676	0.055	0.683	0.086	0.509	0.061	0.712	0.135		
Group 6 (0.5 mg/kg AD05793)	0.721	0.082	0.724	0.079	0.600	0.015	0.696	0.088		
Group 7 (0.5 mg/kg AD05794)	0.652	0.041	0.823	0.047	0.611	0.059	0.736	0.090		
Group 8 (0.5 mg/kg AD05795)	0.757	0.117	0.905	0.189	0.644	0.074	0.817	0.085		
Group 9 (0.5 mg/kg AD05796)	0.610	0.107	0.760	0.062	0.535	0.075	0.696	0.080	0.734	0.117
Group 10 (0.5 mg/kg AD05797)	0.676	0.020	0.688	0.005	0.564	0.037	0.701	0.092	0.790	0.089
Group 11 (0.5 mg/kg AD05798)	0.709	0.030	0.808	0.068	0.719	0.094	0.759	0.117	0.653	0.024
Group 12 (0.5 mg/kg AD05799)	0.666	0.085	0.674	0.039	0.695	0.083	0.667	0.088	0.707	0.033
Group 13 (0.5 mg/kg AD05800)	0.806	0.060	0.796	0.065	0.706	0.075	0.772	0.088	0.801	0.158

Table 52. Average LDL Normalized to Pre-Treatment and Control from Example 10

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.123	1.000	0.172	1.000	0.146	1.000	0.180	1.000	0.202
Group 2 (0.5 mg/kg AD05488)	0.836	0.057	0.969	0.033	0.901	0.129	0.762	0.165	0.863	0.153
Group 3 (0.5 mg/kg AD05790)	0.922	0.174	0.944	0.082	0.916	0.126	0.899	0.153		
Group 4 (0.5 mg/kg AD05791)	0.878	0.119	0.744	0.097	0.848	0.158	0.755	0.145		
Group 5 (0.5 mg/kg AD05792)	0.805	0.060	0.776	0.058	0.623	0.017	0.745	0.082		
Group 6 (0.5 mg/kg AD05793)	0.968	0.181	0.828	0.106	0.768	0.049	0.751	0.200		
Group 7 (0.5 mg/kg AD05794)	0.853	0.057	0.847	0.031	0.677	0.233	0.714	0.156		
Group 8 (0.5 mg/kg AD05795)	1.044	0.073	0.956	0.219	0.805	0.159	0.777	0.040		
Group 9 (0.5 mg/kg AD05796)	0.807	0.143	0.760	0.056	0.606	0.087	0.697	0.055	0.784	0.150
Group 10 (0.5 mg/kg AD05797)	0.786	0.102	0.749	0.013	0.688	0.024	0.689	0.155	0.879	0.225
Group 11 (0.5 mg/kg AD05798)	0.879	0.159	0.896	0.203	0.648	0.039	0.738	0.055	0.762	0.065
Group 12 (0.5 mg/kg AD05799)	0.853	0.104	0.837	0.196	0.678	0.176	0.652	0.138	0.873	0.157
Group 13 (0.5 mg/kg AD05800)	0.951	0.268	0.885	0.041	0.682	0.130	0.826	0.244	1.107	0.298

As indicated in Table 48 above, each of the ANGPTL3 RNAi agents tested showed a significant reduction in ANGPTL3 protein across all time points, and similar trends are seen with respect to reductions in TG levels, total cholesterol levels, and LDL levels.

Example 11. ***In Vivo Testing of ANGPTL3 RNAi Agents in Mice.***

To assess the dose response of ANGPTL3 RNAi agent AD05488, six- to eight-week-old female C57bl/6 mice were used. Pre-dose serum samples were taken at day -1 after a four hour fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl containing the respective mg/kg dose of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or control (D5W) with no RNAi agent, according to the dosing groups recited in Table 53:

Table 53. Dosing Groups of Example 11

Group	RNAi Agent and Dose	Dosing Regimen
1	D5W (no RNAi agent)	Single injection on day 1
2	0.05 mg/kg AD05488	Single injection on day 1
3	0.1 mg/kg AD05488	Single injection on day 1
4	0.5 mg/kg AD05488	Single injection on day 1
5	1.0 mg/kg AD05488	Single injection on day 1
6	2.5 mg/kg AD05488	Single injection on day 1
7	5.0 mg/kg AD05488	Single injection on day 1

The RNAi agent tested (AD05488) included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Four (4) mice in each group were tested (n=4). Serum was collected on days 8, 15, 22, and 29. Mice were fasted for four hours prior to each collection. ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -1) to determine the ratio of expression "normalized to pre-treatment." Expression at a specific time point was then normalized to the D5W control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group. This resulted in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 54-58:

Table 54. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control from Example 11

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.291	1.000	0.170	1.000	0.224	1.000	0.293
Group 2 (0.05 mg/kg AD05488)	0.788	0.110	0.929	0.203	1.106	0.117	0.990	0.175
Group 3 (0.1 mg/kg AD05488)	0.511	0.109	0.757	0.092	0.720	0.069	0.734	0.058
Group 4 (0.5 mg/kg AD05488)	0.207	0.039	0.261	0.050	0.310	0.080	0.349	0.090
Group 5 (1.0 mg/kg AD05488)	0.116	0.038	0.141	0.027	0.171	0.066	0.199	0.054
Group 6 (2.5 mg/kg AD05488)	0.064	0.010	0.047	0.012	0.056	0.009	0.063	0.002
Group 7 (5.0 mg/kg AD05488)	0.018	0.005	0.019	0.004	0.029	0.010	0.031	0.003

Table 55. Average Triglycerides Normalized to Pre-Treatment and Control from Example 11

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.181	1.000	0.120	1.000	0.135	1.000	0.221
Group 2 (0.05 mg/kg AD05488)	1.296	0.168	1.274	0.180	1.202	0.016	1.999	0.356
Group 3 (0.1 mg/kg AD05488)	1.116	0.193	1.141	0.256	1.234	0.184	1.798	0.555
Group 4 (0.5 mg/kg AD05488)	1.028	0.266	1.027	0.283	0.864	0.208	1.855	0.364
Group 5 (1.0 mg/kg AD05488)	0.860	0.186	0.755	0.228	0.720	0.140	1.254	0.195
Group 6 (2.5 mg/kg AD05488)	0.623	0.197	0.556	0.139	0.447	0.075	0.772	0.269

Group 7 (5.0 mg/kg AD05488)	0.923	0.150	0.892	0.105	0.781	0.107	1.128	0.299

Table 56. Average Total Cholesterol Normalized to Pre-Treatment and Control from Example 11

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.061	1.000	0.058	1.000	0.124	1.000	0.069
Group 2 (0.05 mg/kg AD05488)	0.856	0.030	1.090	0.092	0.946	0.081	0.915	0.059
Group 3 (0.1 mg/kg AD05488)	0.820	0.095	0.974	0.097	0.785	0.078	0.945	0.074
Group 4 (0.5 mg/kg AD05488)	0.740	0.061	0.918	0.081	0.897	0.102	0.883	0.071
Group 5 (1.0 mg/kg AD05488)	0.610	0.072	0.816	0.074	0.857	0.099	0.920	0.063
Group 6 (2.5 mg/kg AD05488)	0.647	0.076	0.832	0.119	0.772	0.174	0.694	0.117
Group 7 (5.0 mg/kg AD05488)	0.583	0.086	0.787	0.030	0.790	0.136	0.783	0.176

Table 57. Average HDL Normalized to Pre-Treatment and Control from Example 11

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.054	1.000	0.049	1.000	0.101	1.000	0.070
Group 2 (0.05 mg/kg AD05488)	0.851	0.030	1.052	0.104	0.982	0.083	0.894	0.071
Group 3 (0.1 mg/kg AD05488)	0.807	0.101	0.950	0.077	0.806	0.064	0.910	0.063
Group 4 (0.5 mg/kg AD05488)	0.727	0.066	0.876	0.087	0.906	0.091	0.897	0.084

Group 5 (1.0 mg/kg AD05488)	0.575	0.079	0.785	0.077	0.839	0.113	0.888	0.043
Group 6 (2.5 mg/kg AD05488)	0.618	0.069	0.787	0.109	0.782	0.181	0.701	0.116
Group 7 (5.0 mg/kg AD05488)	0.534	0.082	0.717	0.018	0.760	0.140	0.759	0.156

Table 58. Average LDL Normalized to Pre-Treatment and Control from Example 11

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W)	1.000	0.130	1.000	0.174	1.000	0.216	1.000	0.118
Group 2 (0.05 mg/kg AD05488)	0.798	0.036	1.147	0.107	0.772	0.097	0.822	0.076
Group 3 (0.1 mg/kg AD05488)	0.878	0.160	1.153	0.172	0.732	0.183	0.845	0.202
Group 4 (0.5 mg/kg AD05488)	0.816	0.138	1.137	0.217	0.866	0.011	0.896	0.099
Group 5 (1.0 mg/kg AD05488)	0.760	0.094	1.145	0.101	0.993	0.194	1.123	0.270
Group 6 (2.5 mg/kg AD05488)	0.805	0.101	1.185	0.191	0.915	0.209	0.814	0.049
Group 7 (5.0 mg/kg AD05488)	0.802	0.148	1.170	0.129	0.909	0.076	0.932	0.171

Additionally, ANGPTL3 mRNA levels were also assessed. All of the mice from each respective group were sacrificed on day 29 after serum collection, livers were harvested, and approximately 100 mg liver samples were collected and snap-frozen in liquid nitrogen for RNA isolation. Levels of ANGPTL3 mRNA in the mice livers were then measured by RT-qPCR, the results of which are set forth in the following Table 59:

Table 59. Average ANGPTL3 mRNA Level at Day 29, Normalized to Control from Example 11

	Day 29		
Group ID	**Avg Relative ANGPTL3 mRNA**	**Low Variance (Error)**	**High Variance (Error)**
Group 1 (D5W)	1.000	0.075	0.081
Group 2 (0.05 mg/kg AD05488)	0.798	0.126	0.149
Group 3 (0.1 mg/kg AD05488)	0.563	0.054	0.059
Group 4 (0.5 mg/kg AD05488)	0.277	0.074	0.100
Group 5 (1.0 mg/kg AD05488)	0.123	0.035	0.049
Group 6 (2.5 mg/kg AD05488)	0.036	0.007	0.009
Group 7 (5.0 mg/kg AD05488)	0.038	0.011	0.016

As shown in, among other things, Tables 54 and 59, the administration of ANGPTL3 RNAi agent AD05488 showed a reduction in both ANGPTL3 protein and ANGPTL3 mRNA.

Example 12. ***In Vivo Testing of ANGPTL3 RNAi Agents in LDL Receptor (LDLR) Knockout Mice.***

To evaluate the effect of RNAi agent administration in a disease model, mice having a genetic mutation for the LDL receptor (referred to herein as LDLR KO mice) were commercially obtained (The Jackson Laboratory). The LDLR KO mice are homozygous for the $Ldlr^{tm1Her}$ mutation, and have elevated serum cholesterol levels, particularly when placed on a high fat diet. For three weeks prior to the onset of the study, thirty-nine (39) LDLR KO mice were placed on a high fat diet (Teklad Custom Diets TD.88137). An additional eight (8) LDLR KO mice were placed on a normal chow diet over the same three-week period. Pre-dose serum samples were taken on day -15 and day -1 after a four hour

fast. At day 1, each mouse was given a single subcutaneous administration of 200 µl/30 g animal body weight containing the respective mg/kg dose of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), control (D5W) with no RNAi agent, or a control RNAi agent that included a nucleotide sequence designed to target the Hepatitis B Virus (HBV) genome. A second injection of the same formulation was administered to the animals on day 29. The dosing regimen for the study is recited in the following Table 60:

Table 60. Dosing Groups of Example 12

Group	RNAi Agent and Dose	Diet	Dosing Regimen	Number of LDL KO Mice (n=)
1	D5W (no RNAi agent)	High Fat ("Western") Diet	Injection on day 1, remaining animals received a second injection on day 29	13
2	3.0 mg/kg AD05488	High Fat ("Western") Diet	Injection on day 1, remaining animals received a second injection on day 29	13
3	3.0 mg/kg of a control RNAi agent directed to HBV	High Fat ("Western") Diet	Injection on day 1, remaining animals received a second injection on day 29	13
4	D5W (no RNAi agent)	Normal Chow-Fed Diet	Injection on day 1 and a second single injection on day 29	4
5	3.0 mg/kg AD05488	Normal Chow-Fed Diet	Injection on day 1, and a second injection on day 29	4

Each mouse remained on its respective diet through the duration of study. The RNAi agent tested (AD05488) included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Serum was collected on days 8, 15, 22, 29 (pre-second dose), 36, 43, 50, and 57. LDLR KO mice were fasted for four hours prior to each collection. On day 15, four (4) LDLR KO mice from Groups 1, 2, and 3 (i.e., the groups being administered the high

fat "Western" diet) were sacrificed after serum collection, and on day 29, an additional four (4) LDLR KO mice from Groups 1, 2, and 3 were sacrificed after serum collection, for the purpose of performing mRNA assessments.

ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, respective levels of ANGPTL3 protein, TG, total cholesterol, HDL, or LDL for each animal at a time point was divided by the average pre-treatment level of expression in that animal (in this case the average of day -15 and day -1) to determine the ratio of expression "normalized to pre-treatment."

Expression at a specific time point was then normalized to the D5W control group that was administered the same diet (i.e., either the high fat "Western" diet or the normal chow-fed diet) by dividing the "normalized to pre-treatment" ratio for an individual animal by the mean "normalized to pretreatment" ratio of all mice in the D5W control group on the respective same diet, resulting in expression for each time point normalized to that in the control group.

Data from the study set forth in this Example are shown in the following Tables 61-65:

Table 61. Average ANGPTL3 Protein Normalized to Pre-Treatment and Control (Diet Matched) from Example 12

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (D5W) (High Fat Diet)	1.000	0.130	1.000	0.125	1.000	0.190	1.000	0.127
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.013	0.008	0.009	0.007	0.011	0.006	0.011	0.005
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.958	0.093	1.116	0.126	0.925	0.218	0.962	0.268
Group 4 (D5W) (Normal Diet)	1.000	0.094	1.000	0.061	1.000	0.140	1.000	0.277
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.042	0.013	0.037	0.013	0.043	0.017	0.060	0.026
	Day 36		**Day 43**		**Day 50**		**Day 57**	
Group 1 (D5W) (High Fat Diet)	1.000	0.182	1.000	0.266	1.000	0.174	1.000	0.237
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.005	0.001	0.005	0.002	0.005	0.003	0.007	0.004
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	1.356	0.438	1.371	0.381	1.230	0.331	1.006	0.373
Group 4 (D5W) (Normal Diet)	1.000	0.183	1.000	0.114	1.000	0.047	1.000	0.149
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.024	0.010	0.024	0.008	0.032	0.008	0.037	0.016

Table 62. Average Triglycerides Normalized to Pre-Treatment and Control (Diet Matched) from Example 12

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (D5W) (High Fat Diet)	1.000	0.272	1.000	0.381	1.000	0.276	1.000	0.265
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.121	0.022	0.086	0.027	0.094	0.032	0.096	0.027
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.923	0.345	0.864	0.321	0.735	0.210	0.775	0.174
Group 4 (D5W) (Normal Diet)	1.000	0.172	1.000	0.092	1.000	0.099	1.000	0.138
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.472	0.039	0.503	0.018	0.473	0.086	0.480	0.052
	Day 36		**Day 43**		**Day 50**		**Day 57**	
Group 1 (D5W) (High Fat Diet)	1.000	0.331	1.000	0.363	1.000	0.377	1.000	0.476
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.104	0.031	0.084	0.025	0.091	0.027	0.079	0.025
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.746	0.171	0.520	0.097	0.683	0.104	0.713	0.154
Group 4 (D5W) (Normal Diet)	1.000	0.096	1.000	0.241	1.000	0.043	1.000	0.289
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.548	0.051	0.348	0.053	0.625	0.061	0.438	0.087

Table 63. Average Total Cholesterol Normalized to Pre-Treatment and Control (Diet Matched) from Example 12

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**
Group 1 (D5W) (High Fat Diet)	1.000	0.101	1.000	0.142	1.000	0.187	1.000	0.161
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.524	0.045	0.438	0.034	0.410	0.037	0.410	0.058
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.942	0.125	0.969	0.188	0.980	0.177	1.017	0.198
Group 4 (D5W) (Normal Diet)	1.000	0.072	1.000	0.052	1.000	0.102	1.000	0.088
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.645	0.100	0.683	0.100	0.691	0.122	0.636	0.083
	Day 36		**Day 43**		**Day 50**		**Day 57**	
Group 1 (D5W) (High Fat Diet)	1.000	0.100	1.000	0.158	1.000	0.176	1.000	0.213
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.438	0.042	0.363	0.043	0.374	0.055	0.341	0.058
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	1.029	0.143	0.847	0.105	0.932	0.129	0.860	0.124
Group 4 (D5W) (Normal Diet)	1.000	0.150	1.000	0.180	1.000	0.115	1.000	0.088
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.758	0.114	0.657	0.161	0.824	0.135	0.684	0.096

Table 64. Average HDL Normalized to Pre-Treatment and Control (Diet Matched) from Example 12

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**	**Avg HDL**	**Std Dev (+/-)**
Group 1 (D5W) (High Fat Diet)	1.000	0.061	1.000	0.083	1.000	0.083	1.000	0.072
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.693	0.042	0.735	0.066	0.716	0.062	0.711	0.078
Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.869	0.177	0.870	0.136	0.908	0.122	0.919	0.155
Group 4 (D5W) (Normal Diet)	1.000	0.016	1.000	0.024	1.00	0.053	1.000	0.068
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.645	0.069	0.745	0.088	0.766	0.087	0.737	0.088

Table 65. Average LDL Normalized to Pre-Treatment and Control (Diet Matched) from Example 12

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (D5W) (High Fat Diet)	1.000	0.105	1.000	0.133	1.000	0.195	1.000	0.192
Group 2 (3.0 mg/kg AD05488) (High Fat Diet)	0.547	0.060	0.476	0.041	0.429	0.054	0.419	0.075

Group 3 (3.0 mg/kg HBV Control RNAi agent) (High Fat Diet)	0.959	0.150	1.000	0.207	1.004	0.197	1.063	0.230
Group 4 (D5W) (Normal Diet)	1.000	0.134	1.000	0.088	1.000	0.160	1.000	0.160
Group 5 (3.0 mg/kg AD05488) (Normal Diet)	0.558	0.116	0.606	0.107	0.662	0.147	0.563	0.091

As shown in Tables 61-65, the groups dosed with 3.0 mg/kg of ANGPTL3 RNAi agent AD05488 (i.e., Groups 2 and 5) showed significant reductions in ANGPTL3 protein levels, TG levels, and total cholesterol in this model. The LDLR KO mice on a high fat "Western" diet showed particularly reduced levels, with an approximately 99% reduction in ANGPTL3 protein levels at day 57 (0.007) compared to control from the administration of two 3 mg/kg dose of ANGPTL3 RNAi agent AD05488. It is also noted that Group 3, which included an RNAi agent control that included nucleotide sequences designed to target an HBV mRNA, performed as expected and showed essentially no inhibition of ANGPTL3.

Additionally, ANGPTL3 mRNA levels were also assessed. On day 15, four (4) mice were sacrificed from each of Groups 1, 2, and 3. On day 29, an additional four (4) mice were sacrificed from each of Groups 1, 2, and 3. On day 57, all remaining animals from all Groups were sacrificed. At sacrifice, livers were harvested, and approximately 100 mg liver samples from the median lobes were collected and snap-frozen in liquid nitrogen for RNA isolation. Levels of ANGPTL3 mRNA in the mice livers were then measured by RT-qPCR, and normalized to the mRNA levels of the mice in Group 1 (high fat "Western" diet; D5W administration; day 15 sacrifice), the results of which are set forth in the following Table 66:

Table 66. Average ANGPTL3 mRNA Level at Day 29, Normalized to Control from Example 12

Group ID	Day of Sacrifice	Number of Animals (n=)	Avg Relative ANGPTL3 mRNA	Low Variance (Error)	High Variance (Error)
Group 1 (D5W, high fat diet) (day 15 sacrifice)	15	4	1.000	0.213	0.271
Group 1 (D5W, high fat diet) (day 29 sacrifice)	29	4	1.133	0.074	0.079
Group 1 (D5W, high fat diet) (day 57 sacrifice)	57	5	0.949	0.106	0.119
Group 2 (3.0 mg/kg AD05488, high fat diet) (day 15 sacrifice)	15	4	0.019	0.006	0.009

Group 2 (3.0 mg/kg AD05488, high fat diet) (day 29 sacrifice)	29	4	0.032	0.007	0.009
Group 2B (3.0 mg/kg AD05488, high fat diet) (day 57 sacrifice)	57	5	0.024	0.005	0.006
Group 3 (3.0 mg/kg HBV control RNAi agent, high fat diet) (day 15 sacrifice)	15	4	1.044	0.138	0.159
Group 3 (3.0 mg/kg HBV control RNAi agent, high fat diet) (day 29 sacrifice)	29	4	1.095	0.206	0.254
Group 3 (3.0 mg/kg HBV control RNAi agent, high fat diet) (day 57 sacrifice)	57	5	0.994	0.134	0.155
Group 4 (D5W, normal chow)	57	4	1.397	0.055	0.057
Group 5 (3.0 mg/kg AD05488, normal chow)	57	4	0.060	0.009	0.010

The administration of ANGPTL3 RNAi agent AD05488 showed a significant reduction in ANGPTL3 mRNA levels in both the animals on the high fat "Western" diet and the animals on the normal chow-fed diet.

Example 13. ***In Vivo Testing of ANGPTL3 RNAi Agents in High Fructose Corn Syrup (HFCS) diet-fed Rhesus Monkeys.***

ANGPTL3 RNAi agent AD05488 was further evaluated in high-fructose corn syrup (HFCS) diet-fed Rhesus monkeys. Rhesus monkeys were placed on an HFCS diet 37 days prior to dosing. These animals were known to develop increased plasma triglycerides greater than 180 mg/dL on the HFCS diet. On day 1 and again on day 29, four (4) Rhesus monkeys were administered a subcutaneous injection containing 4.0 mg/kg of ANGPTL3 RNAi agent AD05488 formulated in saline (n=4). Two additional Rhesus monkeys were administered normal saline control. ANGPTL3 RNAi agent AD05488 contained modified nucleotides

and included N-acetyl-galactosamine targeting ligands conjugated to the 5′-terminal end of the sense strand, as shown in Tables 3, 4, and 5.

Fasted blood samples were drawn for analysis, and serum samples were analyzed on days -8 (predose), 8, 15, 21, 29, and 36. ANGPTL3 expression levels, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, the level of ANGPTL3 protein, triglyceride, HDL, and total cholesterol, respectively, for each animal at a time point, was divided by the pre-treatment level of expression in that animal (in this case at day -8) to determine the ratio of expression "normalized to pre-treatment."

Data from the study set forth in this Example are shown in the following Tables 67-71:

Table 67. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 13 (Fasted)

	Day 8		Day 15		Day 21		Day 29		Day 36	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (saline control)	1.087	0.419	1.282	0.436	1.214	0.334	1.363	0.230	1.134	0.248
Group 2 (4.0 mg/kg AD05488)	0.229	0.082	0.154	0.090	0.116	0.080	0.114	0.047	0.064	0.044

Table 68. Average Triglycerides Normalized to Pre-Treatment from Example 13 (Fasted)

	Day 8		Day 15		Day 21		Day 29		Day 36	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (saline control)	0.743	0.055	0.717	0.054	1.017	0.155	0.758	0.263	0.659	0.111
Group 2 (4.0 mg/kg AD05488)	0.351	0.241	0.244	0.094	0.233	0.089	0.302	0.192	0.177	0.076

Table 69. Average Cholesterol Normalized to Pre-Treatment from Example 13 (Fasted)

	Day 8		Day 15		Day 21		Day 29		Day 36	
Group ID	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**

Group 1 (saline control)	0.972	0.050	0.944	0.079	0.957	0.0.18	0.882	0.021	0.894	0.038
Group 2 (4.0 mg/kg AD05488)	0.734	0.200	0.641	0.174	0.579	0.107	0.549	0.090	0.459	0.086

Table 70. Average HDL Normalized to Pre-Treatment from Example 13 (Fasted)

	Day 8		**Day 15**		**Day 21**		**Day 29**		**Day 36**	
Group ID	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**
Group 1 (saline control)	1.082	0.098	1.071	0.111	1.003	0.158	1.025	0.131	1.027	0.071
Group 2 (4.0 mg/kg AD05488)	1.202	0.276	1.091	0.322	0.921	0.296	0.730	0.232	0.798	0.349

Table 71. Average LDL Normalized to Pre-Treatment from Example 13 (Fasted)

	Day 8		**Day 15**		**Day 21**		**Day 29**		**Day 36**	
Group ID	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**	**Avg Chol**	**Std Dev (+/-)**
Group 1 (saline control)	0.892	0.060	0.928	0.046	0.823	0.034	0.804	0.076	0.804	0.172
Group 2 (4.0 mg/kg AD05488)	0.973	0.475	0.909	0.390	0.908	0.437	0.955	0.520	0.710	0.499

The Rhesus monkeys dosed with AD05488 at 4.0 mg/kg dosage levels showed a significant reduction in ANGPTL3 protein compared to pre-treatment measurements across each of the measured time points. Further, reductions in triglyceride and total cholesterol levels were evident.

Example 14. ***In Vivo Testing of ANGPTL3 RNAi Agents and Statins in LDL Receptor (LDLR) Knockout Mice.***

To evaluate the effect of co-administration of RNAi agents and statins in a disease model, LDLR KO mice were commercially obtained (The Jackson Laboratory). For three weeks prior to the onset of the study, forty-one (41) male 7 to 8 week old LDLR KO mice were placed on a high fat ("western") diet (Teklad Custom Diets TD.88137), and remained on that diet throughout the duration of the study. Pre-dose serum samples were taken on study Day 1 after a four hour fast. The dosing regimen for the study is recited in the following Table 72:

Table 72. Dosing Groups of Example 14

Group	Atorvastatin Dose and Dosing Regimen	RNAi Agent and Dose Dosing Regimen	LDL KO Mice (n=)
1	Vehicle oral gavage administered daily starting on Day 1	D5W (no RNAi agent) Injection on Day 23	7
2	N/A	Single 2.5 mg/kg AD05488 Injection on Day 23	7
3	10 mg/kg atorvastatin oral gavage administered daily starting on Day 1	Single 2.5 mg/kg AD05488 Injection on Day 23	8
4	20 mg/kg* atorvastatin oral gavage administered daily starting on Day 1	Single 2.5 mg/kg AD05488 Injection on Day 23	6
5	10 mg/kg atorvastatin oral gavage administered daily starting on Day 1	N/A	7
6	20 mg/kg* atorvastatin oral gavage administered daily starting on Day 1	N/A	6

* Mice were treated at 40 mg/kg for the first 11 days, then switched to 20 mg/kg thereafter.

The vehicle used for the oral gavage in the study was a 1:1 mixture of Ora-Plus®:Ora-Sweet® solution, which were acquired commercially. For the preparation of the atorvastatin oral gavage administrations, the respective desired dose of atorvastatin was first dissolved in sterile water (0.3 mL water per 1 mL of desired formulation) and vortexed until smooth, followed by the addition of a mixture of 1:1 Ora-Plus®:Ora-Sweet® solution (0.7 mL vehicle per 1 mL of desired formulation) and vortexed. On day 1 and for each day thereafter an oral gavage dose was administered for each of the Groups except for Group 2. On Day 23, Groups 1, 2, 3, and 4 received a single subcutaneous administration of 2.5 mg/kg dose (31.25 μg/mL solution) of an ANGPTL3 RNAi agent in D5W (dextrose in 5% water), or vehicle control (D5W) with no RNAi agent.

The RNAi agent tested (AD05488) included a modified sequence and an N-acetyl-galactosamine-containing targeting ligand conjugated to the 5' terminal end of the sense strand. (*See* Tables 3, 4, and 5 for modified sequences and targeting ligand structures). The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Serum was collected on days 8, 15, 22 (pre-RNAi agent injection), 29, 36, 43, and 50. LDLR KO mice were fasted for four hours prior to each collection.

ANGPTL3 protein levels in serum were measured by ELISA assay (R&D Systems), according to the manufacturer's recommendations. Among other biomarkers, triglycerides, total cholesterol, and low-density lipoprotein (LDL) in serum were measured on a Cobas® Integra 400 (Roche Diagnostics), according to the manufacturer's recommendations.

The ANGPTL3 protein levels, triglyceride levels, total cholesterol levels, HDL levels, and LDL levels for each animal were normalized. For normalization, respective levels of ANGPTL3 protein, TG, total cholesterol, or LDL for each animal at a time point was divided by the pre-treatment level of expression in that animal (in this case pre-dose levels on Day 1) to determine the ratio of expression "normalized to pre-treatment." Data from the study set forth in this Example are shown in the following Tables 73-76:

Table 73. Average ANGPTL3 Protein Normalized to Pre-Treatment from Example 14

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**	**Avg ANGPTL3**	**Std Dev (+/-)**
Group 1 (Daily Vehicle Gavage; D5W injection)	1.511	0.236	1.787	0.263	1.680	0.273	1.650	0.237
Group 2 (No gavage; 2.5 mg/kg AD05488)	1.419	0.224	1.584	0.214	1.658	0.295	0.023	0.008
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	1.597	0.344	1.765	0.363	1.681	0.419	0.015	0.005
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	1.683	0.216	1.999	0.282	1.675	0.376	0.022	0.042
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	1.577	0.295	1.839	0.319	1.693	0.270	1.764	0.283
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	1.553	0.673	1.924	0.836	1.871	0.604	1.767	0.770
	Day 36		**Day 43**		**Day 50**			
Group 1 (Daily Vehicle Gavage; D5W injection)	1.604	0.307	1.784	0.460	1.622	0.387		
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.012	0.004	0.020	0.014	0.026	0.005		

Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.005	0.001	0.007	0.003	0.010	0.002	
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.004	0.002	0.005	0.001	0.010	0.004	
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	1.851	0.384	1.758	0.441	1.878	0.342	
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	1.981	0.775	1.935	0.619	1.847	0.610	

Table 74. Average Triglycerides Normalized to Pre-Treatment from Example 14

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**	**Avg TG**	**Std Dev (+/-)**
Group 1 (Daily Vehicle Gavage; D5W injection)	0.711	0.151	0.826	0.193	1.009	0.304	0.753	0.219
Group 2 (No gavage; 2.5 mg/kg AD05488 on day 23)	0.988	0.253	1.247	0.330	1.524	0.189	0.166	0.023
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.613	0.338	0.436	0.213	0.614	0.162	0.072	0.020
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.567	0.233	0.526	0.280	0.748	0.208	0.071	0.021

Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	0.667	0.353	0.601	0.319	1.086	0.546	0.803	0.297
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.477	0.328	0.362	0.200	0.505	0.248	0.552	0.250
	Day 36		**Day 43**		**Day 50**			
Group 1 (Daily Vehicle Gavage; D5W injection)	1.060	0.388	0.986	0.251	0.948	0.239		
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.125	0.037	0.139	0.034	0.153	0.012		
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.058	0.013	0.076	0.019	0.065	0.016		
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.058	0.011	0.089	0.024	0.082	0.016		
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	0.958	0.558	0.862	0.338	0.977	0.634		
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.598	0.260	0.603	0.194	0.677	0.219		

Table 75. Average Total Cholesterol Normalized to Pre-Treatment from Example 14

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**	**Avg Total Chol**	**Std Dev (+/-)**

Group 1 (Daily Vehicle Gavage; D5W injection)	0.996	0.107	1.009	0.192	1.142	0.183	1.087	0.187
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.962	0.109	1.122	0.133	1.261	0.160	0.645	0.092
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.763	0.204	0.620	0.166	0.758	0.108	0.444	0.074
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.682	0.171	0.622	0.200	0.857	0.326	0.448	0.141
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	0.821	0.244	0.774	0.257	0.980	0.359	0.943	0.318
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.586	0.248	0.620	0.190	0.767	0.220	0.761	0.157
	Day 36		**Day 43**		**Day 50**			
Group 1 (Daily Vehicle Gavage; D5W injection)	1.201	0.223	1.237	0.230	1.125	0.309		
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.518	0.085	0.512	0.082	0.515	0.073		
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.362	0.070	0.331	0.054	0.322	0.048		

Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.295	0.045	0.293	0.036	0.310	0.043
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	1.105	0.365	0.998	0.276	0.987	0.376
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.825	0.082	0.767	0.148	0.807	0.114

Table 76. Average LDL Normalized to Pre-Treatment from Example 14

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**	**Avg LDL**	**Std Dev (+/-)**
Group 1 (Daily Vehicle Gavage; D5W injection)	0.951	0.138	0.969	0.238	1.044	0.222	1.093	0.243
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.893	0.107	1.051	0.126	1.113	0.178	0.653	0.119
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.692	0.170	0.537	0.154	0.659	0.112	0.422	0.097
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.572	0.193	0.515	0.222	0.755	0.388	0.434	0.166
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	0.778	0.255	0.695	0.266	0.861	0.365	0.881	0.362

Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.490	0.254	0.556	0.195	0.687	0.209	0.700	0.171
	Day 36		**Day 43**		**Day 50**			
Group 1 (Daily Vehicle Gavage; D5W injection)	1.130	0.237	1.242	0.286	1.050	0.348		
Group 2 (No gavage; 2.5 mg/kg AD05488)	0.492	0.103	0.443	0.166	0.480	0.086		
Group 3 (10 mg/kg daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.312	0.083	0.264	0.055	0.256	0.050		
Group 4 (20 mg/kg* daily oral gavage atorvastatin; 2.5 mg/kg AD05488 on day 23)	0.240	0.060	0.217	0.038	0.242	0.029		
Group 5 (10 mg/kg daily oral gavage atorvastatin; no injection)	0.996	0.357	0.929	0.303	0.872	0.356		
Group 6 (20 mg/kg* daily oral gavage atorvastatin; no injection)	0.716	0.071	0.689	0.153	0.687	0.100		

Mice administered with daily atorvastatin showed approximately 40-60% reduction in triglycerides, approximately 23-40% reduction in total cholesterol, and approximately 30-45% reduction in LDL, respectively. Mice treated with higher doses of atorvastatin typically gave deeper reductions.

Administration with ANGPTL3 RNAi agent AD05488 with the co-administration of atorvastatin (i.e., Groups 3 and 4) showed additive effects on lipid parameters. For example, for Groups that involved co-administration of atorvastatin and RNAi agent, total reductions in triglycerides, total cholesterol, and LDL, were ~95%, ~70%, and ~80%, respectively. Overall lipid parameters profile with the co-administration of atorvastatin was slightly better than administration of ANGPTL3 RNAi agent AD05488 alone. Further, Groups with ANGPTL3 RNAi agent AD05488 showed a clear reduction in ANGPTL3 protein levels, while no reduction in ANGPTL3 protein was seen in groups that did not involve the administration of an ANGPTL3 RNAi agent.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

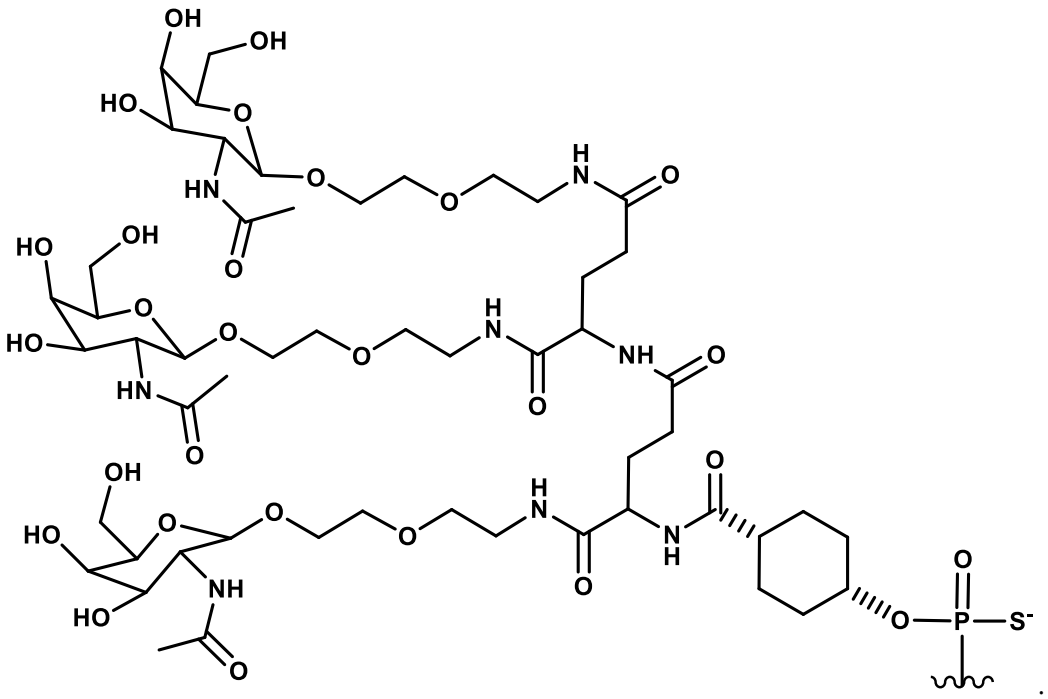
CLAIMS

1. An RNAi agent for inhibiting expression of an ANGPTL3 gene, comprising:

an antisense strand that consists of the modified nucleotide sequence of (5′ → 3′) usAfscsUfgAfuCfaAfaUfaUfgUfuGfaGfsc (SEQ ID NO:2), and

a sense strand that consists of the sequence of (5′ → 3′) (NAG37)s(invAb)sgcucaacaUfAfUfuugaucaguas(invAb) (SEQ ID NO:300);

wherein a is 2′-O-methyl adenosine; c is 2′-O-methyl cytidine; g is 2′-O-methyl guanosine; u is 2′-O-methyl uridine, Af is 2′-fluoro adenosine, Cf is 2′-fluoro cytidine; Gf is 2′-fluoro guanosine; Uf is 2′-fluoro uridine; s is a phosphorothioate linkage; (invAb) is an inverted abasic deoxyribose residue; and (NAG37)s has the following chemical structure:



2. A pharmaceutically acceptable salt of the RNAi agent of claim 1.

3. The pharmaceutically acceptable salt of claim 2, wherein the pharmaceutically acceptable salt is a sodium salt.

4. A pharmaceutical composition comprising the RNAi agent of claim 1 and a pharmaceutically acceptable excipient.

5. The pharmaceutical composition of claim 4, wherein the pharmaceutically acceptable excipient comprises saline.

6. The pharmaceutical composition of claim 4, wherein the pharmaceutically acceptable excipient comprises water for injection.

7. The pharmaceutical composition of claim 6 further comprising a pharmaceutically acceptable buffering agent.

8. The pharmaceutical composition of claim 4, wherein the pharmaceutically acceptable excipient comprises buffered saline.

9. The pharmaceutical composition of claim 4, wherein the pharmaceutical composition is packaged in a pre-filled syringe.

10. The pharmaceutical composition of claim 4, wherein the pharmaceutical composition is packaged in a vial.