RNAi Agents for Dual Inhibition of Expression of Apolipoprotein C-III (APOC3) and Proprotein Convertase Subtilisin Kexin 9 (PCSK9), Compositions Thereof, and Methods of Use

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to United States Provisional Patent Application Serial No. 63/559,682, filed on February 29, 2024, and United States Provisional Patent Application Serial No. 63/685,514, filed on August 21, 2024, the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present disclosure relates to RNA interference (RNAi) agents, e.g., multimeric (for example, "dimer") double stranded RNAi agents such as small interfering RNAs (siRNAs), for dual and concurrent inhibition of Apolipoprotein C-III (APOC3) and Proprotein Convertase Subtilisin Kexin 9 (PCSK9) gene expression, compositions that include APOC3-PCSK9 RNAi agents, and methods of use thereof.

SEQUENCE LISTING

[0003] This application contains a Sequence Listing (in compliance with Standard ST26), which has been submitted in xml format and is hereby incorporated by reference in its entirety. The xml sequence listing file is named 30739-WO_SeqListing.xml, created February 25, 2025, and is 2655 kb in size.

BACKGROUND OF THE INVENTION

[0004] Apolipoprotein C-III, or APOC3, is encoded by the human Apolipoprotein C-III gene and is a promising target for the treatment of diseases associated with hypertriglyceridemia. Elevated serum triglyceride (TG) levels have been identified as an independent risk factor for cardiovascular disease, and as a contributing factor in the development of atherosclerosis. Individuals with severe hypertriglyceridemia (often > 1000 mg/dL) are also at risk of recurrent pancreatitis. Triglycerides are primarily transported in the blood as a major component of very low density lipoprotein (VLDL) and chylomicron particles, which are known as TG-rich lipoproteins. Lipoproteins are composed of a

hydrophobic triacylglycerol and cholesteryl ester core, and a hydrophilic outer layer of phospholipids, cholesterol, and apoproteins. APOC3 is one of these apoproteins. APOC3 is primarily synthesized in the liver (more specifically, in hepatocytes in the liver) and plays an important role in the production, metabolism, and clearance of TG-rich lipoproteins from plasma.

[0005] Certain therapeutics specifically targeting APOC3 for the treatment of diseases associated with hypertriglyceridemia have entered clinical trials. For example, Arrowhead Pharmaceuticals has developed plozasiran, a promising APOC3-targeted RNAi agent, that has shown in clinical trials consistent engagement with durable APOC3 gene silencing, resulting in deep reductions in triglyceride levels of more than 60% while also being generally well-tolerated from a safety perspective. (*See, e.g.*, Arrowhead Pharmaceuticals, Inc. Press Release, *Arrowhead Presents New Phase 2 Data on Plozasiran and Zodasiran at AHA 2023* (November 13, 2023), *available at* https://ir.arrowheadpharma.com/news-releases/news-release-details/arrowhead-presents-new-phase-2-data-plozasiran-and-zodasiran-aha (last visited February 26, 2025)). Currently, there is no regulatory-approved therapy (of any modality) that lowers triglycerides to this extent. Overall, the ongoing clinical trials for plozasiran have indicated a favorable risk/benefit ratio to justify the continued clinical development for at least the treatment of hypertriglyceridemia, chylomicronemia, and mixed dyslipidemia.

[0006] Proprotein convertase subtilisin kexin 9, or PCSK9, has become a therapeutic target for cholesterol-lowering therapy. Due to its key role in plasma cholesterol metabolism, PCSK9 has been identified by scientists in both academia and industry as a target for treating hypercholesterolemia. Low density lipoprotein (LDL) is the major transporter of cholesterol in the bloodstream. LDL-cholesterol (LDL-C) is normally removed from the bloodstream through receptor-mediated endocytosis in the liver via the LDL receptor. Maintaining adequate levels of the LDL receptor is critical to prevent buildup of LDL-C, as an excessively high level of LDL-C in blood (hypercholesterolemia) and tissues is associated with development of atherosclerotic plaques and cardiovascular disease. Mutations in the LDL receptor or mutations that impact binding of LDL to the receptor have been demonstrated to cause hypercholesterolemia. In addition, gain-of-function mutations in PCSK9 have been found to increase LDL levels by promoting degradation of the LDL receptor, and loss-of-function mutations result in hypocholesterolemia and significantly lower risk of coronary heart disease.

[0007] While statins that increase expression of the LDL receptor and antibody treatments that inhibit PCSK9 are already on the market, certain tolerability issues exists with some patients. Further, while various small molecule inhibitors of PCSK9, as well as inhibitors that

block LDL receptor binding, are in clinical development, and a small interfering RNA (siRNA) specific to PCSK9 mRNA has received regulatory approval in the US and Europe, each of these products have certain shortcomings. Given the role of PCSK9 in controlling plasma LDL levels and the severity of disease if hypercholesterolemia remains uncontrolled, further development of additional inhibitory therapeutics could provide needed options to improve the cardiovascular health of patients with elevated LDL-C.

[0008] Importantly, certain diseases, such as Familial Combined Hyperlipidemia (FCH), are characterized by hypercholesterolemia, hypertriglyceridemia, hyperlipoproteinemia, and high rate of coronary artery disease, and is the most common inherited lipid disorder with the prevalence of about 1-2% in general population. For this and similar diseases that have a complexity of disease manifestations involving aspects of both cholesterolemia and triglyceridemia, current treatment relies on polypharmacotherapy, which requires multiple administrations of different drugs (typically with different modalities and mechanisms of action).

[0009] A single or "mono" drug that can effectively treat both hypercholesterolemia and hypertriglyceridemia is highly desirable. While RNAi therapeutics targeting APOC3 and PCSK9 individually have shown great promise for providing a suitable treatment for hypertriglyceridemia and hypercholesterolemia, respectively, in patients, a single RNAi molecule silencing both APOC3 and PCSK9 would be a highly useful therapy for which there exists a need. For example, ensuring that equal amounts of both APOC3-targeted and PCSK9-targeted antisense strand RNAi trigger are administered can promote, among other things, patient compliance over the current polypharmacotherapy regimens. Additionally, because current existing RNAi therapy for PCSK9 and RNAi therapy in development for APOC3 both utilize a single receptor (the asialoglycoprotein receptor (ASGPr)) to internalize in hepatocytes, having a multimeric complex that can internalize two RNAi triggers during one receptor turnover can provide efficiencies in administration.

[0010] However, obtaining suitable delivery of oligonucleotide-based therapeutics such as RNAi agents is and still remains the most pressing challenge to overcome in discovering and identifying viable RNAi therapeutics. While developments over the past few decades have led to an understanding of how to better deliver oligonucleotides to hepatocytes by covalently linking the oligonucleotide payload to a targeting ligand comprised of N-Acetylgalactosamine (NAG or GalNAc), further improvements are needed and desired. Improving delivery can potentially permit less drug to be administered to the patient or subject, which can provide the

benefit of reducing the likelihood of toxicological side-effects and potentially lower the costs of the therapeutic as less material will be required to be manufactured.

[0011] Once such long-proposed concept for potential delivery improvement has been to link two or more RNAi agents together, and to further link the multimeric (e.g., "dimer") complex to a single targeting ligand, thereby forming a multimeric RNAi agent conjugate. (*See, e.g.*, US Patent Application Publication No. 2007/0173473, at Figures 22-24). In theory, this could allow a single delivery targeting ligand to carry double (in the case of two RNAi agents, or an RNAi agent "dimer"), or potentially even more oligonucleotide-based therapeutic cargo payloads per each targeting ligand. However, it has been a long-existing challenge to turn this relatively straightforward concept into a reality that sufficiently delivers an RNAi therapeutic *in vivo* that provides any advantage over monomeric conjugates.

SUMMARY

[0012] Disclosed herein are multimeric RNAi agents for inhibiting expression an Apolipoprotein C-III (APOC3) gene and a Proprotein Convertase Subtilisin Kexin 9 (PCSK9) gene.

[0013] In some embodiments, the RNAi agents are multimeric oligonucleotide-based therapeutics including RNA interference (RNAi) agents (also herein termed RNAi agent, RNAi trigger, or trigger; e.g., double-stranded RNAi agents or small (or short) interfering RNA (siRNAs)), for inhibiting gene expression in hepatic cells *in vivo*. The delivery of multimeric RNAi agents facilitates the selective and efficient inhibition of the expression of genes present in the liver, and specifically the inhibition or silencing of genes expressed in hepatocytes.

[0014] 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.

[0015] Other objects, features, aspects, and advantages of the invention will be apparent from the following detailed description, accompanying figures, and from the claims.

DETAILED DESCRIPTION

[0016] The disclosed RNAi agents, compositions thereof, and methods of use may be understood more readily by reference to the following detailed description, which form a part of this disclosure. It is to be understood that the disclosure is not limited to what is specifically described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting.

[0017] It is to be appreciated that while certain features of the disclosures included herein are, for clarity, described herein in the context of separate embodiments, they may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

Definitions

[0018] As used herein, the terms "oligonucleotide" and "polynucleotide" mean a polymer of linked nucleosides each of which can be independently modified or unmodified.

[0019] As used herein, an "RNAi agent" (also referred to as an "RNAi trigger") means a chemical composition of matter 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. RNAi agents can include one or more modified nucleotides and/or one or more non-phosphodiester linkages.

[0020] As used herein, the term "antisense strand" and "guide strand" are consistent with how those term are used in the art, and can be used to refer to either a first antisense strand or a second antisense strand of a multimeric RNAi agent.

[0021] As used herein, the terms "sense strand" and "passenger strand" are consistent with how those terms are used in the art, and refers to either a first sense strand or a second sense strand of a multimeric RNAi agent.

[0022] As used herein, the term "monomeric RNAi agent" refers to an RNAi agent comprised of one antisense strand and one sense strand. A monomeric RNAi agent is typically designed to have an antisense strand sequence that is designed to inhibit gene expression of a single gene.

[0023] As used herein, the terms "multimeric RNAi agent complex", "multimeric RNAi agents", and "multimeric complex" refer to RNAi agents comprising one or more oligonucleotides that are, independently, at least partially complementary to the mRNA of one or more target genes. For example, multimeric complexes, as described herein, may comprise one or more antisense strands which are hybridized (i.e., have formed base pair hydrogen bonds to form a double helical structure) to a single sense strand. The antisense strands of the multimeric RNAi agent complex may initiate the RNA-induced silencing complex (RISC), to silence expression of the respective targeted gene or genes. In some embodiments, the one or more antisense strands are at least partially complementary to the mRNA of the same gene (e.g., two antisense strands of a single multimeric complex may be at least partially complementary to the mRNA of a single gene at different positions of the gene). In some embodiments, the one or more antisense strands are at least partially complementary to the mRNA of different genes (e.g., two antisense strands of a single multimeric complex may be at least partially complementary to the mRNA of two different genes).

[0024] Exemplary multimeric RNAi complexes can be referred to as "[Gene 1]-[Gene 2] RNAi agents." For example, an "APOC3-PCSK9 RNAi agent" describes a multimeric RNAi complex having an antisense strand for silencing APOC3 gene expression and an antisense strand for silencing PCSK9 gene expression. In some embodiments, a multimeric RNAi agent complex comprising two RNAi agent antisense strands, and can be referred to as a "dimer."

[0025] 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.

[0026] 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.

[0027] 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.

[0028] 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 complex 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.

[0029] 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.

[0030] 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.

[0031] 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.

[0032] 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 a target mRNA.

[0033] As used herein, an "oligonucleotide-based agent" is a nucleotide sequence containing about 10-50 (e.g., 10 to 48, 10 to 46, 10 to 44, 10 to 42, 10 to 40, 10 to 38, 10 to 36, 10 to 34, 10 to 32, 10 to 30, 10 to 28, 10 to 26, 10 to 24, 10 to 22, 10 to 20, 10 to 18, 10 to 16, 10 to 14, 10 to 12, 12 to 50, 12 to 48, 12 to 46, 12 to 44, 12 to 42, 12 to 40, 12 to 38, 12 to 36, 12 to 34, 12 to 32, 12 to 30, 12 to 28, 12 to 26, 12 to 24, 12 to 22, 12 to 20, 12 to 18, 12 to 16, 12 to 14, 14 to 50, 14 to 48, 14 to 46, 14 to 44, 14 to 42, 14 to 40, 14 to 38, 14 to 36, 14 to 34, 14 to 32, 14 to 30, 14 to 28, 14 to 26, 14 to 24, 14 to 22, 14 to 20, 14 to 18, 14 to 16, 16 to 50, 16 to 48, 16 to 46, 16 to 44, 16 to 42, 16 to 40, 16 to 38, 16 to 36, 16 to 34, 16 to 32, 16 to 30, 16 to 28, 16 to 26, 16 to 24, 16 to 22, 16 to 20, 16 to 18, 18 to 50, 18 to 48, 18 to 46, 18 to 44, 18 to 42, 18 to 40, 18 to 38, 18 to 36, 18 to 34, 18 to 32, 18 to 30, 18 to 28, 18 to 26, 18 to 24, 18 to 22, 18 to 20, 20 to 50, 20 to 48, 20 to 46, 20 to 44, 20 to 42, 20 to 40, 20 to 38, 20 to 36, 20 to 34, 20 to 32, 20 to 30, 20 to 28, 20 to 26, 20 to 24, 20 to 22, 22 to 50, 22 to 48, 22 to 46, 22 to 44, 22 to 42, 22 to 40, 22 to 38, 22 to 36, 22 to 34, 22 to 32, 22 to 30, 22 to 28, 22 to 26, 22 to 24, 24 to 50, 24 to 48, 24 to 46, 24 to 44, 24 to 42, 24 to 40, 24 to 38, 24 to 36, 24 to 34, 24 to 32, 24 to 30, 24 to 28, 24 to 26, 26 to 50, 26 to 48, 26 to 46, 26 to 44, 26 to 42, 26 to 40, 26 to 38, 26 to 36, 26 to 34, 26 to 32, 26 to 30, 26 to 28, 28 to 50, 28 to 48, 28 to 46, 28 to 44, 28 to 42, 28 to 40, 28 to 38, 28 to 36, 28 to 34, 28 to 32, to 28 to 30, 30 to 50, 30 to 48, 30 to 46, 30 to 44, 30 to 42, 30 to 40, 30 to 38, 30 to 36, 30 to 34, 30 to 32, 32 to 50, 32 to 48, 32 to 46, 32 to 44, 32 to 42, 32 to 40, 32 to 38, 32 to 36, 32 to 34, 34 to 50, 34 to 48, 34 to 46, 34 to 44, 34 to 42, 34 to 40, 34 to 38, 34 to 36, 36 to 50, 36 to 48, 36 to 46, 36 to 44, 36 to 42, 36 to 40, 36 to 38, 38 to 50, 38 to 48, 38 to 46, 38 to 44, 38 to 42, 38 to 40, 40 to 50, 40 to 48, 40 to 46, 40 to 44, 40 to 42, 42 to 50, 42 to 48, 42 to 46, 42 to 44, 44 to 50, 44 to 48, 44 to 46, 46 to 50, 46 to 48, or 48 to 50) nucleotides or nucleotide base pairs. In some embodiments, an

oligonucleotide-based agent has a nucleobase sequence that is at least partially complementary to a coding sequence in an expressed target nucleic acid or target gene within a cell. In some embodiments, the oligonucleotide-based agent, upon delivery to a cell expressing a gene, are able to inhibit the expression of the underlying gene, and are referred to herein as "expression-inhibiting oligonucleotide-based agents." The gene expression can be inhibited *in vitro* or *in vivo*.

[0034] "Oligonucleotide-based agents" include, but are not limited to: single-stranded oligonucleotides, single-stranded antisense oligonucleotides, short interfering RNAs (siRNAs), double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), ribozymes, interfering RNA molecules, and dicer substrates. In some embodiments, an oligonucleotide-based agent is a single-stranded oligonucleotide, such as an antisense oligonucleotide. In some embodiments, an oligonucleotide-based agent is a double-stranded oligonucleotide. In some embodiments, an oligonucleotide-based agent is a double-stranded oligonucleotide that is an RNAi agent.

[0035] 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.

[0036] 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.

[0037] 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.

[0038] 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."

[0039] 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.

[0040] 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.

[0041] 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.

[0042] As used herein, the term "linked" or "conjugated" when referring to the connection between two compounds or molecules means that two molecules are joined by a covalent bond or are associated via noncovalent bonds (e.g., hydrogen bonds or ionic bonds). In some examples, where the term "linked" or "conjugated" refers to the association between two molecules via noncovalent bonds, the association between the two different molecules has a K_D of less than 1×10^{-4} M (e.g., less than 1×10^{-5} M, less than 1×10^{-6} M, or less than 1×10^{-7} M) in physiologically acceptable buffer (e.g., buffered saline). 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.

[0043] As used herein, a linking group is one or more atoms that connects one molecule or portion of a molecule to another to second molecule or second portion of a molecule. Similarly, as used in the art, the term scaffold is sometimes used interchangeably with a linking group. Linking groups may comprise any number of atoms or functional groups. In some embodiments, linking groups may not facilitate any biological or pharmaceutical response, and merely serve to link two biologically active molecules.

[0044] As used herein, a "metabolically stabilized carbohydrate ligand" is a carbohydrate ligand suitable for binding to the asialoglycoprotein receptor that is abundantly expressed on hepatocytes, wherein the carbohydrate ligand has been chemically modified to provide for a more stable chemical composition in serum. Suitable tests to determine whether such a compound is more metabolically stabilized and can still retain the ability to deliver cargo molecules such as RNAi agents to hepatocytes can be readily determined by persons of skill in the art. In some embodiments, metabolically stabile carbohydrate ligands comprise a sugar moiety. In some embodiments, the sugar moiety is selected from the group consisting of glucose, galactose, and N-Acetylgalactosamine. Non-limiting examples of a metabolically stabilized carbohydrate ligands are the metabolically stabilized N-Acetylgalactosamine ligands of Formula I and Formula II disclosed herein. In some embodiments, the metabolically stabilized carbohydrate ligand is chemically modified at the atom adjacent to the anomeric carbon. In some embodiments, the atom adjacent to the anomeric carbon is a second carbon atom, which may be a methylene (-CH2-) moiety. In other embodiments, the atom adjacent to the anomeric carbon is a sulfur (-S-) atom.

[0045] Unless stated otherwise, 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.

[0046] 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.

[0047] 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.

Modified Nucleotides

[0048] In some embodiments, an 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. 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 RNAi agent or even in a single nucleotide thereof. The 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.

[0049] 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.

[0050] 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

[0051] In some embodiments, one or more nucleotides of an 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.

[0052] In some embodiments, a sense strand of an RNAi agent can contain 1, 2, 3, 4, 5, or 6 phosphorothioate linkages, an antisense strand of an 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 RNAi agent can contain 1, 2, 3, or 4 phosphorothioate linkages, an antisense strand of an 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.

[0053] In some embodiments, an 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 does 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.

[0054] In some embodiments, an 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 17-19, 18-20, 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 RNAi agent contains at least three or four phosphorothioate internucleoside linkages in the antisense strand.

[0055] In some embodiments, an 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.

APOC3-PCSK9 RNAi Agents

[0056] The APOC3-PCSK9 RNAi agents disclosed herein are designed to target specific positions on an APOC3 or a PCSK9 gene (e.g., SEQ ID NOs:1 and 2, respectively).

NM_000040.1 *Homo sapiens* apolipoprotein C3 (APOC3), mRNA transcript (SEQ ID NO: 1), 533 bases:

1 tgctcagttc atccctagag gcagctgctc caggaacaga ggtgccatgc agccccgggt
61 actccttgtt gttgccctcc tggcgctcct ggcctctgcc cgagcttcag aggccgagga
121 tgcctccctt ctcagcttca tgcagggtta catgaagcac gccaccaaga ccgccaagga
181 tgcactgagc agcgtgcagg agtcccaggt ggcccagcag gccaggggct gggtgaccga
241 tggcttcagt tccctgaaag actactggag caccgttaag gacaagttct ctgagttctg
301 ggatttggac cctgaggtca gaccaacttc agccgtggct gcctgagacc tcaatacccc
361 aagtccacct gcctatccat cctgcgagct ccttgggtcc tgcaatctcc agggctgccc
421 ctgtaggttg cttaaaaggg acagtattct cagtgctctc ctaccccacc tcatgcctgg
481 ccccccctcca ggcatgctgg cctcccaata aagctggaca agaagctgct atg

NM_174936.4, *Homo sapiens*, proprotein convertase subtilisin/kexin type 9 (PCSK9), mRNA transcript (SEQ ID NO: 2), 3637 bases:

1 agcgacgtcg aggcgctcat ggttgcaggc gggcgccgcc gttcagttca gggtctgagc
61 ctggaggagt gagccaggca gtgagactgg ctcgggcggg ccgggacgcg tcgttgcagc
121 agcggctccc agctcccagc caggattccg cgcgcccctt cacgcgccct gctcctgaac
181 ttcagctcct gcacagtcct ccccaccgca aggctcaagg cgccgccggc gtggaccgcg
241 cacggcctct aggtctcctc gccaggacag caacctctcc cctggccctc atgggcaccg
301 tcagctccag gcggtcctgg tggccgctgc cactgctgct gctgctgctg ctgctcctgg
361 gtcccgcggg cgcccgtgcg caggaggacg aggacggcga ctacgaggag ctggtgctag
421 ccttgcgttc cgaggaggac ggcctggccg aagcacccga gcacggaacc acagccacct
481 tccaccgctg cgccaaggat ccgtggaggt tgcctggcac ctacgtggtg gtgctgaagg
541 aggagaccca cctctcgcag tcagagcgca ctgcccgccg cctgcaggcc caggctgccc

601 gccggggata cctcaccaag atcctgcatg tcttccatgg ccttcttcct ggcttcctgg
661 tgaagatgag tggcgacctg ctggagctgg ccttgaagtt gccccatgtc gactacatcg
721 aggaggactc ctctgtcttt gcccagagca tcccgtggaa cctggagcgg attacccctc
781 cacggtaccg ggcggatgaa taccagcccc ccgacggagg cagcctggtg gaggtgtatc
841 tcctagacac cagcatacag agtgaccacc gggaaatcga gggcagggtc atggtcaccg
901 acttcgagaa tgtgcccgag gaggacggga cccgcttcca cagacaggcc agcaagtgtg
961 acagtcatgg cacccacctg gcaggggtgg tcagcggccg ggatgccggc gtggccaagg
1021 gtgccagcat gcgcagcctg cgcgtgctca actgccaagg gaagggcacg gttagcggca
1081 ccctcatagg cctggagttt attcggaaaa gccagctggt ccagcctgtg gggccactgg
1141 tggtgctgct gcccctggcg ggtgggtaca gccgcgtcct caacgccgcc tgccagcgcc
1201 tggcgagggc tggggtcgtg ctggtcaccg ctgccggcaa cttccgggac gatgcctgcc
1261 tctactcccc agcctcagct cccgaggtca tcacagttgg ggccaccaat gcccaagacc
1321 agccggtgac cctggggact ttggggacca actttggccg ctgtgtggac ctctttgccc
1381 caggggagga catcattggt gcctccagcg actgcagcac ctgctttgtg tcacagagtg
1441 ggacatcaca ggctgctgcc cacgtggctg gcattgcagc catgatgctg tctgccgagc
1501 cggagctcac cctggccgag ttgaggcaga gactgatcca cttctctgcc aaagatgtca
1561 tcaatgaggc ctggttccct gaggaccagc gggtactgac ccccaacctg gtggccgccc
1621 tgccccccag cacccatggg gcaggttggc agctgttttg caggactgta tggtcagcac
1681 actcggggcc tacacggatg gccacagccg tcgcccgctg cgccccagat gaggagctgc
1741 tgagctgctc cagtttctcc aggagtggga agcggcgggg cgagcgcatg gaggcccaag
1801 ggggcaagct ggtctgccgg gcccacaacg cttttggggg tgagggtgtc tacgccattg
1861 ccaggtgctg cctgctaccc caggccaact gcagcgtcca cacagctcca ccagctgagg
1921 ccagcatggg gacccgtgtc cactgccacc aacagggcca cgtcctcaca ggctgcagct
1981 cccactggga ggtggaggac cttggcaccc acaagccgcc tgtgctgagg ccacgaggtc
2041 agcccaacca gtgcgtgggc cacagggagg ccagcatcca cgcttcctgc tgccatgccc
2101 caggtctgga atgcaaagtc aaggagcatg gaatcccggc ccctcaggag caggtgaccg
2161 tggcctgcga ggagggctgg accctgactg gctgcagtgc cctccctggg acctcccacg
2221 tcctgggggc ctacgccgta gacaacacgt gtgtagtcag gagccgggac gtcagcacta
2281 caggcagcac cagcgaaggg gccgtgacag ccgttgccat ctgctgccgg agccggcacc
2341 tggcgcaggc ctcccaggag ctccagtgac agccccatcc caggatgggt gtctggggag
2401 ggtcaagggc tggggctgag ctttaaaatg gttccgactt gtccctctct cagccctcca
2461 tggcctggca cgaggggatg gggatgcttc cgcctttccg gggctgctgg cctggccctt
2521 gagtggggca gcctccttgc ctggaactca ctcactctgg gtgcctcctc cccaggtgga
2581 ggtgccagga agctccctcc ctcactgtgg ggcatttcac cattcaaaca ggtcgagctg

2641 tgctcgggtg ctgccagctg ctcccaatgt gccgatgtcc gtgggcagaa tgacttttat
2701 tgagctcttg ttccgtgcca ggcattcaat cctcaggtct ccaccaagga ggcaggattc
2761 ttcccatgga taggggaggg ggcggtaggg gctgcaggga caaacatcgt tgggggtga
2821 gtgtgaaagg tgctgatggc cctcatctcc agctaactgt ggagaagccc ctgggggctc
2881 cctgattaat ggaggcttag ctttctggat ggcatctagc cagaggctgg agacaggtgc
2941 gcccctggtg gtcacaggct gtgccttggt ttcctgagcc acctttactc tgctctatgc
3001 caggctgtgc tagcaacacc caaaggtggc ctgcggggag ccatcaccta ggactgactc
3061 ggcagtgtgc agtggtgcat gcactgtctc agccaacccg ctccactacc cggcagggta
3121 cacattcgca cccctacttc acagaggaag aaacctggaa ccagaggggg cgtgcctgcc
3181 aagctcacac agcaggaact gagccagaaa cgcagattgg gctggctctg aagccaagcc
3241 tcttcttact tcacccggct gggctcctca tttttacggg taacagtgag gctgggaagg
3301 ggaacacaga ccaggaagct cggtgagtga tggcagaacg atgcctgcag gcatggaact
3361 ttttccgtta tcacccaggc ctgattcact ggcctggcgg agatgcttct aaggcatggt
3421 cgggggagag ggccaacaac tgtccctcct tgagcaccag ccccacccaa gcaagcagac
3481 atttatcttt tgggtctgtc ctctctgttg ccttttaca gccaactttt ctagacctgt
3541 tttgcttttg taacttgaag atatttattc tgggttttgt agcattttta ttaatatggt
3601 gacttttaa aataaaaaca aacaaacgtt gtcctaa

[0057] As defined herein, an antisense strand sequence is designed to target an APOC3 gene or a PCSK9 gene at a given position on the gene when the 5′ terminal nucleobase of the antisense strand is aligned with a position that is 21 nucleotides downstream (towards the 3′ end) from the position on the gene when base pairing to the gene. For example, as illustrated in Tables 1A, 1B, 2A, and 2B herein, an antisense strand sequence designed to target an PCSK9 gene at position 1322 requires that when base pairing to the gene, the 5′ terminal nucleobase of the antisense strand is aligned with position 1342 of the PCSK9 gene.

[0058] As provided herein, an APOC3-PCSK9 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 APOC3-PCSK9 RNAi agent disclosed herein that is designed to target position 1322 of an PCSK9 gene, the 5′ terminal nucleobase of the antisense strand of the of the PCSK9 RNAi agent is aligned with position 1342 of the gene; however, the 5′ terminal nucleobase of the antisense strand may

be, but is not required to be, complementary to position 1342 of an PCSK9 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 APOC3-PCSK9 RNAi agent (e.g., the position where the APOC3-PCSK9 RNAi agent is designed to target the PCSK9 gene and the position where the APOC3-PCSK9 RNAi agent is designed to target the APOC3 gene) is important to the level of inhibition achieved by the APOC3-PCSK9 RNAi agent.

[0059] In some embodiments, the APOC3-PCSK9 RNAi agents disclosed herein target an APOC3 or PCSK9 gene at or near the positions of the APOC3 or PCSK9 gene sequence shown in Table 1A or Table 1B. In some embodiments, the antisense strands of an APOC3-PCSK9 RNAi agent disclosed herein include a core stretch sequence that is fully, substantially, or at least partially complementary to a target APOC3 or PCSK9 19-mer sequence disclosed in Table 1A and Table 1B.

Table 1A. APOC3 19-mer mRNA Target Sequences (taken from *Homo sapiens* apolipoprotein C3 (APOC3), mRNA, GenBank NM_000040.1 (SEQ ID NO:1)).

SEQ ID No.	APOC3 19-mer Target Sequences (5′ → 3′)	Corresponding Positions of Sequence on SEQ ID NO: 1	Targeted Gene Position (as referred to herein)
3	GGGACAGUAUUCUCAGUGC	438-456	438

Table 1B. PCSK9 19-mer mRNA Target Sequences (taken from *Homo sapiens* proprotein convertase subtilisin/kexin type 9 (PCSK9), mRNA, GenBank NM_174936.4 (SEQ ID NO:2)).

SEQ ID No.	PCSK9 19-mer Target Sequences (5′ → 3′)	Corresponding Positions of Sequence on SEQ ID NO: 2	Targeted Gene Position (as referred to herein)
4	AGCAAGCAGACAUUUAUCU	3470-3488	3468
5	CUCUGUUGCCUUUUUACAG	3503-3521	3501
6	ACUUUUCUAGACCUGUUUU	3525-3543	3523
7	AGACCUGUUUUGCUUUUGU	3533-3551	3531
8	UGUUUUGCUUUUGUAACUU	3538-3556	3536

[0060] In some embodiments, an APOC3-PCSK9 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 1A or Table 1B. In some embodiments, an APOC3-PCSK9 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 1A or Table 1B.

[0061] In some embodiments, an APOC3-PCSK9 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 1A or Table 1B. In some embodiments, an APOC3-PCSK9 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 1A or Table 1B.

[0062] 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 APOC3 or PCSK9 gene, or can be non-complementary to the APOC3 or PCSK9 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.

[0063] In some embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 2-18, 2-19, 2-20, or 2-21 of any of the antisense strand sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C. In some embodiments, an APOC3-PCSK9 RNAi sense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 3-21, 2-21, 1-21, 3-20, 2-20, 1-20, 3-19, 2-19, 1-19, 3-18, 2-18, or 1-18 of any of the sense strand sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C.

[0064] In some embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 2-18, 2-19, 2-20, or 2-21 of any of the antisense strand sequences of Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C. In some embodiments, an APOC3-PCSK9 RNAi sense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 3-21, 2-21, 1-21, 3-20, 2-20, 1-20, 3-19, 2-19,

1-19, 3-18, 2-18, or 1-18 of any of the sense strand sequences of Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C.

[0065] In some embodiments, an APOC3-PCSK9 RNAi agent is comprised of (i) an antisense strand comprising the sequence of nucleotides (from 5′ end → 3′ end) at positions 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) at positions 3-21, 2-21, 1-21, 3-20, 2-20, 1-20, 3-19, 2-19, 1-19, 3-18, 2-18, or 1-18 of any of the sense strand sequences in Table 2A, Table 2B, Table 4A, Table 4B, or Table 4C.

[0066] In some embodiments, the APOC3-PCSK9 RNAi agents include core 19-mer nucleotide sequences shown in the following Table 2A or Table 2B.

Table 2A. APOC3-PCSK9 RNAi Agent Antisense Strand and Sense Strand Core Stretch Base Sequences (N=any nucleobase; I = hypoxanthine (inosine nucleotide); (A^{2N}) = 2-aminoadenine nucleotide), targeting APOC3.

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 of Identified Sequence on SEQ ID NO: 1	Targeted Gene Position
9	GCACUGAGAAUACUGUCCC	38	GGGACAGUAUUCUCAGUGC	438-456	438
10	UCACUGAGAAUACUGUCCC	39	GGGACAGUAUUCUCAGUGA	438-456	438
11	ACACUGAGAAUACUGUCCC	40	GGGACAGUAUUCUCAGUGU	438-456	438
12	NCACUGAGAAUACUGUCCC	41	GGGACAGUAUUCUCAGUGN	438-456	438
13	NCACUGAGAAUACUGUCCN	42	NGGACAGUAUUCUCAGUGN	438-456	438
14	NCACUGAGAAUACUGUCCN	43	NGGACAGUAUUCUCAGUIN	438-456	438
15	UCACUGAGAAUACUGUCCC	44	GGGACAGUAUUCUCAGUIA	438-456	438

Table 2B. APOC3-PCSK9 RNAi Agent Antisense Strand and Sense Strand Core Stretch Base Sequences (N=any nucleobase; I = hypoxanthine (inosine nucleotide); (A^{2N}) = 2-aminoadenine nucleotide), targeting PCSK9.

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 of Identified Sequence on SEQ ID NO: 2	Targeted Gene Position
16	AGAUAAAUGUCUGCUUGCC	45	GGCAAGCAGACAUUUAUCU	3470-3488	3468
17	UGAUAAAUGUCUGCUUGCC	46	GGCAAGCAGACAUUUAUCA	3470-3488	3468
18	NGAUAAAUGUCUGCUUGCC	47	GGCAAGCAGACAUUUAUCN	3470-3488	3468
19	NGAUAAAUGUCUGCUUGCN	48	NGCAAGCAGACAUUUAUCN	3470-3488	3468
20	CUGUAAAAAGGCAACAGAG	49	CUCUGUUGCCUUUUUACAG	3503-3521	3501
21	CUGUAAAAAGGCAACAGGG	50	CCCUGUUGCCUUUUUACAG	3503-3521	3501
22	UUGUAAAAAGGCAACAGGG	51	CCCUGUUGCCUUUUUACAA	3503-3521	3501
23	AUGUAAAAAGGCAACAGGG	52	CCCUGUUGCCUUUUUACAU	3503-3521	3501
24	NUGUAAAAAGGCAACAGGG	53	CCCUGUUGCCUUUUUACAN	3503-3521	3501
25	NUGUAAAAAGGCAACAGGN	54	NCCUGUUGCCUUUUUACAN	3503-3521	3501
26	UAAACAGGUCUAGAAAAGC	55	ACUUUUCUAGACCUGUUUA	3525-3543	3523
27	AAAACAGGUCUAGAAAAGC	56	GCUUUUCUAGACCUGUUUU	3525-3543	3523
28	NAAACAGGUCUAGAAAAGC	57	GCUUUUCUAGACCUGUUUN	3525-3543	3523
29	NAAACAGGUCUAGAAAAGN	58	NCUUUUCUAGACCUGUUUN	3525-3543	3523
30	ACAAAAGCAAAACAGGUCU	59	AGACCUGUUUUGCUUUUGU	3533-3551	3531
31	UCAAAAGCAAAACAGGUCU	60	AGACCUGUUUUGCUUUUGA	3533-3551	3531
32	NCAAAAGCAAAACAGGUCU	61	AGACCUGUUUUGCUUUUGN	3533-3551	3531
33	NCAAAAGCAAAACAGGUCN	62	NGACCUGUUUUGCUUUUGN	3533-3551	3531
34	AAGUUACAAAAGCAAAACA	63	UGUUUUGCUUUUGUAACUU	3538-3556	3536
35	UAGUUACAAAAGCAAAACA	64	UGUUUUGCUUUUGUAACUA	3538-3556	3536
36	NAGUUACAAAAGCAAAACA	65	UGUUUUGCUUUUGUAACUN	3538-3556	3536
37	NAGUUACAAAAGCAAAACN	66	NGUUUUGCUUUUGUAACUN	3538-3556	3536

[0067] The APOC3-PCSK9 RNAi agent sense strands and antisense strands that comprise or consist of the sequences in Table 2A or Table 2B can be modified nucleotides or unmodified nucleotides. In some embodiments, the APOC3-PCSK9 RNAi agents having the sense and antisense strand sequences that comprise or consist of the sequences in Table 2A or Table 2B are all or substantially all modified nucleotides.

[0068] In some embodiments, the antisense strand of an APOC3-PCSK9 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2A or Table 2B. In some embodiments, the sense strand of an APOC3-PCSK9 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2A or Table 2B.

[0069] As used herein, each N listed in a sequence disclosed in Table 2A or Table 2B 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 2A or Table 2B 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 2A or Table 2B 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 2A or Table 2B 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 2A or Table 2B has a nucleobase that is different from the N nucleotide at the corresponding position on the other strand.

[0070] Certain modified APOC3-PCSK9 RNAi agent antisense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 3A or Table 3B. Certain modified APOC3-PCSK9 RNAi agent sense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 4A, Table 4B, or Table 4C. In forming APOC3-PCSK9 RNAi agents, each of the nucleotides in each of the underlying base sequences listed in Tables 3A, 3B, 4A, 4B, and 4C, as well as in Tables 2A and 2B, above, can be a modified nucleotide.

[0071] The APOC3-PCSK9 RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2A, Table 2B, Table 4A, Table 4B, or Table 4C, can be hybridized to any antisense strand

containing a sequence listed in Table 2A, Table 2B, Table 3A, or Table 3B, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

[0072] In some embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2A, Table 2B, Table 3A, or Table 3B.

[0073] In some embodiments, an APOC3-PCSK9 RNAi agent comprises or consists of a complex having the nucleobase sequences of the sense strand and the antisense strand of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, Table 4A, Table 4B, or Table 4C.

[0074] Examples of antisense strands containing modified nucleotides are provided in Table 3A, Table 3B, and Table 5C. Examples of sense strands containing modified nucleotides are provided in Table 4A, Table 4B, Table 4C, and Table 5C.

[0075] As used in Tables 3A, Table 3B, Table 4A, Table 4B, Table 4C, and Table 5C the following notations are used to indicate modified nucleotides and linking groups:

A	=	adenosine-3′-phosphate;
C	=	cytidine-3′-phosphate;
G	=	guanosine-3′-phosphate;
U	=	uridine-3′-phosphate
I	=	inosine-3′-phosphate
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
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
AUNA	=	2′,3′-seco-adenosine-3′-phosphate (*see* Table 6)
AUNAS	=	2′,3′-seco-adenosine-3′-phosphorothioate (*see* Table 6)
CUNA	=	2′,3′-seco-cytidine-3′-phosphate (*see* Table 6)
CUNAS	=	2′,3′-seco-cytidine-3′-phosphorothioate (*see* Table 6)
GUNA	=	2′,3′-seco-guanosine-3′-phosphate (*see* Table 6)
GUNAS	=	2′,3′-seco-guanosine-3′-phosphorothioate (*see* Table 6)
UUNA	=	2′,3′-seco-uridine-3′-phosphate (*see* Table 6)
UUNAS	=	2′,3′-seco-uridine-3′-phosphorothioate (*see* Table 6)
a_2N	=	2′-O-methyl-2-aminoadenosine-3′-phosphate (*see* Table 6)
a_2Ns	=	2′-O-methyl-2-aminoadenosine-3′-phosphorothioate (*see* Table 6)
(invAb)	=	inverted abasic deoxyribonucleotide (*see* Table 6)
(invAb)s	=	inverted abasic deoxyribonucleotide-5′-phosphorothioate (*see* Table 6)
cPrpa	=	5’-cyclopropyl phosphonate-2′-O-methyladenosine-3′-phosphate (*see* Table 6)
cPrpas	=	5’-cyclopropyl phosphonate-2′-O-methyladenosine-3′-phosphorothioate (*see* Table 6)
cPrpu	=	5’-cyclopropyl phosphonate-2′-O-methyluridine-3′-phosphate (*see* Table 6)
cPrpus	=	5’-cyclopropyl phosphonate-2′-O-methyluridine-3′- phosphorothioate (*see* Table 6)
cPrpi	=	5’-cyclopropyl phosphonate-2′-O-methylinosine-3′-phosphate (*see* Table 6)

cPrpis	=	5'-cyclopropyl phosphonate-2'-O-methylinosine-3'-phosphorithioate (*see* Table 6)
dT	=	2'-deoxythymidine-3'-phosphate
dTs	=	2'-deoxythymidine-3'-phosphorothioate
dTss	=	2'-deoxythymidine-3'-phosphorodithioate
dU	=	2'-deoxyuridine-3'-phosphate
dUs	=	2'-deoxyuridine-3'-phosphorothioate
dUss	=	2'-deoxyuridine-3'-phosphorodithioate
dC	=	2'-deoxycytidine-3'-phosphate
dCs	=	2'-deoxycytidine-3'-phosphorothioate
dG	=	2'-deoxyguanosine-3'-phosphate
dGs	=	2'-deoxyguanosine-3'-phosphorothioate
dA	=	2'-deoxyadenosine-3'-phosphate
dAs	=	2'-deoxyadenosine-3'-phosphorothioate
dAss	=	2'-deoxyadenosine-3'-phosphorodithioate
(NAG37)	=	*see* Table 6
(NAG37)s	=	*see* Table 6
(NAG52)	=	*see* Table 6
(NAG52)s	=	*see* Table 6
(NAG47)	=	*see* Table 6
(NAG47)s	=	*see* Table 6
Sp18	=	*see* Table 6
Sp18s	=	*see* Table 6

[0076] 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 or phosphorodithioate linkage as shown in the modified nucleotide sequences disclosed herein replaces the phosphodiester linkage typically present in oligonucleotides. 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*. Additionally, for the various embodiments disclosed herein, when viewing the respective strand 5' → 3', the inverted abasic residues are inserted such that the 3' position of the deoxyribose is linked at the 3' end of the preceding monomer on the respective strand (*see, e.g.*, Table 6). 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 resonance structures (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 APOC3-PCSK9 RNAi agents and compositions of APOC3-PCSK9 RNAi agents disclosed herein.

[0077] Certain examples of targeting ligands, targeting groups, and linking groups used with the APOC3-PCSK9 RNAi agents disclosed herein are provided below in Table 6. More specifically, targeting groups and linking groups (which together can form a targeting ligand) include (NAG52) and (NAG52)s, for which their chemical structures are provided below in Table 6. Each sense strand and/or antisense strand can have any targeting ligands, targeting groups, or linking groups listed herein, as well as other groups, conjugated to the 5′ and/or 3′ end of the sequence.

Table 3A. APOC3-PCSK9 RNAi Agent Antisense Strand Sequences, targeting APOC3

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.
CA004497	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	67	UCACUGAGAAUACUGUCCC	277
CA004500	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	68	UCACUGAGAAUACUGUCCC	277
CA004660	usCfsasCfuGfagaauAfcUfgUfcCfsc	69	UCACUGAGAAUACUGUCCC	277
CA005467	cPrpusCfsaCfuGfagaauAfcUfgUfcsCfsc	70	UCACUGAGAAUACUGUCCC	277
CA005468	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	UCACUGAGAAUACUGUCCC	277
CA009929	cPrpusCfsaCfugagaauAfcUfgUfccsc	72	UCACUGAGAAUACUGUCCC	277
CA011958	cPrpusCfsaCfugAfgaauAfcUfgUfcscsc	73	UCACUGAGAAUACUGUCCC	277
CA012139	cPrpusCfsaCfugagaauAfcugUfcscsc	74	UCACUGAGAAUACUGUCCC	277
CA903799	usCfsasCfuGfagaauAfcUfgUfcCfcGfsu	75	UCACUGAGAAUACUGUCCCGU	278
CA916226	usCfsasCfuGfagaauAfcUfgUfcCfsc	77	UCACUGAGAAUACUGUCCC	277
CA916236	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	UCACUGAGAAUACUGUCCC	277

(A^{2N}) = 2-aminoadenine nucleotide; I = hypoxanthine (inosine) nucleotide

Table 3B. APOC3-PCSK9 RNAi Agent Antisense Strand Sequences, targeting PCSK9

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.
CA004431	cPrpusAfsguuacaaaaGfcAfaAfacsa	79	UAGUUACAAAAGCAAAACA	280
CA004496	cPrpuAfaacaggucuAfgAfaAfagsc	80	UAAACAGGUCUAGAAAAGC	281
CA004499	cPrpusCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	81	UCAAAAGCAAAACAGGUCC	282
CA004659	cPrpasCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	82	ACAAAAGCAAAACAGGUCC	283

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.
CA004945	cPrpasAfsguuacaaaaGfcAfaAfacasg	83	AAGUUACAAAAGCAAAACAG	284
CA005362	cPrpasAfsguuacaaaaGfcAfaAfacgsg	84	AAGUUACAAAAGCAAAACGG	285
CA005538	cPrpusUfsguaa_2NaaaggCfaAfcAfgsgsg	86	UUGUA(A^{2N})AAAGGCAACAGGG	286
CA005543	cPrpasCfsaaaagcaaaAfcAfgGfuscsc	87	ACAAAAGCAAAACAGGUCC	283
CA005546	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88	ACAAAAGCAAAACAGGUCC	283
CA005898	cPrpusUfguaa_2NaaaggCfaAfcAfgsasg	89	UUGUA(A^{2N})AAGGCAACAGAG	287
CA006321	cPrpaCfaaaagcaaaAfcAfgGfucsusasg	90	ACAAAAGCAAAACAGGUCUAG	288
CA006323	cPrpaCfaaaagcaaaAfcAfgGfucsusa	91	ACAAAAGCAAAACAGGUCUA	289
CA006325	cPrpaCfaaaagcaaaAfcAfgGfucscsa	92	ACAAAAGCAAAACAGGUCCA	290
CA006329	cPrpasAfsguuacaaaaGfcAfaAfacsasg	93	AAGUUACAAAAGCAAAACAG	284
CA008250	cPrpisAfsguuacaaaaGfcAfaAfacsa	94	IAGUUACAAAAGCAAAACA	291
CA008251	cPrpasAfsguuacaaaaGfcAfaAfacsi	95	AAGUUACAAAAGCAAAACI	292
CA008252	cPrpisAfsguuacaaaaGfcAfaAfacsi	96	IAGUUACAAAAGCAAAACI	293
CA008492	cPrpaCfaaaagcaaaAfcAfgGfuCfcsAfsg	97	ACAAAAGCAAAACAGGUCCAG	294
CA011440	cPrpasAfsguuacaaaaGfcAfaAfascsa	98	AAGUUACAAAAGCAAAACA	279
CA011957	cPrpasAfsguuacaAfaaGfcAfaAfacsa	99	AAGUUACAAAAGCAAAACA	279
CA012138	cPrpasAfsguuacaaaaGfcAfaaacsa	100	AAGUUACAAAAGCAAAACA	279
CA908055	cPrpasAfsguuacaaaaGfcAfaAfacasgsg	101	AAGUUACAAAAGCAAAACAGG	295
CA916212	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	AAGUUACAAAAGCAAAACG	296
CA916215	cPrpasAfsguuacaaaaGfcAfaAfacsa	103	AAGUUACAAAAGCAAAACA	279
CA916224	cPrpusGfauaaaugucUfgCfuUfgcsc	104	UGAUAAAUGUCUGCUUGCC	297

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.
CA916258	asCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	105	ACAAAAGCAAAACAGGUCC	283
CA916262	cPrpusAfsguuacaaaaGfcAfaAfacsg	106	UAGUUACAAAAGCAAAACG	298

(A^{2N}) = 2-aminoadenine nucleotide; I = hypoxanthine (inosine) nucleotide

Table 4A. APOC3-PCSK9 RNAi Agent Sense Strand Sequences, complementary to an antisense strand targeting APOC3

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.
CS004426-1	gggacaGfUfAfuucucaguia	107	GGGACAGUAUUCUCAGUIA	299
CS004427-2	gsggacaGfUfAfuucucaguia	108	GGGACAGUAUUCUCAGUIA	299
CS004493-1	gggacaGfUfAfuucucaguia	109	GGGACAGUAUUCUCAGUIA	299
CS004653-2	gsggacaGfUfAfuucucaguia	110	GGGACAGUAUUCUCAGUIA	299
CS004654-2	gsggacaGfUfAfuucucaguia	111	GGGACAGUAUUCUCAGUIA	299
CS004655-2	gsggacaGfUfAfuucucaguia	112	GGGACAGUAUUCUCAGUIA	299
CS004656-2	gsggacaGfUfAfuucucaguia	113	GGGACAGUAUUCUCAGUIA	299
CS004658-2	gsggacaGfUfAfuucucaguia	114	GGGACAGUAUUCUCAGUIA	299
CS005046-1	gggacaGfUfAfuucucaguia	115	GGGACAGUAUUCUCAGUIA	299
CS005047-1	gggacaGfUfAfuucucaguisa	116	GGGACAGUAUUCUCAGUIA	299
CS005048-1	gggacaGfUfAfuucucaguia	117	GGGACAGUAUUCUCAGUIA	299
CS005049-1	gggacaGfUfAfuucucaguisa	118	GGGACAGUAUUCUCAGUIA	299
CS005199-2	gsggacaGfUfAfuucucaguia	119	GGGACAGUAUUCUCAGUIA	299
CS005200-2	gsggacaGfUfAfuucucaguia	120	GGGACAGUAUUCUCAGUIA	299
CS006145-1	gggacaGfUfAfuucucaguia	121	GGGACAGUAUUCUCAGUIA	299
CS006146-1	gggacaGfUfAfuucucaguia	122	GGGACAGUAUUCUCAGUIA	299
CS006147-1	gggacaGfUfAfuucucaguia	123	GGGACAGUAUUCUCAGUIA	299
CS006148-1	gggacaGfUfAfuucucaguia	124	GGGACAGUAUUCUCAGUIA	299
CS006149-1	gggacaGfUfAfuucucaguia	125	GGGACAGUAUUCUCAGUIA	299
CS006150-1	gggacaGfUfAfuucucaguia	126	GGGACAGUAUUCUCAGUIA	299
CS006319-2	gsggacaGfUfAfuucucaguia	127	GGGACAGUAUUCUCAGUIA	299
CS006320-2	gsggacaGfUfAfuucucaguia	128	GGGACAGUAUUCUCAGUIA	299

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.
CS006327-1	gggacaGfUfAfuucucaguia	129	GGGACAGUAUUCUCAGUIA	299
CS006328-2	gsggacaGfUfAfuucucaguia	130	GGGACAGUAUUCUCAGUIA	299
CS006331-2	gsggacaGfUfAfuucucaguia	131	GGGACAGUAUUCUCAGUIA	299
CS006332-1	gggacaGfUfAfuucucaguia	132	GGGACAGUAUUCUCAGUIA	299
CS006607-1	gggacaGfuAfuUfcucaguia	133	GGGACAGUAUUCUCAGUIA	299
CS006608-2	gsggacaGfuAfuUfcucaguia	134	GGGACAGUAUUCUCAGUIA	299
CS006609-1	gggacaGfuAfuUfcucaguia	135	GGGACAGUAUUCUCAGUIA	299
CS006610-2	gsggacaGfuAfuUfcucaguia	136	GGGACAGUAUUCUCAGUIA	299
CS006611-1	gggacaGfuAfuUfcucaguia	137	GGGACAGUAUUCUCAGUIA	299
CS006689-2	gsggacaGfuAfuUfcucaguia	138	GGGACAGUAUUCUCAGUIA	299
CS006690-1	gggacaGfuAfuUfcucaguia	139	GGGACAGUAUUCUCAGUIA	299
CS006692-2	gsggacaGfuAfuUfcucaguia	140	GGGACAGUAUUCUCAGUIA	299
CS006693-1	gggacaGfuAfuUfcucaguia	141	GGGACAGUAUUCUCAGUIA	299
CS006694-2	gsggacaGfuAfuUfcucaguia	142	GGGACAGUAUUCUCAGUIA	299
CS006695-1	gggacaGfuAfuUfcucaguia	143	GGGACAGUAUUCUCAGUIA	299
CS008561-2	gsggacaGfuAfuUfcucaguia	144	GGGACAGUAUUCUCAGUIA	299
CS008562-2	sgsggacaGfuAfuUfcucaguia	145	GGGACAGUAUUCUCAGUIA	299
CS009181-2	gsggacaGfuAfuUfcucaguia	146	GGGACAGUAUUCUCAGUIA	299
CS009182-2	gsggacaGfuAfuUfcucaguia	147	GGGACAGUAUUCUCAGUIA	299
CS009888-2	gsggacaGfuAfuUfcucaguia	148	GGGACAGUAUUCUCAGUIA	299
CS011439-2	ascgggacaGfUfAfuucucaguia	149	ACGGGACAGUAUUCUCAGUIA	300
CS011956-2	gsggacaGfuAfuUfcucaguia	150	GGGACAGUAUUCUCAGUIA	299
CS012137-2	gsggacaguAfuUfcucaguia	151	GGGACAGUAUUCUCAGUIA	299

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.
CS916234-1	gggacaGfUfAfuucucaguias	152	GGGACAGUAUUCUCAGUIA	299
CS916235-2	gsggacaGfUfAfuucucaguia	153	GGGACAGUAUUCUCAGUIA	299
CS916251-2	gsggacaGfUfAfuucucaguia	154	GGGACAGUAUUCUCAGUIA	299

(A^{2N}) = 2-aminoadenine nucleotide; I = hypoxanthine (inosine) nucleotide

Table 4B. APOC3-PCSK9 RNAi Agent Sense Strand Sequences, complementary to an antisense strand targeting PCSK9

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.
CS004426-2	csguuuuGfcUfuUfuguaacuu	155	CGUUUUGCUUUUGUAACUU	301
CS004427-1	cguuuuGfcUfuUfuguaacuu	156	CGUUUUGCUUUUGUAACUU	301
CS004493-2	csguuuuGfcUfuUfuguaacuu	157	CGUUUUGCUUUUGUAACUU	301
CS004653-1	ggcaagCfAfGfacauuuauca	158	GGCAAGCAGACAUUUAUCA	302
CS004654-1	gcuuuuCfUfAfgaccuguuua	159	GCUUUUCUAGACCUGUUUA	303
CS004655-1	ggaccuGfUfUfuugcuuuuga	160	GGACCUGUUUUGCUUUUGA	304
CS004656-1	cguuuuGfcUfuUfuguaacua	161	CGUUUUGCUUUUGUAACUA	305
CS004658-1	ggaccuGfUfUfuugcuuuugu	162	GGACCUGUUUUGCUUUUGU	306
CS005046-2	usguuuuGfcUfuUfuguaacua	163	UGUUUUGCUUUUGUAACUA	307
CS005047-2	usguuuuGfcUfuUfuguaacua	164	UGUUUUGCUUUUGUAACUA	307
CS005048-2	usguuuuGfCfUfuuuguaacua	165	UGUUUUGCUUUUGUAACUA	307
CS005049-2	usguuuuGfCfUfuuuguaacua	166	UGUUUUGCUUUUGUAACUA	307
CS005199-1	cguuuuGfcUfuUfuguaacuu	167	CGUUUUGCUUUUGUAACUU	301
CS005200-1	cguuuuGfcUfuUfuguaacuu	168	CGUUUUGCUUUUGUAACUU	301

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.
CS006145-2	gsgaccuGfuUfUfugcuuuugu	169	GGACCUGUUUUGCUUUUGU	306
CS006146-2	ggaccuGfUfUfuugcuuuugu	170	GGACCUGUUUUGCUUUUGU	306
CS006147-2	csccuguUfGfCfcuuuuuacaa	171	CCCUGUUGCCUUUUUACAA	308
CS006148-2	cccuguUfGfCfcuuuuuacaa	172	CCCUGUUGCCUUUUUACAA	308
CS006149-2	csuguuuuGfCfUfuuuguaaacuu	173	CUGUUUUGCUUUUGUAACUU	309
CS006150-2	cuguuuuGfCfUfuuuguaaacuu	174	CUGUUUUGCUUUUGUAACUU	309
CS006319-1	gsgaccuGfuUfUfugcuuuugu	175	GGACCUGUUUUGCUUUUGU	306
CS006320-1	asgaccuGfuUfUfugcuuuugu	176	AGACCUGUUUUGCUUUUGU	310
CS006327-2	csuguuuuGfcUfuUfuguaaacuu	177	CUGUUUUGCUUUUGUAACUU	309
CS006328-1	uguuuuGfcUfuUfuguaaacuu	178	UGUUUUGCUUUUGUAACUU	311
CS006331-1	csuguuuuGfcUfuUfuguaaacuu	179	CUGUUUUGCUUUUGUAACUU	309
CS006332-2	gsgaccuGfuUfUfugcuuuugu	180	GGACCUGUUUUGCUUUUGU	306
CS006607-2	gsgaccuGfuUfUfugcuuuugu	181	GGACCUGUUUUGCUUUUGU	306
CS006608-1	ggaccuGfuUfUfugcuuuugu	182	GGACCUGUUUUGCUUUUGU	306
CS006609-2	cucuguUfgCfcUfuuuuacaa	183	CUCUGUUGCCUUUUUACAA	312
CS006610-1	cucuguUfgCfcUfuuuuacaa	184	CUCUGUUGCCUUUUUACAA	312
CS006611-2	csucuguUfgCfcUfuuuuacaa	185	CUCUGUUGCCUUUUUACAA	312
CS006689-1	uguuuuGfcUfuUfuguaacua	186	UGUUUUGCUUUUGUAACUA	307
CS006690-2	usguuuuGfcUfuUfuguaaacua	187	UGUUUUGCUUUUGUAACUA	307
CS006692-1	cguuuuGfcUfuUfuguaacuu	188	CGUUUUGCUUUUGUAACUU	301
CS006693-2	csguuuuGfcUfuUfuguaacuu	189	CGUUUUGCUUUUGUAACUU	301
CS006694-1	uguuuuGfcUfuUfuguaacuu	190	UGUUUUGCUUUUGUAACUU	311
CS006695-2	usguuuuGfcUfuUfuguaacuu	191	UGUUUUGCUUUUGUAACUU	311

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.
CS008561-1	usguuuuGfcUfuUfuguaacuu	192	UGUUUUGCUUUUGUAACUU	311
CS008562-1	usguuuuGfcUfuUfuguaacuus	193	UGUUUUGCUUUUGUAACUU	311
CS009181-1	ccuguuuuGfcUfuUfuguaacuu	194	CCUGUUUUGCUUUUGUAACUU	313
CS009182-1	ccuguuuuGfcUfuUfuguaacuu	195	CCUGUUUUGCUUUUGUAACUU	313
CS009888-1	uguuuuGfcUfuUfuguaacuu	196	UGUUUUGCUUUUGUAACUU	311
CS011439-1	cuggaccuGfuUfUfugcuuuugu	197	CUGGACCUGUUUUGCUUUUGU	314
CS011956-1	uguuuuGfcUfuuuguaacuu	198	UGUUUUGCUUUUGUAACUU	311
CS012137-1	uguuuuGfcUfuUfuguaacuu	199	UGUUUUGCUUUUGUAACUU	311
CS916234-2	csguuuuGfcUfuUfuguaacuu	200	CGUUUUGCUUUUGUAACUU	301
CS916235-1	cguuuuGfcUfuUfuguaacuu	201	CGUUUUGCUUUUGUAACUU	301
CS916251-1	cguuuuGfcUfuUfuguaacuu	202	CGUUUUGCUUUUGUAACUU	301

(A^{2N}) = 2-aminoadenine nucleotide; I = hypoxanthine (inosine) nucleotide

Table 4C. APOC3-PCSK9 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.
CS004426	(NAG43)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	203	GGGACAGUAUUCUCAGUIACGUUUUGCUUUUGUAACUU	315
CS004427	(NAG43)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	204	CGUUUUGCUUUUGUAACUUGGGACAGUAUUCUCAGUIA	316
CS004493	(TriAlk14)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	205	GGGACAGUAUUCUCAGUIACGUUUUGCUUUUGUAACUU	315

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.
CS004653	(NAG47)sggcaagCfAfGfacauuuaucas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	206	GGCAAGCAGACAUUUAUCAGGGA CAGUAUUCUCAGUIA	317
CS004654	(NAG47)sgcuuuuCfUfAfgaccuguuuas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	207	GCUUUUCUAGACCUGUUUAGGGA CAGUAUUCUCAGUIA	318
CS004655	(NAG47)sggaccuGfUfUfuugcuuuugas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	208	GGACCUGUUUUGCUUUUGAGGGA CAGUAUUCUCAGUIA	319
CS004656	(NAG47)scguuuuGfcUfuUfuguaacuas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	209	CGUUUUGCUUUUGUAACUAGGGA CAGUAUUCUCAGUIA	320
CS004658	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210	GGACCUGUUUUGCUUUUGUGGGA CAGUAUUCUCAGUIA	321
CS005046	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaacuas(invAb)sC6-NH2	211	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUA	322
CS005047	(NAG52)sgggacaGfUfAfuucucaguisa-Sp18-usguuuuGfcUfuUfuguaacuas(invAb)	212	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUA	322
CS005048	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-usguuuuGfCfUfuuuguaaacuas(invAb)sC6-NH2	213	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUA	322
CS005049	(NAG52)sgggacaGfUfAfuucucaguisa-Sp18-usguuuuGfCfUfuuuguaaacuas(invAb)	214	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUA	322
CS005199	(NAG37)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	215	CGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	316
CS005200	(NAG42)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	216	CGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	316
CS006145	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-ggaccuGfuUfUfugcuuuugus(invAb)	217	GGGACAGUAUUCUCAGUIAGGACC UGUUUUGCUUUUGU	323
CS006146	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-(invAb)sggaccuGfUfUfuugcuuuugus(invAb)	218	GGGACAGUAUUCUCAGUIAGGACC UGUUUUGCUUUUGU	323
CS006147	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-csccuguUfGfCfcuuuuuacaas(invAb)	219	GGGACAGUAUUCUCAGUIACCCUG UUGCCUUUUUACAA	324

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.
CS006148	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-(invAb)scccuguUfGfCfcuuuuuacaas(invAb)	220	GGGACAGUAUUCUCAGUIACCCUG UUGCCUUUUUUACAA	324
CS006149	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csuguuuuGfCfUfuuuguaacuus(invAb)	221	GGGACAGUAUUCUCAGUIACUGUU UUGCUUUUUGUAACUU	325
CS006150	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-(invAb)scuguuuuGfCfUfuuuguaacuus(invAb)	222	GGGACAGUAUUCUCAGUIACUGUU UUGCUUUUUGUAACUU	325
CS006319	(NAG52)sgsgaccuGfuUfUfugcuuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)	223	GGACCUGUUUUUGCUUUUUGUGGGA CAGUAUUCUCAGUIA	321
CS006320	(NAG52)sasgaccuGfuUfUfugcuuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)	224	AGACCUGUUUUUGCUUUUUGUGGGA CAGUAUUCUCAGUIA	326
CS006327	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csuguuuuGfcUfuUfuguaacuus(invAb)	225	GGGACAGUAUUCUCAGUIACUGUU UUGCUUUUUGUAACUU	325
CS006328	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)	226	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS006331	(NAG52)scsuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)	227	CUGUUUUGCUUUUGUAACUUGGG ACAGUAUUCUCAGUIA	328
CS006332	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-gsgaccuGfuUfUfugcuuuuugus(invAb)sC6-NH2	228	GGGACAGUAUUCUCAGUIAGGACC UGUUUUGCUUUUGU	323
CS006607	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-gsgaccuGfuUfUfugcuuuuugus(invAb)sC6-NH2	229	GGGACAGUAUUCUCAGUIAGGACC UGUUUUGCUUUUGU	323
CS006608	(NAG52)sggaccuGfuUfUfugcuuuuugus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	230	GGACCUGUUUUUGCUUUUUGUGGGA CAGUAUUCUCAGUIA	321
CS006609	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-cucuguUfgCfcUfuuuuacaas(invAb)sC6-NH2	231	GGGACAGUAUUCUCAGUIACUCUG UUGCCUUUUUUACAA	329
CS006610	(NAG52)scucuguUfgCfcUfuuuuacaas(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	232	CUCUGUUGCCUUUUUUACAAGGGAC AGUAUUCUCAGUIA	330
CS006611	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-csucuguUfgCfcUfuuuuacaas(invAb)sC6-NH2	233	GGGACAGUAUUCUCAGUIACUCUG UUGCCUUUUUUACAA	329

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.
CS006689	(NAG52)suguuuuGfcUfuUfuguaacuas(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	234	UGUUUUGCUUUUGUAACUAGGGA CAGUAUUCUCAGUIA	331
CS006690	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaacuas(invAb)sC6-NH2	235	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUA	322
CS006692	(NAG52)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	236	CGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	316
CS006693	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	237	GGGACAGUAUUCUCAGUIACGUUU UGCUUUUGUAACUU	315
CS006694	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS006695	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	239	GGGACAGUAUUCUCAGUIAUGUUU UGCUUUUGUAACUU	332
CS008561	usguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	240	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS008562	usguuuuGfcUfuUfuguaacuus(invAb)s-Sp18-sgsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	241	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS009181	(NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	242	CCUGUUUUGCUUUUGUAACUUGG GACAGUAUUCUCAGUIA	333
CS009182	(NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)	243	CCUGUUUUGCUUUUGUAACUUGG GACAGUAUUCUCAGUIA	333
CS009888	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)	244	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS011439	(NAG52)scuggaccuGfuUfUfugcuuuugus(invAb)-Sp18-ascgggacaGfUfAfuucucaguias(invAb)	245	CUGGACCUGUUUUGCUUUUGUAC GGGACAGUAUUCUCAGUIA	334
CS011956	(NAG52)suguuuuGfcUfuuuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	246	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327
CS012137	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaguAfuUfcucaguias(invAb)sC6-NH2	247	UGUUUUGCUUUUGUAACUUGGGA CAGUAUUCUCAGUIA	327

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.
CS916234	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	248	GGGACAGUAUUCUCAGUIACGUUUUGCUUUUUGUAACUU	315
CS916235	(NAG52)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	249	CGUUUUGCUUUUUGUAACUUGGGACAGUAUUCUCAGUIA	316
CS916251	(TriAlk14)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	250	CGUUUUGCUUUUUGUAACUUGGGACAGUAUUCUCAGUIA	316

(A^{2N}) = 2-aminoadenine nucleotide; I = hypoxanthine (inosine) nucleotide

[0078] The APOC3-PCSK9 RNAi agents described herein are formed by annealing each antisense strand to a sense strand sequence having sufficient complementarity to the antisense strand. A sense strand sequence listed in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C can be hybridized to any antisense strand containing a sequence listed in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C provided the two sequences have a region of at least 85% complementarity over a contiguous 15, 16, 17, 18, 19, 20, or 21 nucleotide sequence.

[0079] In some embodiments, an antisense strand of an APOC3-PCSK9 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 3A, Table 3B, or Table 5C. In some embodiments, a sense strand sequence of an APOC3-PCSK9 RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 4A, Table 4B, Table 4C or Table 5C. In some embodiments, a sense strand complementary to an antisense strand targeting APOC3 is covalently linked to a sense strand complementary to an antisense strand targeting PCSK9, and each antisense strand is then annealed to the APOC3-PCSK9 RNAi agent sense strand.

[0080] In some embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C. In some embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 1-17, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, or 2-21, of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C. In certain embodiments, an APOC3-PCSK9 RNAi agent antisense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 3A, Table 3B, or Table 5C.

[0081] In some embodiments, an APOC3-PCSK9 RNAi agent sense strand comprises the nucleotide sequence of any of the sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C. In some embodiments, an APOC3-PCSK9 RNAi agent sense strand comprises the sequence of nucleotides (from 5′ end → 3′ end) at positions 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, or 4-21, of any of the sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C. In certain embodiments, an APOC3-PCSK9 RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 4A, Table 4B, Table 4C, or Table 5C.

[0082] For the APOC3-PCSK9 RNAi agents disclosed herein, the nucleotide at position 1 of the antisense strand (from 5′ end → 3′ end) can be perfectly complementary to an APOC3 gene or a PCSK9 gene, or can be non-complementary to an APOC3 gene or a PCSK9 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.

[0083] A sense strand containing a sequence listed in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C can be hybridized to any antisense strand containing a sequence listed in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C, 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 APOC3-PCSK9 RNAi agent has a sense strand consisting of the modified sequence of any of the modified sequences in Table 4A, Table 4B, Table 4C, or Table 5C, and an antisense strand consisting of the modified sequence of any of the modified sequences in Table 3A, Table 3B, or Table 5C. Certain representative sequence pairings are exemplified by the Complex ID Nos. shown in Tables 5A, 5B, and 5C.

[0084] In some embodiments, an APOC3-PCSK9 RNAi agent comprises, consists of, or consists essentially of a complex represented by any one of the Complex ID Nos. presented herein. In some embodiments, an APOC3-PCSK9 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the complexes represented by any of the Complex ID NOs. presented herein. In some embodiments, an APOC3-PCSK9 RNAi agent comprises the sense strand and antisense strand nucleotide sequences of any of the complexes represented by any of the Complex 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 APOC3-PCSK9 RNAi agent includes the sense strand and antisense strand modified nucleotide sequences of any of the Complex ID NOs. presented herein. In some embodiments, an APOC3-PCSK9 RNAi agent comprises the sense strand and antisense strand modified nucleotide sequences of any of the Complex 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.

[0085] In some embodiments, an APOC3-PCSK9 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense

strand complexes of Table 2A, Table 2B, Table 5A, Table 5B, or 5C, and further comprises a targeting group or targeting ligand. In some embodiments, an APOC3-PCSK9 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand complexes of Table 2A, Table 2B, Table 5A, Table 5B, Table 5C, and further comprises an asialoglycoprotein receptor ligand targeting group.

[0086] 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 Table 2A, Table 2B, Table 3A, Table 3B, Table 4A, Table 4B, Table 4C, or Table 5C. 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 Table 2A, Table 2B, Table 3A, Table 3B, Table 4A, Table 4B, Table 4C, or Table 5C.

[0087] In some embodiments, an APOC3-PCSK9 RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand complexes of Table 2A, Table 2B, Table 5A, Table 5B, or Table 5C, and further comprises a targeting ligand selected from the group consisting of: (NAG52) and (NAG52)s, each as defined in Table 6.

[0088] In some embodiments, an APOC3-PCSK9 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 3A, Table 3B, Table 4A, Table 4B, or Table 4C.

[0089] In some embodiments, an APOC3-PCSK9 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 complexes Tables 5A, 5B, and 5C, and further comprises an asialoglycoprotein receptor ligand targeting group.

[0090] In some embodiments, an APOC3-PCSK9 RNAi agent comprises, consists of, or consists essentially of any of the complexes of Tables 5A, 5B, and 5C.

Table 5A. APOC3-PCSK9 RNAi Agents Complexes with Corresponding Sense and Antisense Strand ID Numbers and Sequence ID numbers for the modified and unmodified nucleotide sequences.

Complex ID	AS1 ID	AS1 modified SEQ ID NO:	AS1 unmodified SEQ ID NO:	AS2 ID	AS2 modified SEQ ID NO:	AS2 unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC003782	CA916212	102	296	CA916236	78	277	CS004427	204	316
AC003783	CA916236	78	277	CA916212	102	296	CS004426	203	315
AC003784	CA916224	104	297	CA916236	78	277	CS004653	206	317
AC003785	CA004496	80	281	CA916236	78	277	CS004654	207	318
AC003786	CA004499	81	282	CA916236	78	277	CS004655	208	319
AC003787	CA916262	106	298	CA916236	78	277	CS004656	209	320
AC003791	CA916212	102	296	CA004497	67	277	CS916235	249	316
AC003792	CA004659	82	283	CA916226	77	277	CS004658	210	321
AC004089	CA916236	78	277	CA004431	79	280	CS005046	211	322
AC004090	CA916236	78	277	CA004431	79	280	CS005047	212	322
AC004091	CA916236	78	277	CA004431	79	280	CS005048	213	322
AC004092	CA916236	78	277	CA004431	79	280	CS005049	214	322
AC004187	CA916212	102	296	CA916226	77	277	CS005199	215	316
AC004188	CA916212	102	296	CA916236	78	277	CS005200	216	316
AC004988	CA005468	71	277	CA004431	79	280	CS005048	213	322
AC004989	CA004659	82	283	CA004660	69	277	CS004658	210	321
AC004990	CA005362	84	285	CA005468	71	277	CS916235	249	316
AC004991	CA005543	87	283	CA005467	70	277	CS004658	210	321
AC004992	CA005546	88	283	CA005468	71	277	CS004658	210	321
AC004999	CA005468	71	277	CA005546	88	283	CS006145	217	323

Complex ID	AS1 ID	AS1 modified SEQ ID NO:	AS1 unmodified SEQ ID NO:	AS2 ID	AS2 modified SEQ ID NO:	AS2 unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC005000	CA005468	71	277	CA005543	87	283	CS006146	218	323
AC005001	CA005468	71	277	CA005538	86	286	CS006147	219	324
AC005002	CA005468	71	277	CA005538	86	286	CS006148	220	324
AC005003	CA005468	71	277	CA004945	83	284	CS006149	221	324
AC005004	CA005468	71	277	CA004945	83	284	CS006150	222	325
AC005025	CA916258	105	283	CA004497	67	277	CS004658	210	321
AC005054	CA916258	105	283	CA005467	70	277	CS004658	210	321
AC005153	CA005546	88	283	CA005468	71	277	CS006319	223	321
AC005154	CA006321	90	288	CA005468	71	277	CS006320	224	326
AC005155	CA006323	91	289	CA005468	71	277	CS006320	224	326
AC005156	CA006325	92	290	CA005468	71	277	CS006319	223	321
AC005157	CA005468	71	277	CA004945	83	284	CS006327	225	325
AC005158	CA006329	93	284	CA005468	71	277	CS006328	226	327
AC005159	CA908055	101	295	CA005468	71	277	CS006328	226	327
AC005160	CA005468	71	277	CA908055	101	295	CS006327	225	325
AC005161	CA004945	83	284	CA005468	71	277	CS006331	227	328
AC005162	CA005468	71	277	CA005546	88	283	CS006332	228	323
AC005190	CA916258	105	283	CA005468	71	277	CS004658	210	321
AC005432	CA005468	71	277	CA005546	88	283	CS006607	229	323
AC005433	CA005546	88	283	CA005468	71	277	CS006608	230	321
AC005434	CA005468	71	277	CA005898	89	287	CS006609	231	329
AC005435	CA005898	89	287	CA005468	71	277	CS006610	232	330
AC005436	CA005468	71	277	CA005898	89	287	CS006611	233	329

Complex ID	AS1 ID	AS1 modified SEQ ID NO:	AS1 unmodified SEQ ID NO:	AS2 ID	AS2 modified SEQ ID NO:	AS2 unmodified SEQ ID NO:	SS ID	SS modified SEQ ID NO:	SS unmodified SEQ ID NO:
AC005489	CA004431	79	280	CA005468	71	277	CS006689	234	331
AC005490	CA005468	71	277	CA004431	79	280	CS006690	235	322
AC005493	CA916212	102	296	CA005468	71	277	CS006692	236	316
AC005494	CA005468	85	277	CA916212	102	296	CS006693	237	315
AC005495	CA916215	103	279	CA005468	71	277	CS006694	238	327
AC005496	CA005468	71	277	CA916215	103	279	CS006695	239	332
AC005898	CA004659	82	283	CA004497	67	277	CS004658	210	321
AC006921	CA008250	94	291	CA005468	71	277	CS006694	238	327
AC006922	CA008251	95	292	CA005468	71	277	CS006694	238	327
AC006923	CA008252	96	293	CA005468	71	277	CS006694	238	327
AC007209	CA916215	103	279	CA005468	71	277	CS008561	240	327
AC007210	CA916215	103	279	CA005468	71	277	CS008562	241	327
AC007745	CA908055	101	295	CA005468	71	277	CS009181	242	333
AC007746	CA908055	101	295	CA005468	71	277	CS009182	243	333
AC008307	CA916215	103	279	CA005468	71	277	CS009888	244	327
AC009718	CA008492	97	294	CA903799	75	278	CS011439	245	334
AC009719	CA011440	98	279	CA009929	72	277	CS006694	238	327
AC010270	CA011957	99	279	CA011958	73	277	CS011956	246	327
AC010444	CA012138	100	279	CA012139	74	277	CS012137	247	327
AC912751	CA004500	68	277	CA916212	102	296	CS916234	248	315
AC912776	CA004500	68	277	CA916212	102	296	CS004493	205	315
AC912777	CA916212	102	296	CA004497	67	277	CS916251	250	316

Table 5B. APOC3-PCSK9 RNAi Agents Complexes with Corresponding Sense and Antisense Strand ID Numbers Referencing Position Targeted on APOC3 or PCSK9 Gene (SEQ ID NOs:1 and 2).

Complex ID	Antisense Strand 1 ID	Antisense Strand 2 ID	Sense Strand ID	Antisense Strand 1 Target Gene	Antisense Strand 2 Target Gene	Targeted APOC3 Gene Position (Of SEQ ID NO:1)	Targeted PCSK9 Gene Position (Of SEQ ID NO:2)
AC003782	CA916212	CA916236	CS004427	PCSK9	APOC3	438	3536
AC003783	CA916236	CA916212	CS004426	APOC3	PCSK9	438	3536
AC003784	CA916224	CA916236	CS004653	PCSK9	APOC3	438	3468
AC003785	CA004496	CA916236	CS004654	PCSK9	APOC3	438	3523
AC003786	CA004499	CA916236	CS004655	PCSK9	APOC3	438	3531
AC003787	CA916262	CA916236	CS004656	PCSK9	APOC3	438	3536
AC003791	CA916212	CA004497	CS916235	PCSK9	APOC3	438	3536
AC003792	CA004659	CA916226	CS004658	PCSK9	APOC3	438	3531
AC004089	CA916236	CA004431	CS005046	APOC3	PCSK9	438	3536
AC004090	CA916236	CA004431	CS005047	APOC3	PCSK9	438	3536
AC004091	CA916236	CA004431	CS005048	APOC3	PCSK9	438	3536
AC004092	CA916236	CA004431	CS005049	APOC3	PCSK9	438	3536
AC004187	CA916212	CA916226	CS005199	PCSK9	APOC3	438	3536
AC004188	CA916212	CA916236	CS005200	PCSK9	APOC3	438	3536
AC004988	CA005468	CA004431	CS005048	APOC3	PCSK9	438	3536
AC004989	CA004659	CA004660	CS004658	PCSK9	APOC3	438	3531
AC004990	CA005362	CA005468	CS916235	PCSK9	APOC3	438	3536
AC004991	CA005543	CA005467	CS004658	PCSK9	APOC3	438	3531
AC004992	CA005546	CA005468	CS004658	PCSK9	APOC3	438	3531
AC004999	CA005468	CA005546	CS006145	APOC3	PCSK9	438	3531
AC005000	CA005468	CA005543	CS006146	APOC3	PCSK9	438	3531
AC005001	CA005468	CA005538	CS006147	APOC3	PCSK9	438	3501
AC005002	CA005468	CA005538	CS006148	APOC3	PCSK9	438	3501

Complex ID	Antisense Strand 1 ID	Antisense Strand 2 ID	Sense Strand ID	Antisense Strand 1 Target Gene	Antisense Strand 2 Target Gene	Targeted APOC3 Gene Position (Of SEQ ID NO:1)	Targeted PCSK9 Gene Position (Of SEQ ID NO:2)
AC005003	CA005468	CA004945	CS006149	APOC3	PCSK9	438	3536
AC005004	CA005468	CA004945	CS006150	APOC3	PCSK9	438	3536
AC005025	CA916258	CA004497	CS004658	PCSK9	APOC3	438	3531
AC005054	CA916258	CA005467	CS004658	PCSK9	APOC3	438	3531
AC005153	CA005546	CA005468	CS006319	PCSK9	APOC3	438	3531
AC005154	CA006321	CA005468	CS006320	PCSK9	APOC3	438	3531
AC005155	CA006323	CA005468	CS006320	PCSK9	APOC3	438	3531
AC005156	CA006325	CA005468	CS006319	PCSK9	APOC3	438	3531
AC005157	CA005468	CA004945	CS006327	APOC3	PCSK9	438	3536
AC005158	CA006329	CA005468	CS006328	PCSK9	APOC3	438	3536
AC005159	CA908055	CA005468	CS006328	PCSK9	APOC3	438	3536
AC005160	CA005468	CA908055	CS006327	APOC3	PCSK9	438	3536
AC005161	CA004945	CA005468	CS006331	PCSK9	APOC3	438	3536
AC005162	CA005468	CA005546	CS006332	APOC3	PCSK9	438	3531
AC005190	CA916258	CA005468	CS004658	PCSK9	APOC3	438	3531
AC005432	CA005468	CA005546	CS006607	APOC3	PCSK9	438	3531
AC005433	CA005546	CA005468	CS006608	PCSK9	APOC3	438	3531
AC005434	CA005468	CA005898	CS006609	APOC3	PCSK9	438	3501
AC005435	CA005898	CA005468	CS006610	PCSK9	APOC3	438	3501
AC005436	CA005468	CA005898	CS006611	APOC3	PCSK9	438	3501
AC005489	CA004431	CA005468	CS006689	PCSK9	APOC3	438	3536
AC005490	CA005468	CA004431	CS006690	APOC3	PCSK9	438	3536
AC005493	CA916212	CA005468	CS006692	PCSK9	APOC3	438	3536
AC005494	CA005468	CA916212	CS006693	APOC3	PCSK9	438	3536
AC005495	CA916215	CA005468	CS006694	PCSK9	APOC3	438	3536
AC005496	CA005468	CA916215	CS006695	APOC3	PCSK9	438	3536
AC005898	CA004659	CA004497	CS004658	PCSK9	APOC3	438	3531

Complex ID	Antisense Strand 1 ID	Antisense Strand 2 ID	Sense Strand ID	Antisense Strand 1 Target Gene	Antisense Strand 2 Target Gene	Targeted APOC3 Gene Position (Of SEQ ID NO:1)	Targeted PCSK9 Gene Position (Of SEQ ID NO:2)
AC006921	CA008250	CA005468	CS006694	PCSK9	APOC3	438	3536
AC006922	CA008251	CA005468	CS006694	PCSK9	APOC3	438	3536
AC006923	CA008252	CA005468	CS006694	PCSK9	APOC3	438	3536
AC007209	CA916215	CA005468	CS008561	PCSK9	APOC3	438	3536
AC007210	CA916215	CA005468	CS008562	PCSK9	APOC3	438	3536
AC007745	CA908055	CA005468	CS009181	PCSK9	APOC3	438	3536
AC007746	CA908055	CA005468	CS009182	PCSK9	APOC3	438	3536
AC008307	CA916215	CA005468	CS009888	PCSK9	APOC3	438	3536
AC009718	CA008492	CA903799	CS011439	PCSK9	APOC3	438	3531
AC009719	CA011440	CA009929	CS006694	PCSK9	APOC3	438	3536
AC010270	CA011957	CA011958	CS011956	PCSK9	APOC3	438	3536
AC010444	CA012138	CA012139	CS012137	PCSK9	APOC3	438	3536
AC912751	CA004500	CA916212	CS916234	APOC3	PCSK9	438	3536
AC912776	CA004500	CA916212	CS004493	APOC3	PCSK9	438	3536
AC912777	CA916212	CA004497	CS916251	PCSK9	APOC3	438	3536

Table 5C. APOC3-PCSK9 RNAi Agent Complexes Showing Chemically Modified Antisense Strand and Sense Strand Sequences

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
AC003782	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	(NAG43)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	204
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC003783	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	(NAG43)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	203
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacsg	102		
AC003784	AS1	cPrpusGfauaaaugucUfgCfuUfgcsc	104	(NAG47)sggcaagCfAfGfacauuuaucas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	206
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC003785	AS1	cPrpuAfaacaggucuAfgAfaAfagsc	80	(NAG47)sgcuuuuCfUfAfgaccuguuuas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	207
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC003786	AS1	cPrpusCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	81	(NAG47)sggaccuGfUfUfuugcuuuugas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	208
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC003787	AS1	cPrpusAfsguuacaaaaGfcAfaAfacsg	106	(NAG47)scguuuuGfcUfuUfuguaacuas(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	209
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC003791	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	(NAG52)scguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	249
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	67		
AC003792	AS1	cPrpasCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	82	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	usCfsasCfuGfagaauAfcUfgUfcCfsc	77		
AC004089	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaaacuas(invAb)sC6-NH2	211
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		
AC004090	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	(NAG52)sgggacaGfUfAfuucucaguisa-Sp18-usguuuuGfcUfuUfuguaaacuas(invAb)	212
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		
AC004091	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-usguuuuGfCfUfuuuguaaacuas(invAb)sC6-NH2	213
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
AC004092	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78	(NAG52)sgggacaGfUfAfuucucaguisa-Sp18-usguuuuGfCfUfuuguaaacuas(invAb)	214
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		
AC004187	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	(NAG37)scguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	215
	AS2	usCfsasCfuGfagaauAfcUfgUfcCfsc	77		
AC004188	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	(NAG42)scguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	216
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	78		
AC004988	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-usguuuuGfCfUfuuguaaacuas(invAb)sC6-NH2	213
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		
AC004989	AS1	cPrpasCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	82	(NAG52)sgggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	usCfsasCfuGfagaauAfcUfgUfcCfsc	69		
AC004990	AS1	cPrpasAfsguuacaaaaGfcAfaAfacgsg	84	(NAG52)scguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	249
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC004991	AS1	cPrpasCfsaaaagcaaaAfcAfgGfuscsc	87	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	cPrpusCfsaCfuGfagaauAfcUfgUfcsCfsc	70		
AC004992	AS1	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC004999	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-ggaccuGfuUfUfugcuuuugus(invAb)	217
	AS2	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88		
AC005000	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-(invAb)sggaccuGfUfUfuugcuuuugus(invAb)	218
	AS2	cPrpasCfsaaaagcaaaAfcAfgGfuscsc	87		
AC005001	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-csccuguUfGfCfcuuuuuacaas(invAb)	219
	AS2	cPrpusUfsguaa_2NaaaggCfaAfcAfgsgsg	86		
AC005002	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		220

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
	AS2	cPrpusUfsguaa_2NaaaggCfaAfcAfgsgsg	86	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-(invAb)scccuguUfGfCfcuuuuuacaas(invAb)	
AC005003	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-	221
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacasg	83	csuguuuuGfCfUfuuuguaacuus(invAb)	
AC005004	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-	222
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacasg	83	(invAb)scuguuuuGfCfUfuuuguaacuus(invAb)	
AC005025	AS1	asCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	105	(NAG52)sggaccuGfUfUfugcuuuugus(invAb)-Sp18-	210
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	67	gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	
AC005054	AS1	asCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	105	(NAG52)sggaccuGfUfUfugcuuuugus(invAb)-Sp18-	210
	AS2	cPrpusCfsaCfuGfagaauAfcUfgUfcsCfsc	70	gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	
AC005153	AS1	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88	(NAG52)sgsgaccuGfuUfUfugcuuuugus(invAb)-Sp18-	223
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	
AC005154	AS1	cPrpaCfaaaagcaaaAfcAfgGfucsusasg	90	(NAG52)sasgaccuGfuUfUfugcuuuugus(invAb)-Sp18-	224
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	
AC005155	AS1	cPrpaCfaaaagcaaaAfcAfgGfucsusa	91	(NAG52)sasgaccuGfuUfUfugcuuuugus(invAb)-Sp18-	224
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	
AC005156	AS1	cPrpaCfaaaagcaaaAfcAfgGfucscsa	92	(NAG52)sgsgaccuGfuUfUfugcuuuugus(invAb)-Sp18-	223
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	
AC005157	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sggggacaGfUfAfuucucaguias(invAb)-Sp18-	225
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacasg	83	csuguuuuGfcUfuUfuguaacuus(invAb)	
AC005158	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsasg	93	(NAG52)suguuuuuGfcUfuUfuguaacuus(invAb)-Sp18-	226
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	
AC005159	AS1	cPrpasAfsguuacaaaaGfcAfaAfacasgsg	101	(NAG52)suguuuuuGfcUfuUfuguaacuus(invAb)-Sp18-	226
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	gsggacaGfUfAfuucucaguias(invAb)	

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
AC005160	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csuguuuuGfcUfuUfuguaaacuus(invAb)	225
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacasgsg	101		
AC005161	AS1	cPrpasAfsguuacaaaaGfcAfaAfacasg	83	(NAG52)scsuguuuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)	227
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005162	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-gsgaccuGfuUfUfugcuuuugus(invAb)sC6-NH2	228
	AS2	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88		
AC005190	AS1	asCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	105	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005432	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sggggacaGfuAfuUfcucaguias(invAb)-Sp18-ggaccuGfuUfUfugcuuuugus(invAb)sC6-NH2	229
	AS2	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88		
AC005433	AS1	cPrpaCfaaaagcaaaAfcAfgGfuscsc	88	(NAG52)sggaccuGfuUfUfugcuuuugus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	230
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005434	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-cucuguUfgCfcUfuuuuacaas(invAb)sC6-NH2	231
	AS2	cPrpusUfguaa_2NaaaggCfaAfcAfgsasg	89		
AC005435	AS1	cPrpusUfguaa_2NaaaggCfaAfcAfgsasg	89	(NAG52)scucuguUfgCfcUfuuuuacaas(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	232
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005436	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-csucuguUfgCfcUfuuuuacaas(invAb)sC6-NH2	233
	AS2	cPrpusUfguaa_2NaaaggCfaAfcAfgsasg	89		
AC005489	AS1	cPrpusAfsguuacaaaaGfcAfaAfacsa	79	(NAG52)suguuuuuGfcUfuUfuguaaacuas(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	234
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005490	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaaacuas(invAb)sC6-NH2	235
	AS2	cPrpusAfsguuacaaaaGfcAfaAfacsa	79		
AC005493	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102		236

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)scguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	
AC005494	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	85	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	237
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacsg	102		
AC005495	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsa	103	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC005496	AS1	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71	(NAG52)sgggacaGfuAfuUfcucaguias(invAb)-Sp18-usguuuuGfcUfuUfuguaacuus(invAb)sC6-NH2	239
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacsa	103		
AC005898	AS1	cPrpasCfsasAfaAfgCfaAfaAfcAfgGfuCfsc	82	(NAG52)sggaccuGfUfUfuugcuuuugus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	210
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	67		
AC006921	AS1	cPrpisAfsguuacaaaaGfcAfaAfacsa	94	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC006922	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsi	95	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC006923	AS1	cPrpisAfsguuacaaaaGfcAfaAfacsi	96	(NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC007209	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsa	103	usguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	240
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC007210	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsa	103	usguuuuGfcUfuUfuguaaacuus(invAb)s-Sp18-sgsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	241
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC007745	AS1	cPrpasAfsguuacaaaaGfcAfaAfacasgsg	101	(NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	242
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC007746	AS1	cPrpasAfsguuacaaaaGfcAfaAfacasgsg	101	(NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)	243
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		

Complex ID		Modified Antisense Strand (5′ → 3′)	SEQ ID NO.	Modified Sense Strand (5′ → 3′)	SEQ ID NO.
AC008307	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsa	103	(NAG52)suguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)	244
	AS2	cPrpusCfsaCfugagaauAfcUfgUfcscsc	71		
AC009718	AS1	cPrpaCfaaaagcaaaAfcAfgGfuCfcsAfsg	97	(NAG52)scuggaccuGfuUfUfugcuuuugus(invAb)-Sp18-ascgggacaGfUfAfuucucaguias(invAb)	245
	AS2	usCfsasCfuGfagaauAfcUfgUfcCfcGfsu	75		
AC009719	AS1	cPrpasAfsguuacaaaaGfcAfaAfascsa	98	(NAG52)suguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	238
	AS2	cPrpusCfsaCfugagaauAfcUfgUfccsc	72		
AC010270	AS1	cPrpasAfsguuacaAfaaGfcAfaAfacsa	99	(NAG52)suguuuuGfcUfuuuguaaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb)sC6-NH2	246
	AS2	cPrpusCfsaCfugAfgaauAfcUfgUfcscsc	73		
AC010444	AS1	cPrpasAfsguuacaaaaGfcAfaaacsa	100	(NAG52)suguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaguAfuUfcucaguias(invAb)sC6-NH2	247
	AS2	cPrpusCfsaCfugagaauAfcugUfcscsc	74		
AC912751	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	68	(NAG52)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaaacuus(invAb)sC6-NH2	248
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacsg	102		
AC912776	AS1	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	68	(TriAlk14)sgggacaGfUfAfuucucaguias(invAb)-Sp18-csguuuuGfcUfuUfuguaaacuus(invAb)sC6-NH2	205
	AS2	cPrpasAfsguuacaaaaGfcAfaAfacsg	102		
AC912777	AS1	cPrpasAfsguuacaaaaGfcAfaAfacsg	102	(TriAlk14)scguuuuGfcUfuUfuguaaacuus(invAb)-Sp18-gsggacaGfUfAfuucucaguias(invAb)sC6-NH2	250
	AS2	cPrpusCfsasCfuGfagaauAfcUfgUfcCfsc	67		

[0091] In some embodiments, an APOC3-PCSK9 RNAi agent is prepared or provided as a salt, mixed salt, or a free-acid. The multimeric RNAi agents described herein, upon delivery to a cell expressing an APOC3 and/or PCSK9 gene, inhibit or knockdown expression of one or more APOC3 and/or PCSK9 genes *in vivo* and/or *in vitro*.

Targeting Ligands and Targeting Groups

[0092] As disclosed herein, the multimeric RNAi agent conjugate delivery platform is comprised of one or more targeting groups. 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 molecule, cell receptor ligands, hapten, 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 in some instances can serve as linkers.

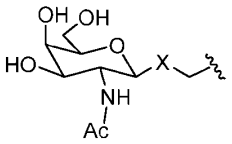
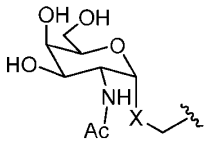
[0093] In some embodiments, a targeting group is covalently linked to the 3′ and/or 5′ end of either the sense strand and/or the antisense strand of an RNAi agent. In some embodiments, a targeting ligand is linked to the 3′ and/or 5′ end of the sense strand of one of the RNAi agents. In some embodiments, a targeting group is linked to the 5′ end of an RNAi agent sense strand of one RNAi agent. In some embodiments, a targeting group is linked internally to one or more nucleotides of an RNAi agent sense strand. In some embodiments, a targeting ligand is positioned between two RNAi agents in the multimeric RNAi agent conjugate. A targeting group may be linked directly or indirectly to the RNAi agent via a linker/linking group. In some embodiments, a targeting group is linked to the RNAi agent via a metabolically stabilized bond or linkage.

[0094] In some embodiments, a targeting group comprises an asialoglycoprotein receptor ligand. As used herein, an asialoglycoprotein receptor ligand is a ligand that contains a moiety 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-Acetylgalactosamine, 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), as well as metabolically stabilized glycosidic linked N-Acetylgalactosamine. 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).

[0095] 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, also referred to as monovalent or monodentate) 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 galactose derivative or galactose derivative cluster can also be attached internally to one or more nucleotides the sense or antisense strand of the RNAi agent using methods known in the art.

In some embodiments, the targeting ligand is comprised of one or more metabolically stabilized N-Acetylgalactosamine (NAG or GalNAc) targeting ligands that includes the structure of the following Formulae:

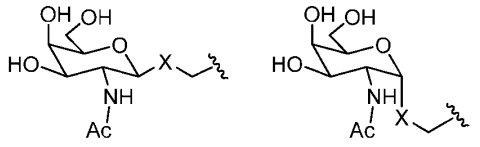
[0096]  Formula I or  Formula II , wherein X = CH_2 or S.

[0097] In some embodiments, the metabolically stabilized NAG targeting ligand is a trimer (also referred to as tri-antennary or tri-valent), wherein three moieties of Formula I or Formula II are attached through a centralized branch point. (*See, e.g.*, the chemical structure referred to herein of NAG52). In some embodiments, the targeting ligand is a cluster of four metabolically stabilized NAG moieties thereby forming a tetramer (also referred to as tetra-antennary or tetra-

valent) targeting ligand. In some embodiments, the metabolically stabilized NAG targeting ligand is a bi-antennary or bi-valent), wherein two moieties of Formula I or Formula II are attached through a centralized branch point.

[0098] As used herein, a metabolically stabilized NAG targeting ligand contains one or more moieties of Formula I or Formula II, each linked to a central branch point. In some embodiments, the targeting ligands 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, e.g., U.S. Patent No. 5,885,968; Biessen et al. J. Med. Chem. 1995 Vol. 39 p. 1538-1546). The branch point can be any small molecule which permits attachment of three galactose derivatives and further permits attachment of the branch point to an 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.

[0099] In some embodiments, a delivery platform disclosed herein comprises one or more targeting ligands that include a compound of Formula I or Formula II:

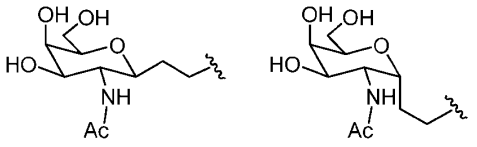


Formula I or Formula II ,

or a pharmaceutically acceptable salt thereof,

wherein X = CH_2 or S.

[00100] In some embodiments, a delivery platform disclosed herein comprises one or more targeting ligands that include a compound of Formula 1a or Formula 1b:



Formula Ia or Formula IIa .

[00101] Methods of making compounds of Formula Ia are described in the Examples below.

[00102] In some embodiments, compounds that may be conjugated to RNAi agents to synthesize a delivery platform for an RNAi agent are shown in Table 6 below, or a pharmaceutically acceptable salt thereof.

Linking Groups

[00103] In some embodiments, an RNAi agent contains or is conjugated to one or more non-nucleotide groups including, but not limited to 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 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 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 RNAi agent sense strand. A non-nucleotide group can 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.

[00104] 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 conjugate. In some embodiments, a non-nucleotide group enhances endocytosis of the RNAi agent.

[00105] The 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.

[00106] 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 ligand, targeting group, PK/PD modulator, 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 may further add flexibility and/or length to the linkage. Spacers include, but are not 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.

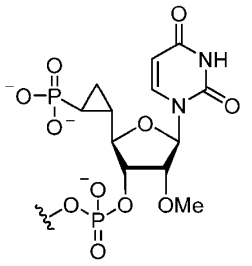
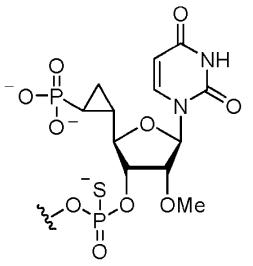
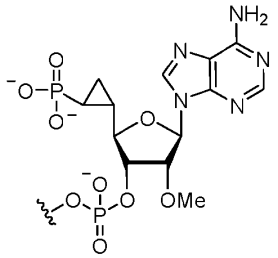
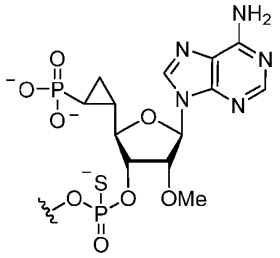
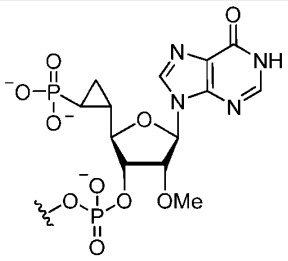
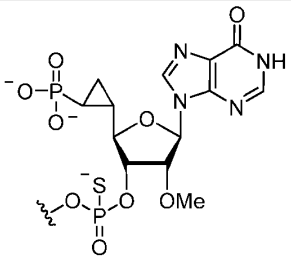
[00107] In some embodiments, targeting groups are linked to RNAi agents without the use of an additional linker. In some embodiments, the targeting group is designed having a linker readily present to facilitate the linkage to an RNAi agent. In some embodiments, when two or more RNAi agents are included in a composition, the two or more RNAi agents can be linked to their respective targeting groups using the same linkers. In some embodiments, when two or

more RNAi agents are included in a composition, the two or more RNAi agents are linked to their respective targeting groups using different linkers.

[00108] In some embodiments, a linking group may be conjugated synthetically to the 5' or 3' end of the sense strand of an RNAi agent described herein. In some embodiments, a linking group is conjugated synthetically to the 5' end of the sense strand of an RNAi agent. In some embodiments, a linking group conjugated to an RNAi agent may be a trialkyne linking group.

[00109] Examples of certain modified nucleotides and linking groups, are provided in Table 6.

[00110] **Table 6.** Structures Representing Various Modified Nucleotides and Linking Groups

 cPrpu	 cPrpus
 cPrpa	 cPrpas
 cPrpi	 cPrpis

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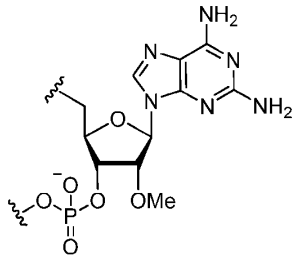
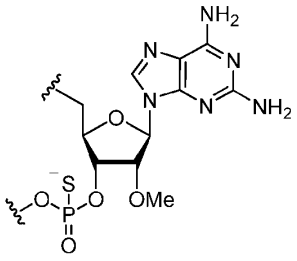
<tr><td>
a_2N</td><td>
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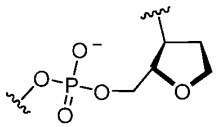
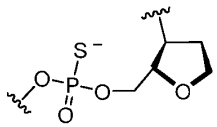
linkage towards 5' end

linkage towards 3' end
(invAb)</td><td>linkage towards 5' end

linkage towards 3' end
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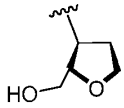
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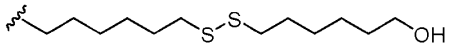
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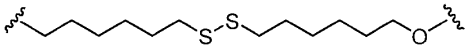
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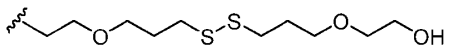
<tr><td colspan="2">When position at the 3' terminal end:
linkage towards 5' end

(6-SS-6)</td></tr>
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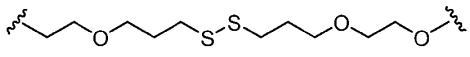
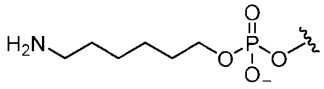
When positioned internally:

linkage towards 5' end linkage towards 3' end



(6-SS-6)



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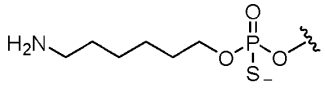
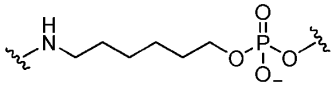
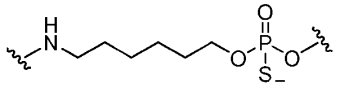


(NH2-C6)s



-C6-



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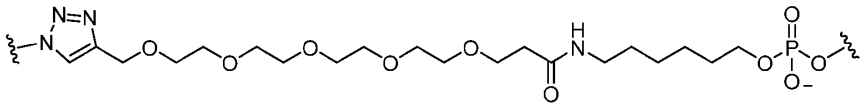
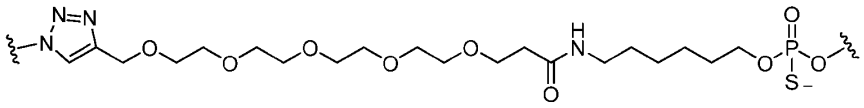


-L6-C6-



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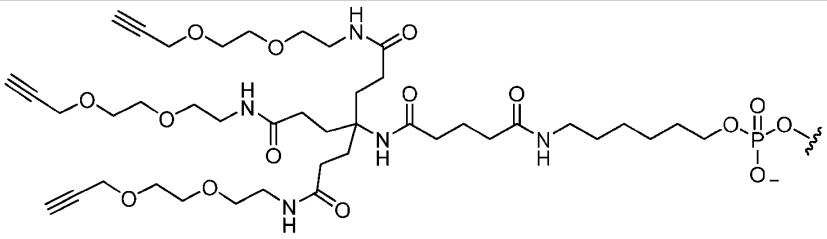

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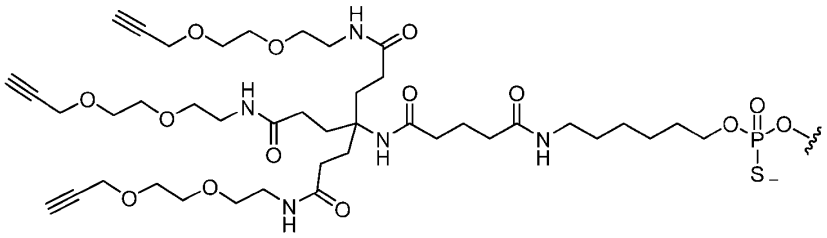

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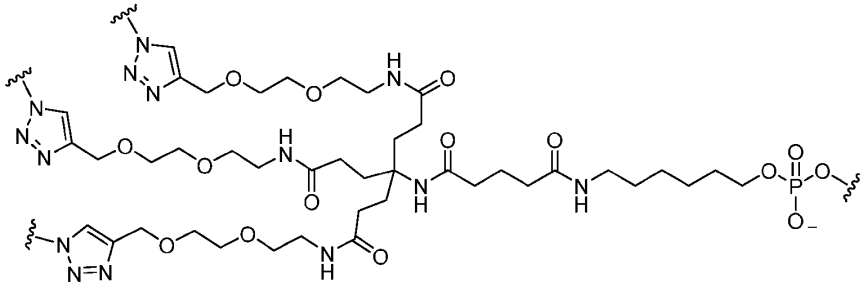

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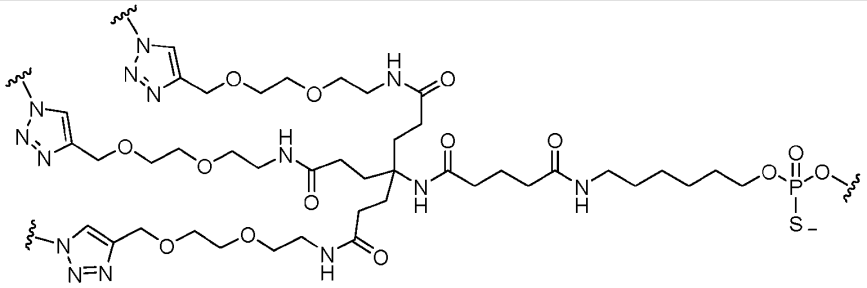

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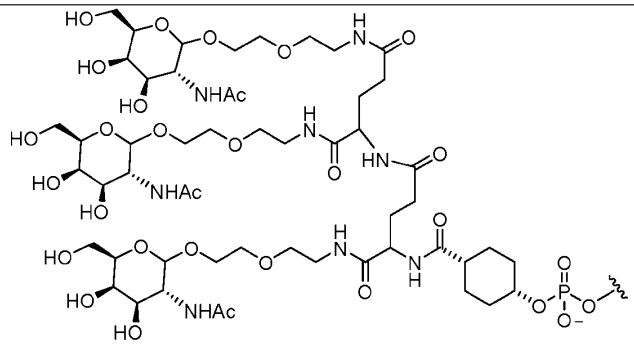

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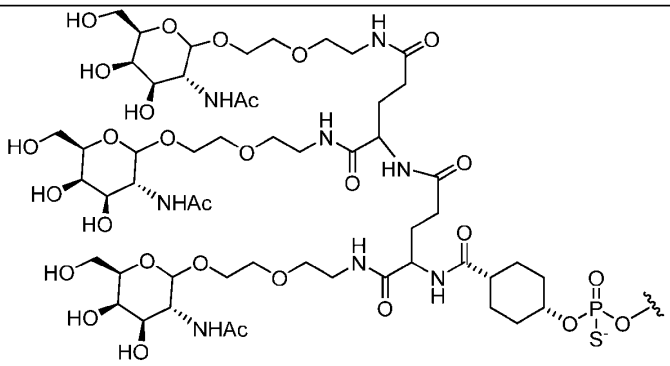

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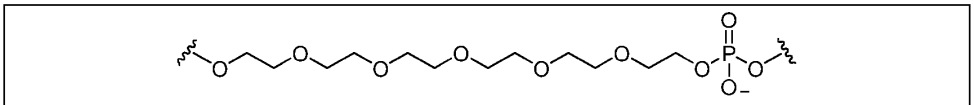

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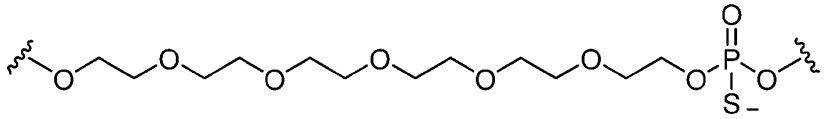

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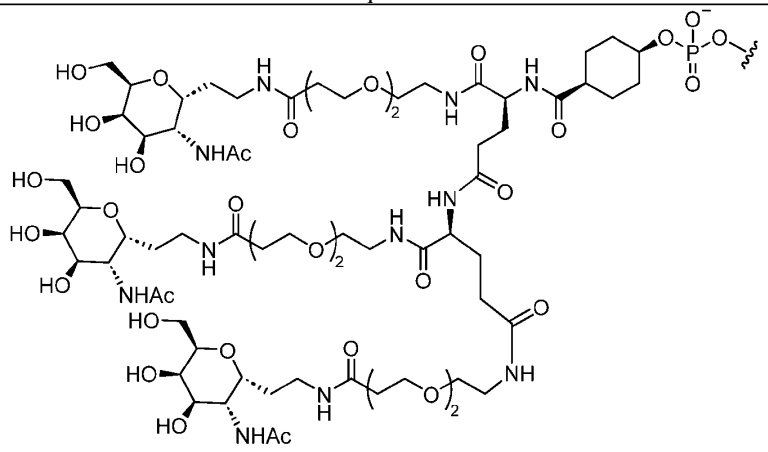

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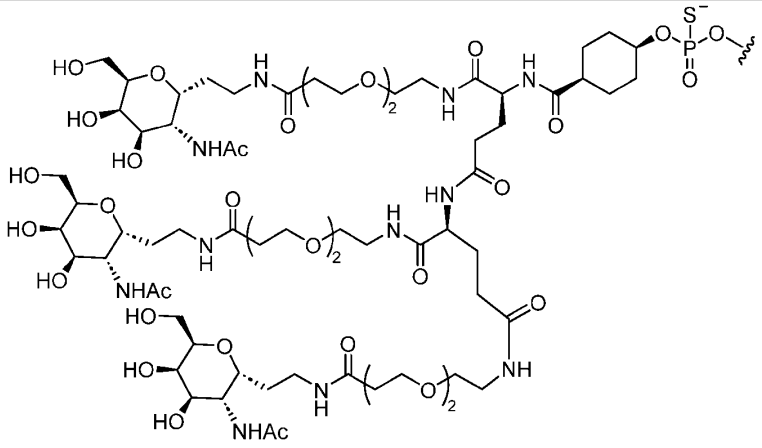

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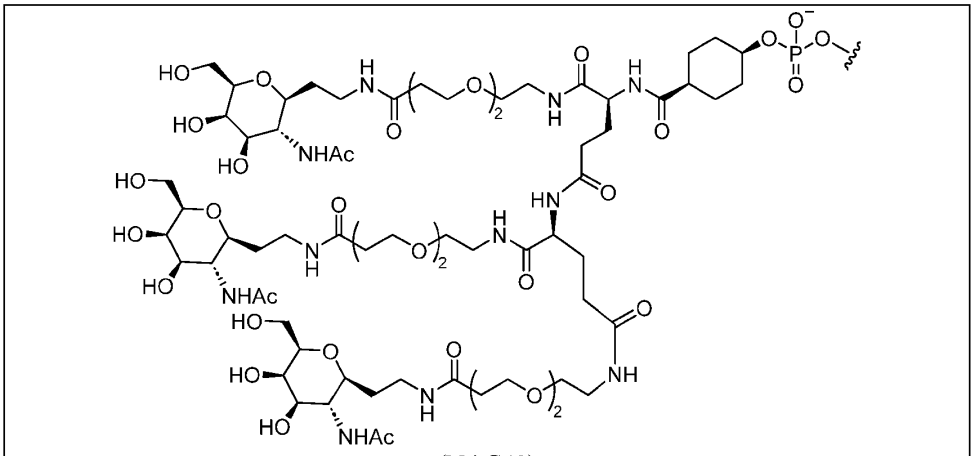

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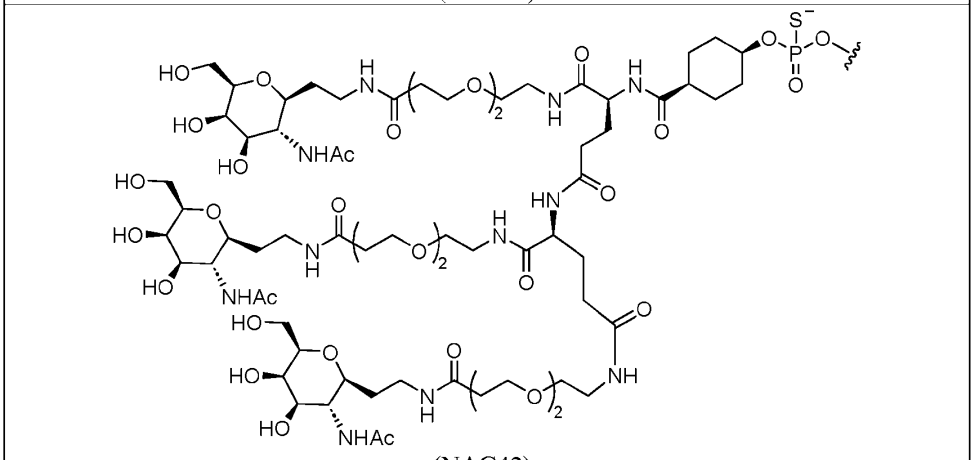

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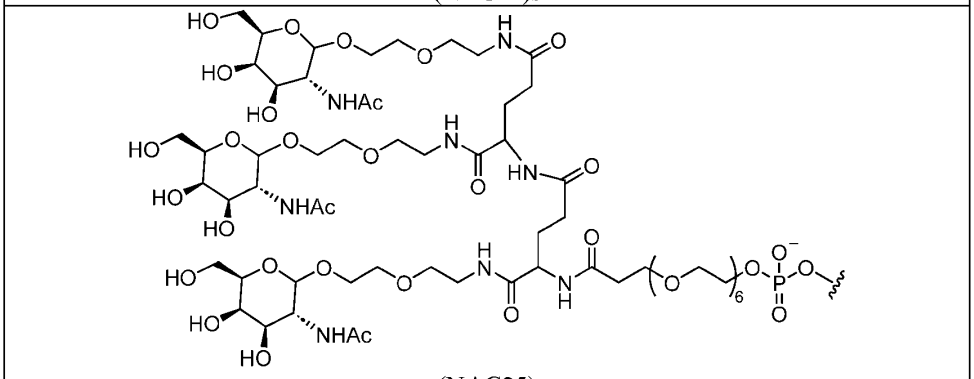

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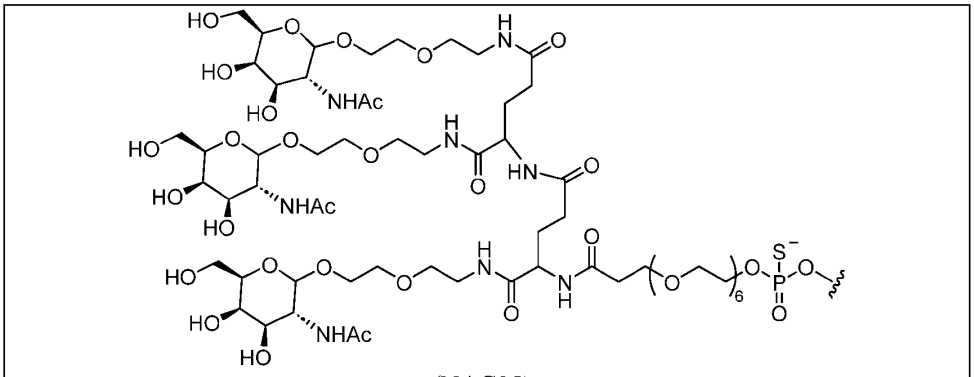

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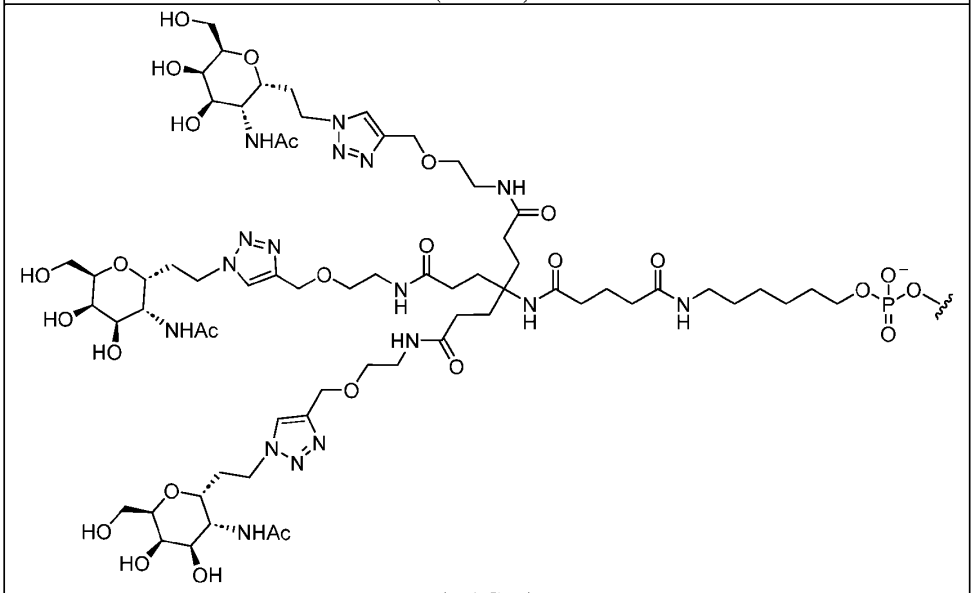

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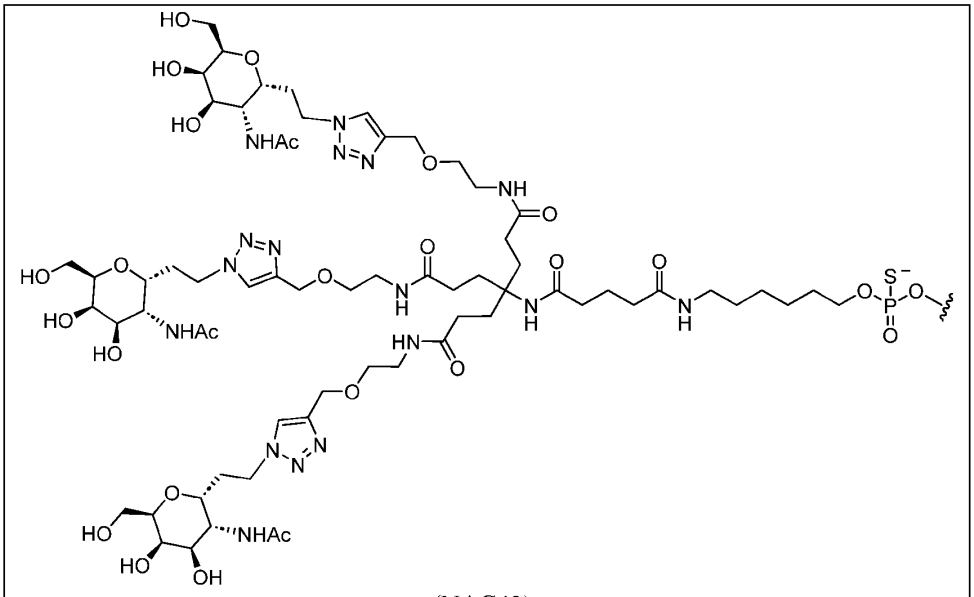

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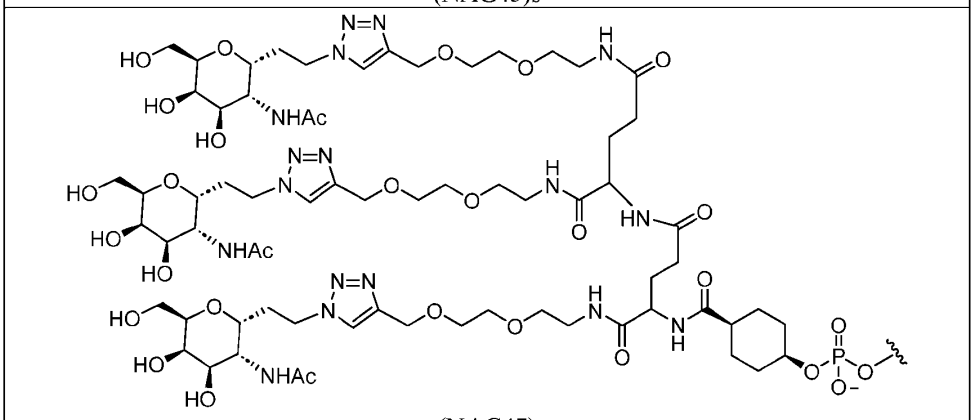

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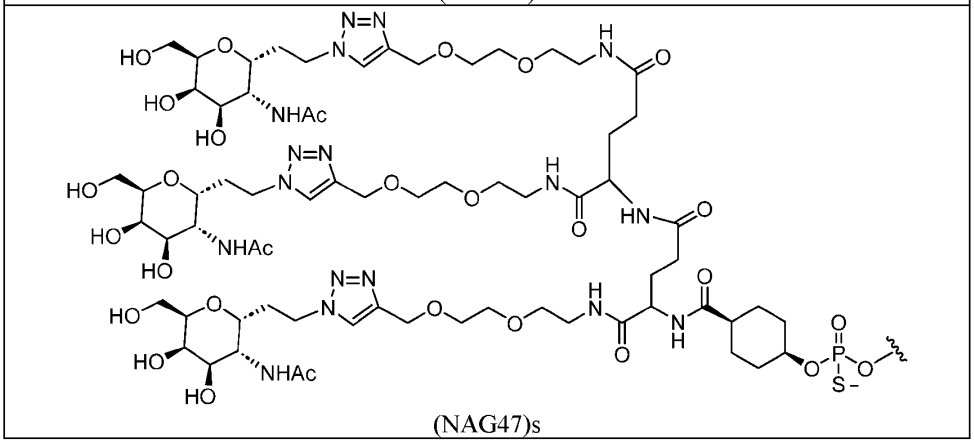


(NAG47)s

[00111] Alternatively, other linking groups known in the art may be used.

[00112] In addition or alternatively to linking an RNAi agent to one or more targeting ligands, targeting groups, and/or PK/PD modulators, in some embodiments, a delivery vehicle may be used to deliver an RNAi agent to a cell or tissue. A delivery vehicle is a compound that can improve delivery of the RNAi agent to a cell or tissue, and 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.

[00113] 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 2008/022309, 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

[00114] The APOC3-PCSK9 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 APOC3-PCSK9 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.

[00115] 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 APOC3 and/or PCSK9 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, symptom, 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 APOC3-PCSK9 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 APOC3-PCSK9 RNAi agent, thereby forming a pharmaceutical formulation or medicament suitable for *in vivo* delivery to a subject, including a human.

[00116] The pharmaceutical compositions that include an APOC3-PCSK9 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 APOC3-PCSK9 RNAi agent, thereby inhibiting the expression of APOC3 and/or PCSK9 mRNA in the subject. In some embodiments, the subject has been previously identified as having a pathogenic upregulation of the target gene in hepatocytes. In some embodiments, the subject has been previously identified or diagnosed as having obesity, diabetes, liver inflammation, dyslipidemia, or metabolic disease. In some embodiments, the subject has been suffering from symptoms associated with diseases such as obesity, diabetes, liver inflammation, dyslipidemia, or metabolic disease. In some embodiments, the subject would benefit from a reduction of APOC3 and/or PCSK9 gene expression in the subject's liver.

[00117] In some embodiments, the described pharmaceutical compositions including an APOC3-PCSK9 RNAi agent are used for treating or managing clinical presentations associated with Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome (FCS), familial partial lipodystrophy, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD), atherosclerosis, other APOC3-related disease, or other PCSK9-related disease.. 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 APOC3-PCSK9 RNAi agents can be used to decrease the number, severity, and/or frequency of symptoms of a disease in a subject.

[00118] In some embodiments, the subject is administered a therapeutically effective amount of one or more pharmaceutical compositions that include an APOC3-PCSK9 RNAi agent thereby treating the symptom. In other embodiments, the subject is administered a prophylactically effective amount of one or more APOC3-PCSK9 RNAi agents, thereby preventing or inhibiting the at least one symptom.

[00119] The route of administration is the path by which an APOC3-PCSK9 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 APOC3-PCSK9 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.

[00120] In some embodiments, the herein described pharmaceutical compositions are administered via subcutaneous injection.

[00121] The pharmaceutical compositions including an APOC3-PCSK9 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.

[00122] 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.

[00123] 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.*, APOC3-PCSK9 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.

[00124] 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.

[00125] 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.

[00126] 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.

[00127] In some embodiments, pharmaceutical formulations that include the APOC3-PCSK9 RNAi agents disclosed herein suitable for subcutaneous administration can be prepared

in an aqueous sodium phosphate buffer (e.g., the APOC3-PCSK9 RNAi agent formulated in 0.5 mM sodium phosphate monobasic, 0.5 mM sodium phosphate dibasic, in water). In some embodiments, pharmaceutical formulations that include the APOC3-PCSK9 RNAi agents disclosed herein suitable for subcutaneous administration can be prepared in water for injection (sterile water). APOC3-PCSK9 RNAi agents disclosed herein suitable for subcutaneous administration can be prepared in isotonic saline (0.9%).

[00128] 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.

[00129] Formulations suitable for oral administration of the APOC3-PCSK9 RNAi agents disclosed herein can also be prepared. In some embodiments, the APOC3-PCSK9 RNAi agents disclosed herein are administered orally. In some embodiments, the APOC3-PCSK9 RNAi agents disclosed herein are formulated in a capsule for oral administration.

[00130] 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.

[00131] The APOC3-PCSK9 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.

[00132] 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.

[00133] 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 APOC3-PCSK9 RNAi agent (e.g., an APOC3-PCSK9 RNAi agent that targets a different sequence within the APOC3 and/or PCSK9 target). In other embodiments, the second therapeutic can be a small molecule drug, an antibody, an antibody fragment, or an aptamer.

[00134] In some embodiments, the described APOC3-PCSK9 RNAi agent(s) are optionally combined with one or more additional therapeutics. The APOC3-PCSK9 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 APOC3-PCSK9 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 APOC3-PCSK9 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 obesity, diabetes, liver inflammation, dyslipidemia, or metabolic disease. In some embodiments, the described APOC3-PCSK9 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 APOC3-PCSK9 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 APOC3-PCSK9 RNAi agents, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

[00135] Generally, an effective amount of an APOC3-PCSK9 RNAi agent will be in the range of from about 0.1 to about 100 mg/kg of body weight/dose, e.g., from about 1.0 to about 50 mg/kg of body weight/dose. 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. In some embodiments, an effective amount of an APOC3-PCSK9 RNAi agent may be a fixed dose. In some embodiments, the fixed dose is in the range of from about 5 mg to about 1,000 mg of APOC3-PCSK9 RNAi agent. In some embodiments, the fixed does is in the range of 50 to 400 mg of APOC3-PCSK9 RNAi agent. Dosing may be weekly, bi-weekly, monthly, quarterly, or at any other interval depending on the dose of APOC3-PCSK9 RNAi agent administered, the activity level of the particular APOC3-PCSK9 RNAi agent, and the desired level of inhibition for the particular subject. The Examples herein show suitable levels for inhibition in certain animal species. The amount administered will depend on such variables as the overall health status of the patient or subject, 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.

[00136] For treatment of disease or for formation of a medicament or composition for treatment of a disease, the pharmaceutical compositions described herein including an APOC3-PCSK9 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.

[00137] The described APOC3-PCSK9 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, pen injectors, autoinjectors, infusion bags/devices, or vials.

Methods of Treatment and Inhibition of Expression

[00138] The APOC3-PCSK9 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 multimeric 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 APOC3 and/or PCSK9 mRNA and/or APOC3 and/or PCSK9

protein levels, a subject that has been diagnosed with or is suffering from symptoms related to diseases such as obesity, diabetes, liver inflammation, dyslipidemia, or metabolic disease.

[00139] In some embodiments, the subject is administered a therapeutically effective amount of any one or more APOC3-PCSK9 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 APOC3-PCSK9 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.

[00140] The APOC3-PCSK9 RNAi agents described herein can be used to treat at least one symptom in a subject having an APOC3- and/or PCSK9-related disease or disorder, or having a disease or disorder that is mediated at least in part by APOC3 and/or PCSK9 gene expression. In some embodiments, the APOC3-PCSK9 RNAi agents are used to treat or manage a clinical presentation of a subject with a disease or disorder that would benefit from or be mediated at least in part by a reduction in APOC3 and/or PCSK9 mRNA. The subject is administered a therapeutically effective amount of one or more of the APOC3-PCSK9 RNAi agents or APOC3-PCSK9 RNAi agent-containing compositions described herein. In some embodiments, the methods disclosed herein comprise administering a composition comprising an APOC3-PCSK9 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 APOC3-PCSK9 RNAi agents, thereby treating the subject by preventing or inhibiting the at least one symptom.

[00141] In certain embodiments, the present disclosure provides methods for treatment of diseases, disorders, conditions, or pathological states mediated at least in part by APOC3 and/or PCSK9 gene expression, in a patient in need thereof, wherein the methods include administering to the patient any of the APOC3-PCSK9 RNAi agents described herein.

[00142] In some embodiments, the 5' end of the sense strand is coupled to a targeting ligand comprising the structure of (NAG52)s.

[00143] In some embodiments, the gene expression level and/or mRNA level of an APOC3 and/or PCSK9 gene in a subject to whom a described APOC3-PCSK9 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 APOC3-PCSK9 RNAi agent or to a subject not receiving the APOC3-PCSK9 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 APOC3 and/or PCSK9 gene expression is inhibited by at least about 30%, 35%, 40%, 45% 50%, 55%, 60%, 65%, or greater than 65% in the cytoplasm of hepatocytes relative to the subject prior to being administered the APOC3-PCSK9 RNAi agent or to a subject not receiving the APOC3-PCSK9 RNAi agent.

[00144] In some embodiments, the APOC3 and/or PCSK9 protein expression level in a subject to whom a described APOC3-PCSK9 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 APOC3-PCSK9 RNAi agent or to a subject not receiving the APOC3-PCSK9 RNAi agent. The protein expression level in the subject may be reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject.

[00145] A reduction in APOC3 and/or PCSK9 mRNA expression levels and APOC3 and/or PCSK9 protein expression levels can be assessed by any methods known in the art. As used herein, a reduction or decrease in APOC3 and/or PCSK9 mRNA level and/or protein level are collectively referred to herein as a reduction or decrease in APOC3 and/or PCSK9 or inhibiting or reducing the gene expression of APOC3 and/or PCSK9. The Examples set forth herein illustrate known methods for assessing inhibition of APOC3 and/or PCSK9 gene expression. The person of ordinary skill in the art would further know suitable methods for assessing inhibition of APOC3 and/or PCSK9 gene expression *in vivo* and/or *in vitro*.

[00146] In some embodiments, disclosed herein are methods of treatment (including prophylactic or preventative treatment) of diseases, disorders, or symptoms caused by diseases such as Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome (FCS), familial partial lipodystrophy, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD), atherosclerosis, other APOC3-related disease, or other PCSK9-related disease, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an APOC3-PCSK9 RNAi agent that includes an antisense strand that is at least partially complementary to the portion of the

APOC3 and/or PCSK9 mRNA having the sequence in Table 1A or Table 1B. In some embodiments, disclosed herein are methods of treatment (including prophylactic or preventative treatment) of diseases or symptoms caused by diseases such as Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome (FCS), familial partial lipodystrophy, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD), atherosclerosis, other APOC3-related disease, or other PCSK9-related disease, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an APOC3-PCSK9 RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B or 5C, and a sense strand that comprises any of the sequences in Tables 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C 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 diseases such as obesity, diabetes, liver inflammation, dyslipidemia, or metabolic disease, wherein the methods include administering to a subject in need thereof a therapeutically effective amount of an APOC3-PCSK9 RNAi agent that includes a sense strand that comprises any of the sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C, and an antisense strand comprising the sequence of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C that is at least partially complementary to the sense strand.

[00147] In some embodiments, the 5' end of the sense strand is coupled to a targeting ligand comprising the structure of (NAG52)s.

[00148] In some embodiments, disclosed herein are methods for inhibiting expression of an APOC3 and/or PCSK9 gene in a cell, wherein the methods include administering to the cell an APOC3-PCSK9 RNAi agent that includes an antisense strand that is at least partially complementary to the portion of the APOC3 and/or PCSK9 mRNA having the sequence in Table 1A or Table 1B. In some embodiments, disclosed herein are methods of inhibiting expression of an APOC3 and/or PCSK9 gene in a cell, wherein the methods include administering to a cell an APOC3-PCSK9 RNAi agent that includes an antisense strand

comprising the sequence of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C and a sense strand that comprises any of the sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C that is at least partially complementary to the antisense strand. In some embodiments, disclosed herein are methods of inhibiting expression of an APOC3 and PCSK9 gene in a cell, wherein the methods include administering an APOC3-PCSK9 RNAi agent that includes a sense strand that comprises any of the sequences in Table 2A, Table 2B, Table 4A, Table 4B, Table 4C, or Table 5C, and an antisense strand that includes the sequence of any of the sequences in Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C that is at least partially complementary to the sense strand.

[00149] In some embodiments, the APOC3-PCSK9 RNAi agents are administered to a subject in need thereof as a first line therapy. In some embodiments, the APOC3-PCSK9 RNAi agents are administered to a subject in need thereof as a second line therapy. In certain embodiments, the APOC3-PCSK9 RNAi agents are administered as a second line therapy to patients who have failed one or more first line standard of care therapies. In certain embodiments, the APOC3-PCSK9 RNAi agents are administered as a maintenance therapy following the administration of one or more prior therapies. In certain embodiments, the APOC3-PCSK9 RNAi agents administered as a maintenance therapy following the administration of one or more standard of care therapies. In some embodiments, the APOC3-PCSK9 RNAi agents administered in combination with one or more additional therapies. In some embodiments, the one or more additional therapies is a standard of care therapy. In some embodiments, the one or more additional therapies is an oral therapy.

[00150] The use of APOC3-PCSK9 RNAi agents provides methods for therapeutic (including prophylactic) treatment of diseases/disorders associated with diseases such as Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome (FCS), familial partial lipodystrophy, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD), atherosclerosis, other APOC3-related disease, or other PCSK9-related disease. The described APOC3-PCSK9 RNAi agents mediate RNA interference to inhibit the expression of one or more genes necessary for production of APOC3 protein and/or PCSK9

protein. APOC3-PCSK9 RNAi agents can also be used to treat or prevent various diseases, disorders, or conditions, including diseases such as Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome (FCS), familial partial lipodystrophy, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD), atherosclerosis, other APOC3-related disease, or other PCSK9-related disease.. Furthermore, compositions for delivery of APOC3-PCSK9 RNAi agents to liver cells, and specifically to hepatocytes, *in vivo*, are described.

Cells, Tissues, Organs, and Non-Human Organisms

[00151] Cells, tissues, organs, and non-human organisms that include at least one of the APOC3-PCSK9 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.

ILLUSTRATIVE EMBODIMENTS

[00152] Provided here are illustrative embodiments of the disclosed technology. These embodiments are illustrative only and do not limit the scope of the present disclosure or of the claims attached hereto.

1. An RNAi agent, for inhibiting the expression of an Apolipoprotein C-III (APOC3) gene and a Proprotein Convertase Subtilisin Kexin 9 (PCSK9) gene, comprising:

(i) a first antisense strand that comprises at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any of the sequences of Table 2A, or Table 2B, Table 3A, Table 3B, or Table 5C, and

(ii) a second antisense strand that comprises at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any of the sequences of Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C, and

(iii) one or more sense strands,

wherein the one or more sense strands comprise a nucleotide sequence that is at least partially complementary to the first antisense strand and the second antisense strand.

2. The RNAi agent of embodiment 1, wherein the one or more sense strands comprise a nucleotide sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C.

3. The RNAi agent of embodiment 1 or embodiment 2, wherein the RNAi agent comprises a first sense strand sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C and a second sense strand sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C.

4. The RNAi agent of any one of embodiments 1-3, wherein the RNAi agent comprises a first antisense strand sequence of any one of the sequences of Table 2A, Table 3A, or Table 5C, and a second sense strand sequence of any one of the sequences of Table 2B, Table 3B, or Table 5C.

5. The RNAi agent of any one of embodiments 1-3, wherein the RNAi agent comprises a first antisense strand sequence of any one of the sequences of Table 2B, Table 3B, or Table 5C, and a second sense strand sequence of any one of the sequences of Table 2A, Table 3A, or Table 5C.

6. The RNAi agent of any one of embodiments 1-5, wherein at least one nucleotide of the RNAi agent is a modified nucleotide or includes a modified internucleoside linkage.

7. The RNAi agent of any one of embodiments 1-6, wherein all or substantially all of the nucleotides of the sense and/or antisense strand of the RNAi agent are modified nucleotides.

8. The RNAi agent of any one of embodiments 1-7, wherein the modified nucleotide is selected from the group consisting of: 2′-O-methyl nucleotide, 2′-fluoro nucleotide, 2′-deoxy nucleotide, 2′,3′-seco nucleotide mimic, locked nucleotide, 2′-F-arabino nucleotide, 2′-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2′-O-methyl nucleotide, inverted 2′-deoxy nucleotide, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholine nucleotide, vinyl phosphonate containing nucleotide, cyclopropyl phosphonate containing nucleotide, and 3′-O-methyl nucleotide.

9. The RNAi agent of embodiment 8, wherein all or substantially all of the modified nucleotides are 2′-O-methyl nucleotides, 2′-fluoro nucleotides, or combinations thereof.

10. The RNAi agent of any one of embodiments 1-9, wherein the first antisense strand sequence is selected from any one of the sequences of Table 3A, and the second antisense strand sequence is selected from any one of the sequences of Table 3B.

11. The RNAi agent of any one of embodiments 1-9, wherein the first antisense strand sequence is selected from any one of the sequences of Table 3B, and the second antisense strand sequence is selected from any one of the sequences of Table 3A.

12. The RNAi agent of any one of embodiments 1-11, wherein:
(a) the one or more sense strands comprise between 30 and 60 nucleotides, or
(b) the first antisense strand is between 18 and 30 nucleotides in length, or
(c) the second antisense strand is between 18 and 30 nucleotides in length, or
(d) any combination of (a) through (c) above.

13. The RNAi agent of embodiment 12, wherein:
(a) the one or more sense strands comprise between 36 and 54 nucleotides, or
(b) the first antisense strand is between 18 and 27 nucleotides in length, or
(c) the second antisense strand is between 18 and 27 nucleotides in length, or
(d) any combination of (a) through (c) above.

14. The RNAi agent of embodiment 13, wherein:
(a) the one or more sense strands comprise between 36 and 48 nucleotides, or
(b) the first antisense strand is between 18 and 24 nucleotides in length, or
(c) the second antisense strand is between 18 and 24 nucleotides in length, or
(d) any combination of (a) through (c) above.

15. The RNAi agent of embodiment 14, wherein:
(a) the one or more sense strands comprise between 38 and 42 nucleotides, or
(b) the first antisense strand is between 19 and 21 nucleotides in length, or
(c) the second antisense strand is between 19 and 21 nucleotides in length, or

(d) any combination of (a) through (c) above.

16. The RNAi agent of embodiment 15, wherein:
(a) the one or more sense strands comprises 38 or 42 nucleotides, or
(b) the second antisense strand is 19 nucleotides in length, or
(c) the second antisense strand is 21 nucleotides in length, or
(d) any combination of (a) through (c) above.

17. The RNAi agent of embodiment 1, wherein the one or more sense strands comprises the structure:

SS_1——L——SS_2

wherein SS_1 comprises a first sense strand sequence;
SS_2 comprises a second sense strand sequence; and
L is a linker, or a bond.

18. The RNAi agent of embodiment 17, wherein L is a nucleotide linker.

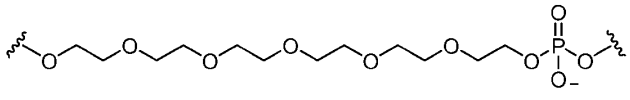
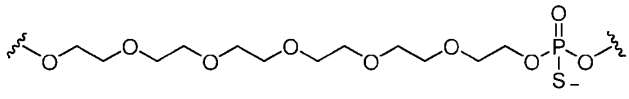
19. The RNAi agent of embodiment 17, wherein L is a non-nucleotide linker.

20. The RNAi agent of embodiment 19, wherein L is polyethylene glycol (PEG).

21. The RNAi agent of any one of embodiments 17-20, wherein SS_1 comprises any one of the sense strand sequences listed in Table 4A or Table 4B.

22. The RNAi agent of any one of embodiments 17-21, wherein SS_2 comprises any one of the sense strand sequences listed in Table 4A or Table 4B.

23. The RNAi agent of embodiment 17, wherein SS_1 and SS_2 each independently comprise any one of the sense strand sequences listed in Table 4A or Table 4B, wherein at least one sense strand sequence is selected from Table 4A and the other sense strand sequence is selected from Table 4B.

24. The RNAi agent of any one of embodiments 17-23, wherein L comprises the structure:  (Sp18), or  (Sp18s).

25. The RNAi agent of embodiment 17, wherein SS_1——L——SS_2 is selected from any one of the sense strands listed in Table 4C.

26. The RNAi agent of any one of embodiments 17-25, wherein the first antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3A, and the second antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3B.

27. The RNAi agent of any one of embodiments 17-26, wherein the first antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3B, and the second antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3A.

28. The RNAi agent of any one of embodiments 1-27, wherein the one or more sense strands consists of, consists essentially of, or comprises the nucleotide sequence of any of the modified sense strand sequences of Table 4A, Table 4B, Table 4C, or Table 5C.

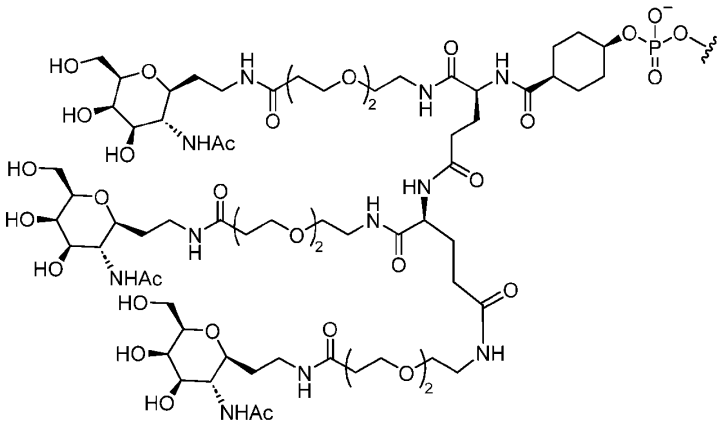
29. The RNAi agent of any one of embodiments 1-28, wherein the RNAi agent is linked to a targeting ligand.

30. The RNAi agent of embodiment 29, wherein the targeting ligand comprises N-acetylgalactosamine.

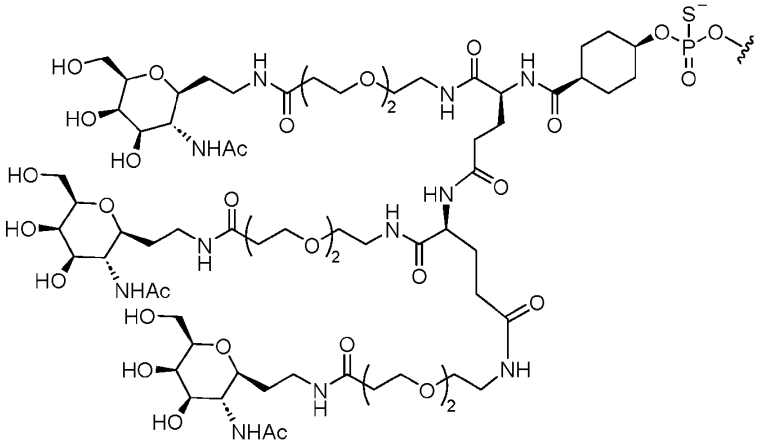
31. The RNAi agent of embodiment 29, wherein the targeting ligand comprises a metabolically stabilized carbohydrate ligand.

32. The RNAi agent of embodiment 31, wherein the targeting ligand comprises a metabolically stabilized N-acetylgalactosamine ligand.

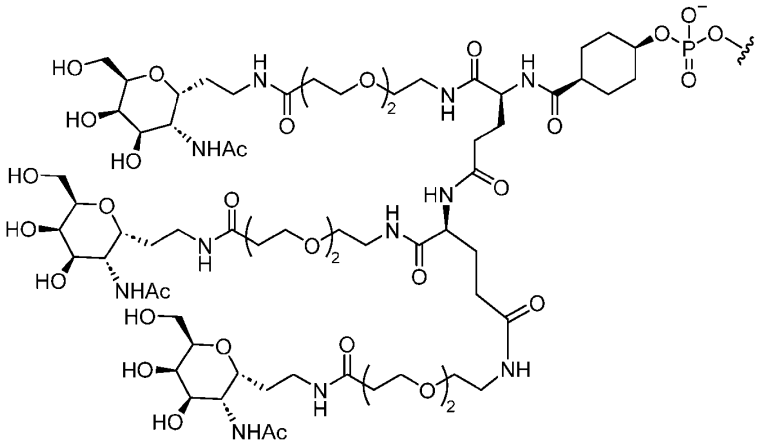
33. The RNAi agent of any one of embodiments 1-32, comprising



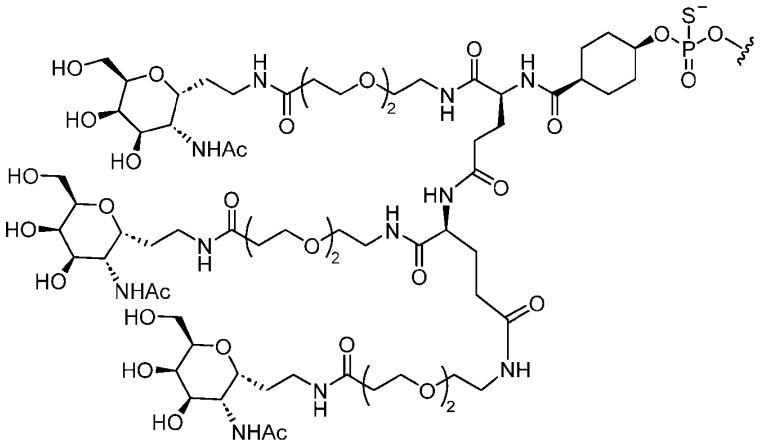
(NAG42),



(NAG42)s,



(NAG52),



(NAG52)s, or a pharmaceutically acceptable salt thereof.

34. The RNAi agent of any one of embodiments 29-33, wherein the targeting ligand is linked to the one or more sense strands.

35. The RNAi agent of embodiment 34, wherein the targeting ligand is linked to the 5' terminal end of the one or more sense strands.

36. The RNAi agent of any of embodiments 1-35, wherein the RNAi agent has two blunt ends.

37. The RNAi agent of any of embodiments 1-36, wherein the one or more sense strands comprise one or two terminal caps.

38. The RNAi agent of any of embodiments 1-37, wherein the one or more sense strands comprise one or two inverted abasic residues.

39. The RNAi agent of embodiment 1, wherein the RNAi agent forms a complex of any of the complexes set forth in Table 5A, Table 5B, or Table 5C.

40. The RNAi agent of any one of embodiments 1-39, wherein the one or more sense strands comprises inverted abasic residues at the 3' terminal end of the nucleotide sequence, at the 5' end of the nucleotide sequence, or at both.

41. The RNAi agent of embodiment 1, wherein:

the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

AAGUUACAAAAGCAAAACA (SEQ ID NO: 279); and

the second antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

UCACUGAGAAUACUGUCCC (SEQ ID NO: 277).

42. The RNAi agent of embodiment 41, wherein the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

AAGUUACAAAAGCAAAACAGG (SEQ ID NO: 295).

43. The RNAi agent of embodiment 41 or 42, wherein:

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

UGUUUUGCUUUUGUAACUU (SEQ ID NO: 311); and

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

GGGACAGUAUUCUCAGUIA (SEQ ID NO: 299).

44. The RNAi agent of embodiment 43, wherein the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: CCUGUUUUGCUUUUGUAACUU (SEQ ID NO: 313).

45. The RNAi agent of any one of embodiments 41-44, wherein all or substantially all of the nucleotides are modified nucleotides.

46. The RNAi agent of embodiment 1, wherein:

the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: cPrpasAfsguuacaaaaGfcAfaAfaca (SEQ ID NO: 342); and

the second antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO: 71);

wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5'-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5'-cyclopropyl phosphonate-2′-O-methyluridine; s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the first sense strand or second sense strand are modified nucleotides.

47. The RNAi agent of embodiment 46, wherein the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 101).

48. The RNAi agent of embodiment 46 or 47, wherein:

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: uguuuuGfcUfuUfuguaacuu (SEQ ID NO: 178); and

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

gsggacaGfuAfuUfcucaguia (SEQ ID NO: 134)

wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the first sense strand or second sense strand are modified nucleotides.

49. The RNAi agent of embodiment 48, wherein the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: ccuguuuuGfcUfuUfuguaacuu (SEQ ID NO: 194).

50. The RNAi agent of any one of embodiments 41-49, wherein the one or more sense strands further comprises inverted abasic residues at the 3' terminal end of the nucleotide sequence, at the 5' end of the nucleotide sequence, or at both.

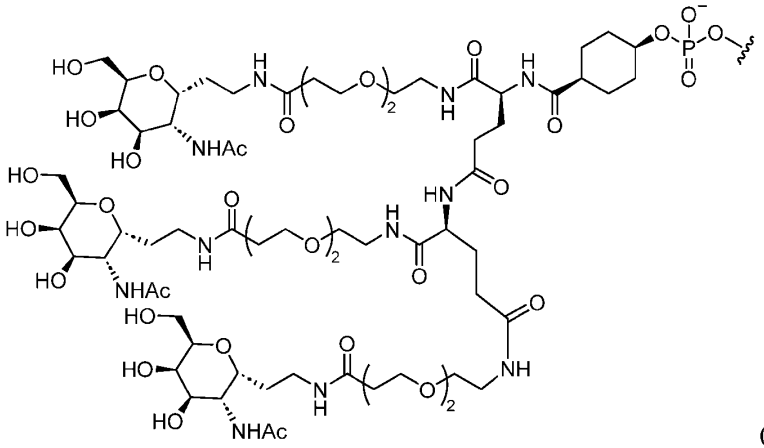
51. The RNAi agent of embodiment 50, wherein the first sense strand further includes an inverted abasic residue at the 3' terminal end, and wherein the second sense strand further comprises an inverted abasic residue at the 3' terminal end.

52. The RNAi agent of any one of embodiments 41-51, wherein the one or more sense strands comprises a linker.

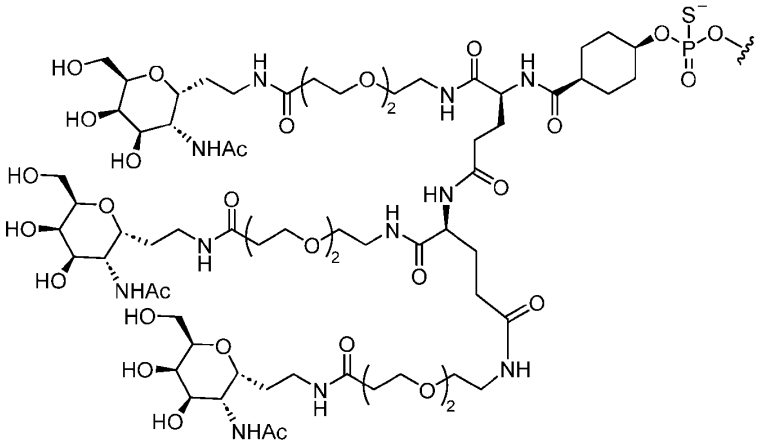
53. The RNAi agent of embodiment 52, wherein the linker comprises the structure: (Sp18).

54. The RNAi agent of any one of embodiments 41-53, wherein the RNAi agent comprises a targeting ligand.

55. The RNAi agent of any one of embodiments 41-54 comprising:

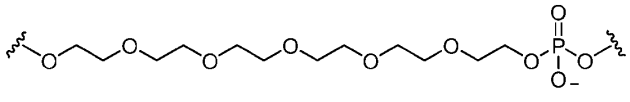


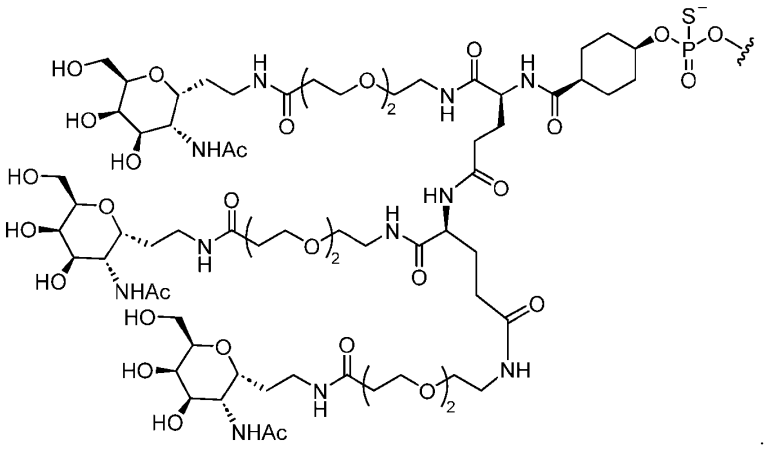
(NAG52),

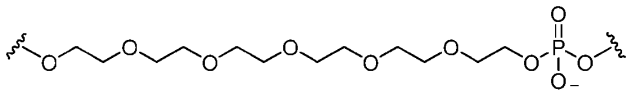


(NAG52)s, or a pharmaceutically acceptable salt of either (NAG52) or (NAG52)s.

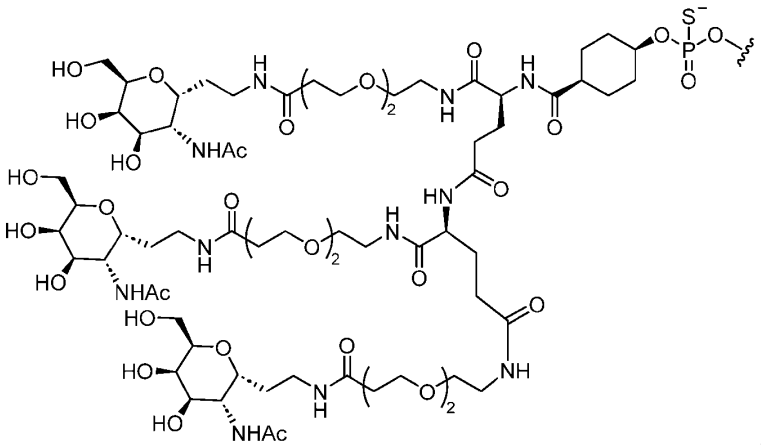
56. The RNAi agent of any one of embodiments 41-55, wherein the sense strand has the structure: (NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb) (SEQ ID NO: 243), and wherein the first antisense strand has the structure: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 101), and the second antisense strand has the structure: cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO: 71), wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5’-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5’-cyclopropyl phosphonate-2′-O-methyluridine; s

represents a phosphorothioate linkage; (invAb) is an inverted abasic residue, Sp18 represents the structure:  , and (NAG52)s represents the structure:



57. The RNAi agent of any one of embodiments 41-55, wherein the sense strand has the structure: (NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb) (SEQ ID NO: 244), and wherein the first antisense strand has the structure: cPrpasAfsguuacaaaaGfcAfaAfacsa (SEQ ID NO: 103), and the second antisense strand has the structure: cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO: 71), wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5’-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5’-cyclopropyl phosphonate-2′-O-methyluridine; s represents a phosphorothioate linkage; (invAb) is an inverted abasic residue, Sp18 represents the structure:  , and (NAG52)s

represents the structure:



58. The RNAi agent of any one of embodiments 1-57, wherein the RNAi agent is a pharmaceutically acceptable salt.

59. A composition comprising the RNAi agent of any one of embodiments 1-58, wherein the composition comprises a pharmaceutically acceptable excipient.

60. The composition of embodiment 59, wherein the pharmaceutically acceptable excipient is sodium phosphate buffer.

61. The composition of embodiment 59, wherein the pharmaceutically acceptable excipient is isotonic saline or water for injection.

62. A method for inhibiting expression of an APOC3 and a PCSK9 gene in a cell, the method comprising introducing into a cell an effective amount of an RNAi agent of any one of embodiments 1-57 or the composition of any one of embodiments 59-61.

63. The method of embodiment 62, wherein the cell is within a subject.

64. The method of embodiment 63, wherein the subject is a human subject.

65. The method of any one of embodiments 62-64, wherein APOC3 gene expression is inhibited by at least about 30% and PCSK9 gene expression of the subject is inhibited by at least about 30%, as measured by reductions in mRNA and/or protein levels.

66. The method of any one of embodiments 62-65, wherein APOC3 protein levels are reduced by about 50% and PCSK9 protein levels are reduced by about 50%.

67. A method of treating an APOC3-related and/or PCSK9-related disease, disorder, or symptom, the method comprising administering to a human subject in need thereof a therapeutically effective amount of the composition of any one of embodiments 59-61.

68. The method of embodiment 67, wherein the disease is Familial Combined Hyperlipidemia (FCH) hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome, familial partial lipodystrophy, hypercholesterolemia, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, or cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD).

69. The method of any one of embodiments 62-68, wherein the RNAi agent is administered at a dose of about 0.05 mg/kg to about 5.0 mg/kg of body weight of the human subject.

70. The method of any one of embodiments 62-69, wherein the RNAi agent is administered in two or more doses.

71. The RNAi agent of any one of embodiments 1-58 or the composition of any one of embodiments 59-61, for use in the treatment of a disease, disorder, or symptom that is mediated at least in part by a reduction in APOC3 gene expression and/or PCSK9 gene expression.

72. The RNAi agent or composition of embodiment 71, wherein the disease is Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome, familial partial lipodystrophy, hypercholesterolemia, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD).

73. The RNAi agent or composition of embodiment 71 or embodiment 72, wherein the RNAi agent is administered at a dose of about 0.05 mg/kg to about 10.0 mg/kg of body weight of the human subject.

73. Use of the RNAi agent of any one of embodiments 1-58 or the composition of any one of embodiments 59-61, for the preparation of a pharmaceutical composition for treating a disease, disorder, or symptom that is mediated at least in part by a reduction in APOC3 mRNA and/or protein levels and/or a reduction in PCSK9 mRNA and/or protein levels.

[00153] The above provided embodiments and items are now illustrated with the following, non-limiting examples.

EXAMPLES

[00154] The following examples are not limiting and are intended to illustrate certain embodiments disclosed herein.

Example 1. Synthesis of RNAi agents and Multimeric RNAi agents conjugates.

[00155] The following describes the general procedures for the syntheses of certain RNAi agents, and conjugates thereof, including the multimeric RNAi conjugates that are illustrated in the non-limiting Examples set forth herein.

[00156] *Synthesis of RNAi Agents.* RNAi agents can be synthesized using methods generally known in the art. For the synthesis of the RNAi agents illustrated in the Examples set forth herein, 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, a MerMade96E® (Bioautomation), a MerMade12® (Bioautomation), or an Oligopilot 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) or polystyrene (obtained from Kinovate, Oceanside, CA, USA). All RNA and 2′-modified RNA phosphoramidites were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA), ChemGenes (Wilmington, MA, USA), or Hongene Biotech (Morrisville, NC, USA). Specifically, the 2′-O-methyl phosphoramidites that were used include 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 and 2′-O-propargyl phosphoramidites carried the same protecting groups as the 2′-O-methyl phosphoramidites. 5′-dimethoxytrityl-2′-O-methyl-inosine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from Glen Research (Virginia). The inverted abasic (3′-O-dimethoxytrityl-2′-deoxyribose-5′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were purchased from ChemGenes. The following UNA phosphoramidites that were used included the following: 5′-(4,4'-Dimethoxytrityl)-N6-(benzoyl)-2′,3′-seco-adenosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, 5′-(4,4'-Dimethoxytrityl)-N-acetyl-2′,3′-seco-cytosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-

diiso-propyl)]-phosphoramidite, 5′-(4,4'-Dimethoxytrityl)-N-isobutyryl-2′,3′-seco-guanosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite, and 5′-(4,4'-Dimethoxy-trityl)-2′,3′-seco-uridine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N- diiso-propyl)]-phosphoramidite. 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 or a 200mM solution of xanthane hydride (TCI America, Portland, OR, USA) in pyridine was employed.

[00157] TFA aminolink phosphoramidites were also commercially purchased (ThermoFisher) to introduce the (NH2-C6) reactive group linkers. TFA aminolink phosphoramidite was dissolved in anhydrous acetonitrile (50 mM) 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 10 min (RNA), 90 sec (2′ O-Me), and 60 sec (2′ F). Trialkyne-containing phosphoramidites were synthesized to introduce the respective (TriAlk#) linkers. When used in connection with the RNAi agents presented in certain Examples herein, trialkyne-containing phosphoramidites were dissolved in anhydrous dichloromethane or anhydrous acetonitrile (50 mM), while all other amidites were dissolved in anhydrous acetonitrile (50 mM), 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 10 min (RNA), 90 sec (2′ O-Me), and 60 sec (2′ F).

[00158] For some RNAi agents, a linker, such as a C6-SS-C6 or a 6-SS-6 group, C6-SS(Me)-C5 was introduced at the 3' terminal end of the sense strand. Pre-loaded resin was commercially acquired with the respective linker. Alternatively, for some sense strands, a dT resin was used and the respectively linker was then added via standard phosphoramidite synthesis.

[00159] *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% to 31% 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).

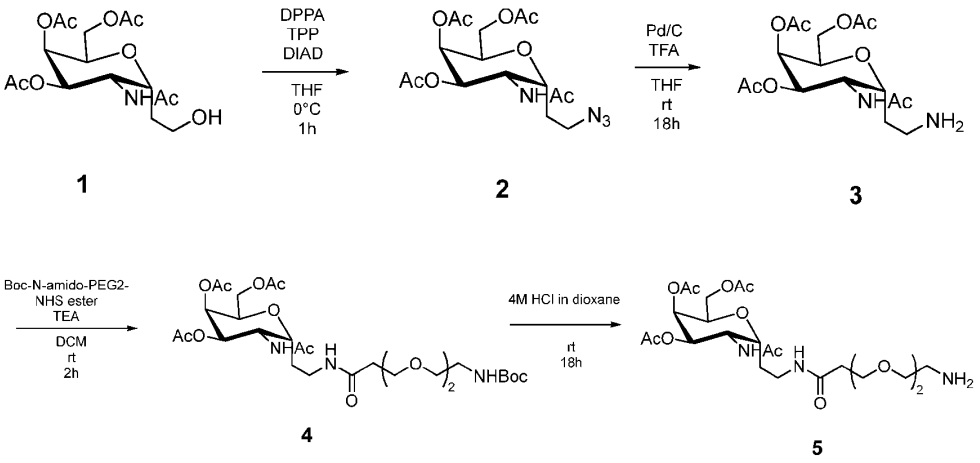
[00160] *Purification.* Crude oligomers were purified by anionic exchange HPLC using a TSKgel SuperQ-5PW 13µm column and Shimadzu LC-8 system. Buffer A was 20 mM Tris, 5 mM EDTA, pH 9.0 and contained 20% Acetonitrile and buffer B was the same as buffer A with the addition of 1.5 M sodium chloride. UV traces at 260 nm were recorded. Appropriate

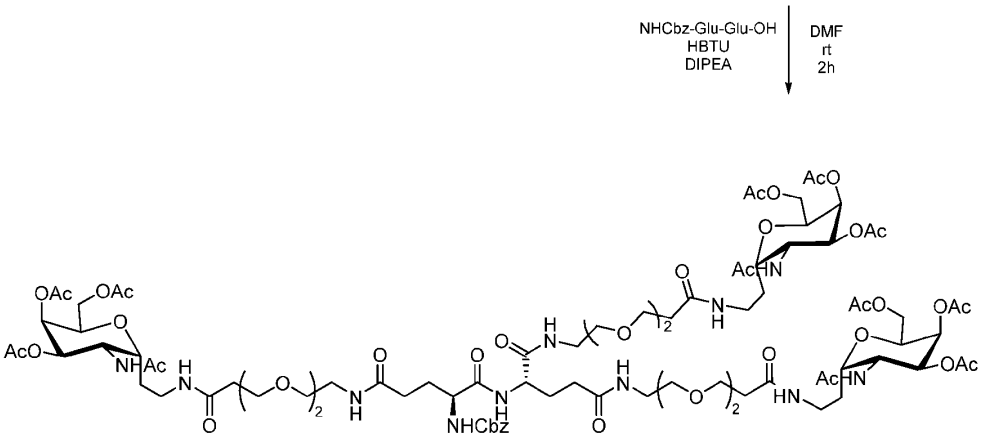
fractions were pooled then run on size exclusion HPLC using a GE Healthcare XK 16/40 column packed with Sephadex G25 fine with a running buffer of 100mM ammonium bicarbonate, pH 6.7 and 20% Acetonitrile or filtered water.

[00161] *Annealing.* Complementary strands were mixed by combining equimolar RNA solutions (sense and antisense) in 1× PBS (Phosphate-Buffered Saline, 1×, Corning, Cellgro) to form the RNAi agents. Some RNAi agents were lyophilized and stored at −15 to −25 °C. Complex concentration was determined by measuring the solution absorbance on a UV-Vis spectrometer in 1× PBS. The solution absorbance at 260 nm was then multiplied by a conversion factor and the dilution factor to determine the complex concentration. The conversion factor used was either 0.050 mg/(mL·cm) or was calculated from an experimentally determined extinction coefficient.

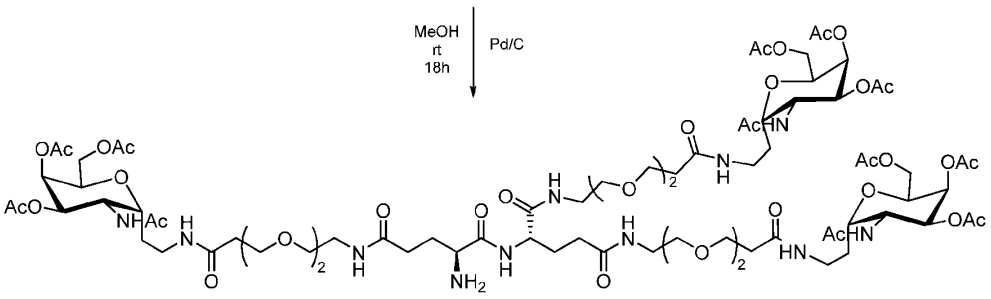
Example 2. ***Synthesis of Targeting Ligands***

[00162] ***Synthesis of NAG52 Phosphoramidite (compound 9)***

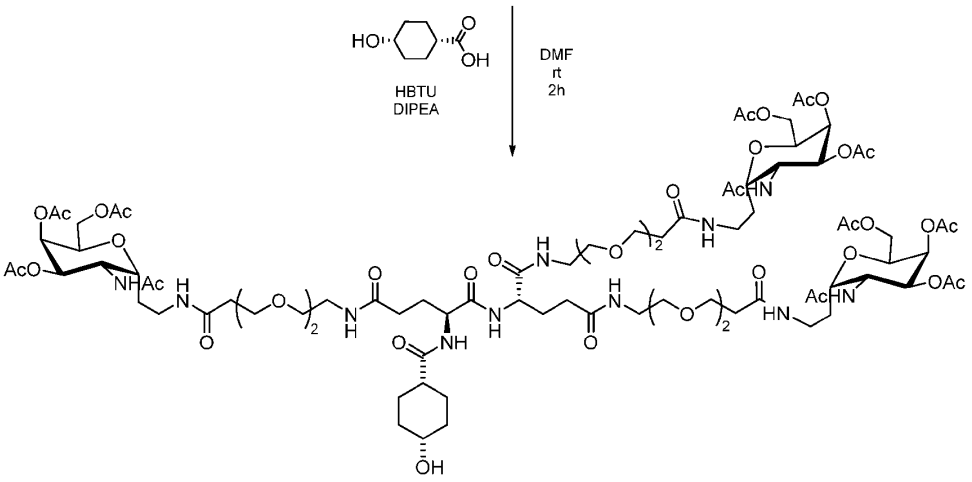



NHCbz-Glu-Glu-OH
HBTU
DIPEA
DMF
rt
2h

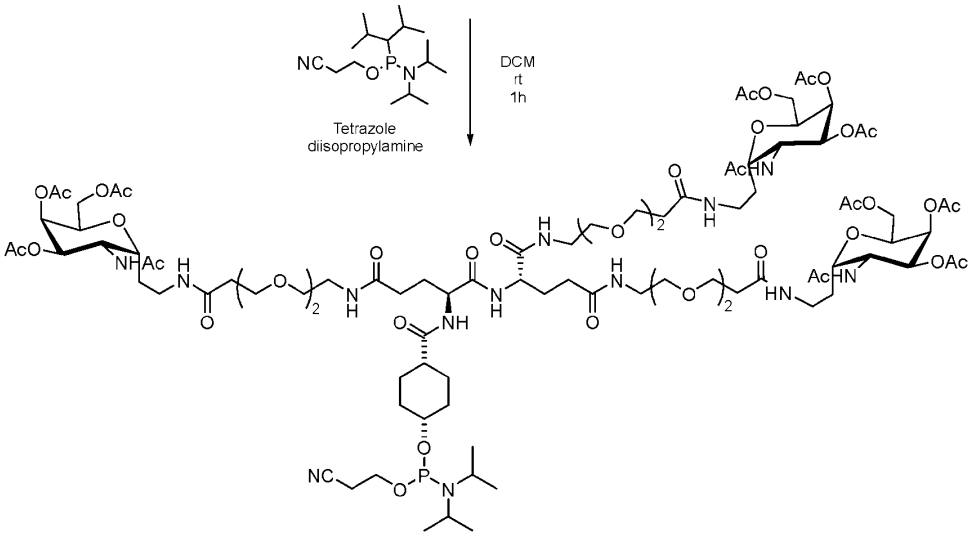
6


MeOH
rt
18h
Pd/C

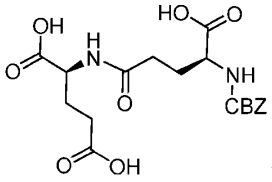
7

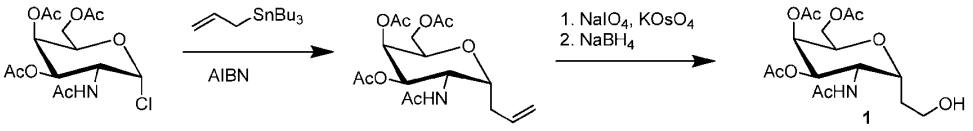

HBTU
DIPEA
DMF
rt
2h

8

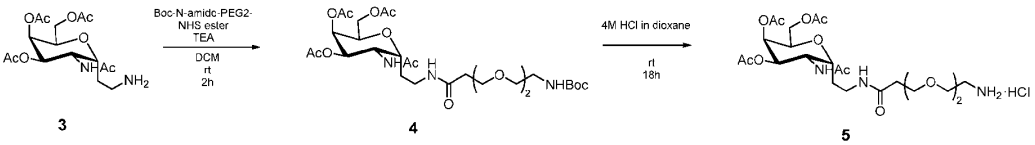


9

[00163] Compounds **1**, **2** and **3** were each synthesized in accordance with previously published procedures (*see, e.g.*, U.S. Patent Application Publication No. 2002/0107224 A1), and Cbz-NH-Glu-Glu-OH:  was synthesized in accordance with the procedure described in International Patent Application Publication No: WO 2017/156012 to Arrowhead Pharmaceuticals, Inc.), the contents of those references are incorporated by reference as if fully set forth herein. More specifically, **1** was synthesized from the following synthetic route:



[00164] **Synthesis of 5**



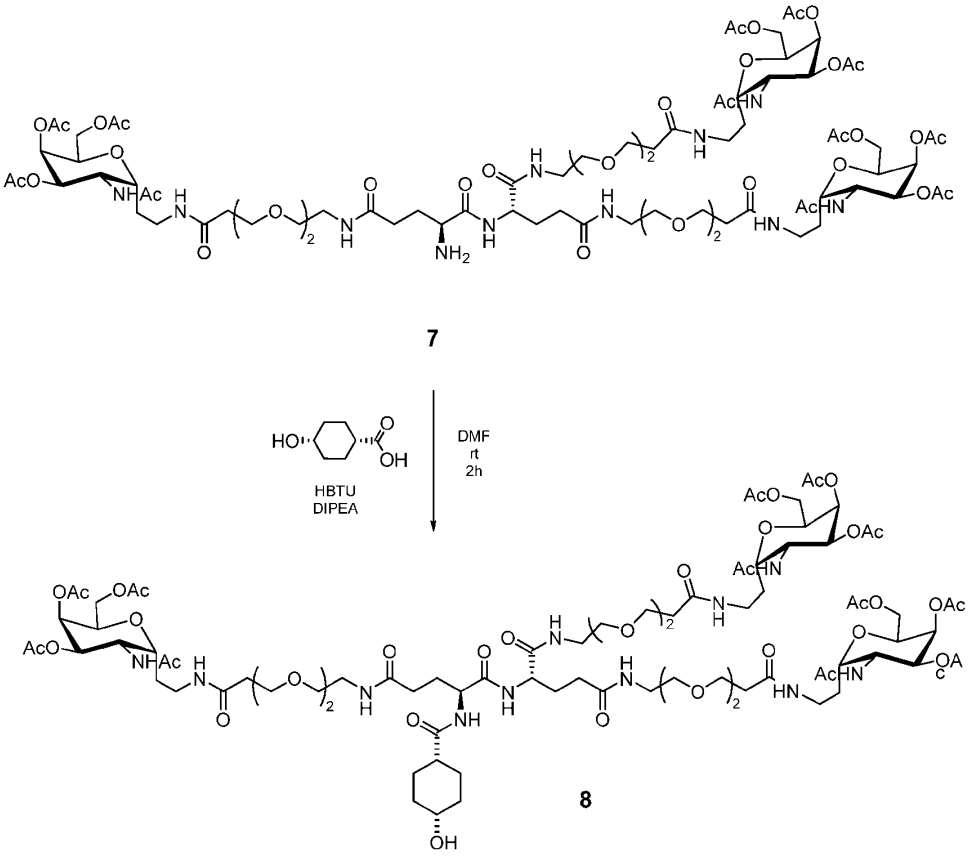
[00165] **Compound 3** (4.61 g, 12.3 mmol) and Boc-N-amido-PEG2-NHS ester (CAS 2183440-73-3, 4.61 g, 12.3 mmol) were dissolved in anhydrous DCM (100 mL) followed by

addition of triethylamine (3.4 mL, 24.6 mmol). The reaction mixture was stirred at room temperature (rt) for 2h, the solution was concentrated down to 30 mL under reduced pressure and diluted with chloroform (300 mL). The resulting solution was first washed with brine/citric acid (1:1, 30 mL) and then with brine/saturated bicarbonate solution (1:1, 30 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and purified on a silica column (100% DCM to 20% MeOH in DCM). Fractions containing the desired product **4** were combined, the solvent was removed under reduced pressure and the resulting foaming residue was redissolved in 4M HCl in 1,4-dioxane (100 mL). The reaction mixture was stirred at rt for 1h, and the solvent was removed under reduced pressure. The resulting residue was suspended in toluene and dried under reduced pressure giving the desired product **5** as an HCl salt (5.30 g, 9.30 mmol, 76% yield). Calculated MW 533.26 Found ESI MS^+ m/z = 534.23 [$M+H^+$].

[00166] Synthesis of 7

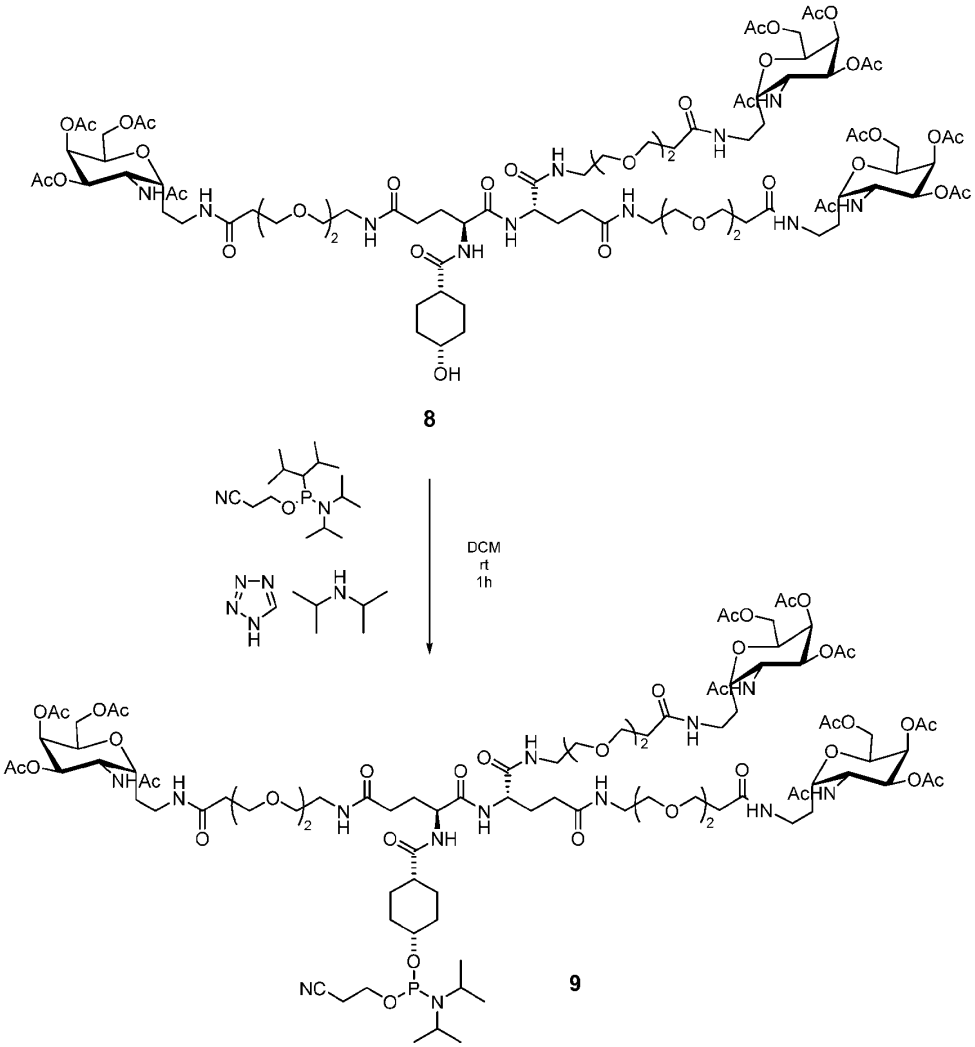
5 (5.30 g, 9.94 mmol) was dissolved in anhydrous DMF followed by addition of DIPEA (4.4 mmol, 45.97 mmol). Z-BisGlu (1.12 g, 2.73 mmol) and TBTU (3.03 g, 7.99 mmol) was added upon vigorous stirring. The solution turned light maroon, darkening over time. The reaction mixture was left stirring at rt for 2h when no unreacted starting material could be detected by LC-MS. The solvent was removed by co-evaporation with toluene, and the residue was redissolved in chloroform (400 mL). The resulting solution was first washed with brine/water (1:1, 60 mL) and then with brine/bicarbonate solution (1:1, 60 mL). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and purified on a silica column (100% DCM to 20% MeOH in DCM). Fractions containing the desired product **6** were combined, the solvent was removed under reduced pressure and the resulting foaming residue was redissolved in methanol (200 mL). Pd/C (0.70 g) was added to the solution, the suspension was hydrogenated under 1 atm overnight. The reaction mixture was stirred under hydrogen at rt overnight. The solution was filtered through a celite pad and concentrated under reduced pressure to yield the desired product **7** that was used as is in the next step (3.80 g, 2.09 mmol, 77% yield). Calculated MW 1821.84 Found ESI MS^+ m/z = 912.13 [$M+2H^+$].

[00167] Synthesis of 8



7 (3.80 g, 2.09 mmol) was dissolved in anhydrous DMF (30 mL) and was slowly added to a solution containing 4-hydroxycyclohexane carboxylic acid (0.35 g, 2.43 mmol), TBTU (0.82 g, 2.16 mmol) and DIPEA (1.12 mL, 6.44 mmol) in anhydrous DMF (30 mL). The reaction mixture was stirred at rt for 2h. The solvent was removed by co-evaporation with toluene, and the residue was redissolved in chloroform (300 mL). The solution was washed with brine/5% citric acid (1:1, 30 mL), dried over Na_2SO_4 and concentrated under reduced pressure and purified on a silica column (100% DCM to 30% MeOH in DCM) to yield the desired product **8** (2.40 g, 1.23 mmol, 59% yield). Calculated MW 1947.90 Found ESI MS^+ m/z = 975.46 $[M+2H^+]$.

[00168] Synthesis of 9



8 (2.40 g, 1.23 mmol) was thoroughly dried by co-evaporating DCM with toluene and dried in vacuo for 30 min. A round-bottom flask was charged with a stir bar and pre-treated molecular sieves, and was purged with nitrogen. The flask was filled with DCM (100 mL), and the molecular sieves were gently stirred for 10 min. Diisopropylammonium tetrazolide (1.40 g, 8.19 mmol) was added to the solution, and the reaction mixture was stirred for another 30 min. 2-Cyanoethyl N,N,N',N'-tetraisopropylphosphorodiamidite (0.55 mL, 1.73 mmol) was added, and the reaction mixture was stirred for 1h at rt when no unreacted starting material could be detected by LC-MS. The solution was filtered through a celite pad to remove molecular sieves

and diluted with saturated bicarbonate solution (100 mL) upon stirring. After 15 min, the organic layer was separated, and the aqueous layer was extracted with chloroform (2 x 200 mL). The organic fractions were combined, dried over Na_2SO_4, concentrated under reduced pressure and purified on a silica column (100% DCM (+0.1% triethylamine) to 10%MeOH in DCM (+0.1% triethylamine)). The fractions containing the desired product **9** were combined, concentrated under reduced pressure, and the product was co-evaporated twice with toluene to remove any residual triethylamine to give the desired product as an off-white solid (2.4 g, 1.16 mmol, 94% yield).

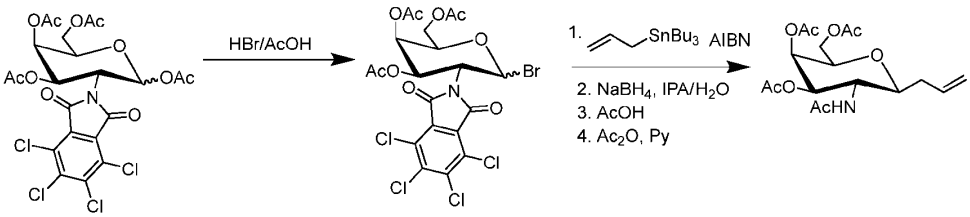
Tris (αCNAGAc3Peg2)BisGluNHCO-CH-PA:

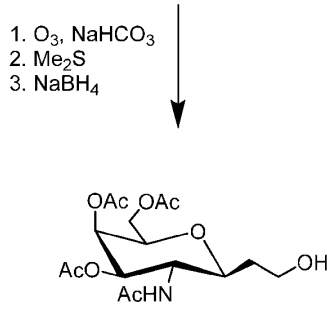
^{1}H NMR (DMSO-d6): 1.14 d (12H), 1.44 m (7H), 1.62-1.90 m (11H), 1.80 s (9H), 1.94 s (9H), 2.00 s (9H), 2.07 s (9H), 2.03-2.16 m (4H), 2.20-2.31 m (6H), 2.76 t (2H), 2.88-2.98 m (3H), 3.12- 3.23 m (10H), 3.34-3.42 m (6H), 3.46 s (12 H), 3.57 t (8H), 3.62- 3.76 m (2H), 3.98-4.20 m (15H), 4.20-4.30 m (3H), 4.96 dd (3H), 5.28, d(3H), 7.56- 8.00 m (8H), 8.12 d (3H).

^{31}P NMR (DMSO-d6): 145.84, 146.01

[00169] ***Synthesis Scheme of NAG42 Phosphoramidite (compound 9B)***

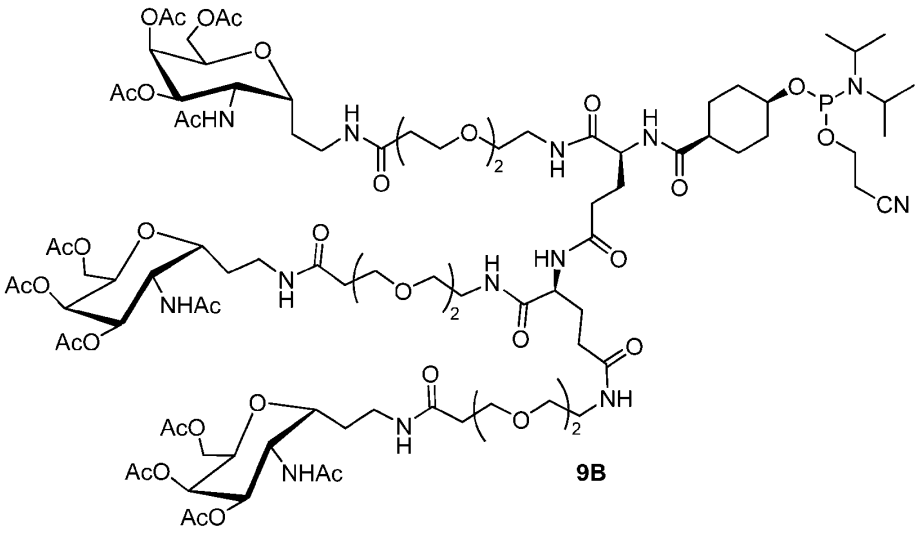
The synthesis of NAG42 follows the same synthetic route as NAG52 described above, with the only change being that it employs a beta anomeric stabilized linkage instead of an alpha anomeric linkage. More specifically, compound **1B** having a beta anomeric linkage can be synthesized as follows:





1B

The remaining synthesis follows what is described above for the synthesis of NAG52, with **1B** replacing **1**, and resulting in compound **9B:**



9B

Tris (βCNAGAc3Peg2)BisGluNHCO-CH-PA:

^{1}H NMR (DMSO-d6): 1.14 d (12H), 1.36-1.54 m (7H), 1.60-1.86 m (11H), 1.79 s (9H), 1.89 s (9H), 1.99 s (9H), 2.10 s (9H), 2-02-2.16 m (4H), 2.24-2.30 m (6H), 2.76 t (2H), 2.98-3.08 m (3H), 3.12-3.24 m (10H), 3.30-3.42 m (8H), 3.47 s (12H), 3.58 t (8H), 3.62-3.76 m (2H), 3.80-4.06 m (14H), 4.10-4.20 m (2H), 4.88 dd (3H), 5.26 d (3H), 7.55-8.00 m (11H).

^{31}P NMR (DMSO-d6): 145.84, 145.89

Example 3. Conjugation of Linkers and Targeting Ligands to RNAi agents

[00170] A. Conjugation of Activated Ester Linkers

[00171] One potential method for conjugation of linkers is by the coupling of activated esters. In some embodiments, the following procedures may be used to conjugate linking groups having terminal propargyl groups to an RNAi agent with an amine-functionalized sense strand, such as C6-NH2, NH2-C6, or (NH2-C6)s, as shown in Table 2, above. An annealed RNAi Agent dried by lyophilization is dissolved in DMSO and 10% water (v/v%) at 25 mg/mL. Then 50-100 equivalents of TEA and 3 equivalents of activated ester linker are added to the solution. The solution is allowed to react for 1-2 hours, while monitored by RP-HPLC-MS (mobile phase A 100 mM HFIP, 14 mM TEA; mobile phase B: acetonitrile on an XBridge C18 column, Waters Corp.)

[00172] The product can then be precipitated by adding 12 mL acetonitrile and 0.4 mL PBS and centrifuging the solid to a pellet. The pellet is then re-dissolved in 0.4 mL of 1XPBS and 12 mL of acetonitrile. The resulting pellet is dried on high vacuum for one hour.

[00173] B. Conjugation of Targeting Ligands to Propargyl Linkers

[00174] Similarly, another acceptable method to couple targeting ligands of the disclosed compounds herein is through their conjugation to propargyl linkers. In some embodiments, either prior to or after annealing, a 5′ or 3′ tridentate alkyne functionalized sense strand can be conjugated to the NAG ligand. The following describes one possible method for the conjugation of α/β-anomeric metabolically stabilized NAG to an annealed complex: Stock solutions of 0.5M Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA), 0.5M of Cu(II) sulfate pentahydrate ($Cu(II)SO_4 \cdot 5\ H_2O$) and 2M solution of sodium ascorbate are prepared in deionized water. A 75 mg/mL solution in DMSO of NAG ligand azide is made. In a 1.5 mL centrifuge tube containing tri-alkyne functionalized complex (3mg, 75μL, 40mg/mL in deionized water, approximately 15,000 g/mol), 25 μL of 1M Hepes pH 8.5 buffer is added. After vortexing, 35 μL of DMSO is added and the solution is vortexed. The ligand can then be added to the reaction (e.g., 6 eq/complex, 2 eq/alkyne, approximately 15μL) and the solution is vortexed. Using pH paper, pH is checked and confirmed to be pH approximately 8. In a separate 1.5 mL centrifuge tube, 50 μL of 0.5M THPTA is mixed with 10uL of 0.5M $Cu(II)SO_4 \cdot 5\ H_2O$, vortexed, and incubated at room temp for 5 min. After 5 min, THPTA/Cu solution (7.2 μL, 6 eq 5:1 THPTA:Cu) is added to the reaction vial, and vortexed. Immediately afterwards, 2M ascorbate (5 μL, 50 eq per complex, 16.7 per alkyne) is added to the reaction

vial and vortexed. Once the reaction was complete (typically complete in 0.5-1h), the reaction mixture is immediately purified by non-denaturing anion exchange chromatography.

[00175] C. Conjugation of Targeting Ligands to Amine-Functionalized Sense Strand

[00176] In some embodiments, the following procedure may be used to conjugate an activated ester-functionalized targeting ligand such as a metabolically stabilized carbohydrate ligand to an amine functionalized RNAi agent comprising an amine, such as C6-NH2, NH2-C6, or (NH2-C6)s, as shown in Table 2: An annealed, lyophilized RNAi agent is dissolved in DMSO and 10% water (v/v%) at 25 mg/mL. Then 50-100 equivalents TEA and three equivalents of activated ester targeting ligand are added to the mixture. The reaction mixture is allowed to stir for 1-2 hours while monitored by RP-HPLC-MS (mobile phase A: 100 mM HFIP, 14 mM TEA; mobile phase B: Acetonitrile; column: XBridge C18). After the reaction mixture is complete, 12 mL of acetonitrile was added followed by 0.4 mL of PBS and then the mixture is centrifuged. The solid pellet is collected and dissolved in 0.4 mL of 1xPBS and then 12 mL of acetonitrile is added. The resulting pellet is collected and dried under vacuum for 1 hour.

[00177] D. Addition of Targeting Ligands by Phosphoramidite Synthesis or On Resin.

[00178] Other acceptable methods to couple targeting ligands are to prepare the desired ligand as a phosphoramidite compound, which may be added to the 5' end of the strand using standard solid phase synthesis, or to prepare the targeting ligand on resin which can be placed at the 3' end of the strand after cleavage, again using standard solid phase oligonucleotide synthesis.

Example 4. ***Synthesis of APOC3-PCSK9 RNAi Agents.***

[00179] APOC3-PCSK9 RNAi agent complexes shown in Tables 5A, 5B, and 5C, above, were synthesized in accordance with the following general procedures:

A. *Synthesis.*

[00180] The sense and antisense strands of the RNAi agents were synthesized according to phosphoramidite technology on solid phase used in oligonucleotide synthesis. Such standard synthesis is generally known in the art. 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). The monomer positioned at the 3' end of the respective strand was attached to the solid support as a starting point for synthesis. 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 cyclopropyl phosphonate phosphoramidites were synthesized in accordance with International Patent Application Publication No. WO 2017/214112 (*see also* Altenhofer et. al., Chem. Communications (Royal Soc. Chem.), 57(55):6808-6811 (July 2021)). The inverted 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). 5’-O-dimethoxytrityl-N^2,N^6-(phenoxyacetate)-2’-O-methyl-diaminopurine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidites were obtained from ChemGenes or Hongene Biotech.

[00181] 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 APOC3-PCSK9 RNAi agent complexes 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.

[00182] 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.

[00183] 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.

[00184] 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. Complex 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 complex concentration. The conversion factor used was either 0.050 mg/(mL·cm) or was calculated from an experimentally determined extinction coefficient.

Example 5. ***APOC3-SEAP Mouse Model.***

[00185] Six to eight week old female C57BL/6 albino mice were transiently transfected *in vivo* with plasmid by hydrodynamic tail vein injection, administered at least 15 days prior to administration of an APOC3 RNAi agent or control. The plasmid contains the APOC3 cDNA sequence (GenBank NM_000040.1 (SEQ ID NO:1)) inserted into the 3' UTR of the SEAP (secreted human placental alkaline phosphatase) reporter gene. 50 µg of the plasmid containing the APOC3 cDNA sequence in Ringer's Solution in a total volume of 10% of the animal's body weight was injected into mice via the tail vein to create APOC3-SEAP model mice. The solution was injected through a 27-gauge needle in 5-7 seconds as previously described (Zhang G et al., "High levels of foreign gene expression in hepatocytes after tail vein injection of naked plasmid DNA." Human Gene Therapy 1999 Vol. 10, p1735-1737.). Inhibition of expression

of APOC3 by an APOC3 RNAi agent results in concomitant inhibition of SEAP expression, which is measured. At day -1, SEAP expression levels in serum were measured by the Phospha-Light™ SEAP Reporter Gene Assay System (Invitrogen), and the mice were grouped according to average SEAP levels.

[00186] *Analyses*: SEAP levels may be measured at various times, both before and after administration of APOC3 RNAi agents.

[00187] *i) Serum collection*: Mice were anesthetized with 2-3% isoflurane and blood samples were collected from the submandibular area into serum separation tubes (Sarstedt AG & Co., Nümbrecht, Germany). Blood was allowed to coagulate at ambient temperature for 20 min. The tubes were centrifuged at 8,000 ×g for 3 min to separate the serum and stored at 4°C.

[00188] *ii) Serum SEAP levels*: Serum was collected and measured by the Phospha-Light™ SEAP Reporter Gene Assay System (Invitrogen) according to the manufacturer's instructions. Serum SEAP levels for each animal was normalized to the control group of mice injected with saline in order to account for the non-treatment related decline in APOC3 expression with this model. First, the SEAP level for each animal at a time point was divided by the pre-treatment level of expression in that animal (Day −1) in order to determine the ratio of expression "normalized to pre-treatment". Expression at a specific time point was then normalized to the control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the average "normalized to pre-treatment" ratio of all mice in the normal saline control group. Alternatively, in some Examples set forth herein, the serum SEAP levels for each animal were assessed by normalizing to pre-treatment levels only.

Example 6. ***In Vivo Testing of APOC3-PCSK9 RNAi Agents in APOC3-SEAP Mice.***

[00189] The APOC3-SEAP mouse model as described in Example 5, above, was used.

[00190] On Day -21, female C57BL/6 albino mice were administered, via hydrodynamic tail vein injection (HTV), 50 ug of plasmid containing the APOC3 cDNA sequence (GenBank NM_000040.1 (SEQ ID NO:1)) inserted into the 3' UTR of the SEAP (secreted human placental alkaline phosphatase) reporter gene.

[00191] On Day 1, six (n=6) mice were dosed with saline, four (n=4) mice were dosed with either monomeric APOC3 RNAi agents or multimeric APOC3-PCSK9 RNAi agents formulated in saline (at 0.25 mg/kg or 0.5 mg/kg), via subcutaneous (SQ) injection, at 250 μL

per 25 g body weight injection volume. The dosing regimen was in accordance with Table 7 below.

[00192] **Table 7.** Dosing Groups of Example 6.

Group ID	RNAi Agent	Dose	Dosing Regimen	# Animals (n =)
1	Saline	N/A	Single SQ injection on Day 1	n = 6
2	AD09370	0.25 mg/kg	Single SQ injection on Day 1	n = 4
3	AC912744	0.25 mg/kg	Single SQ injection on Day 1	n = 4
4	AC912745	0.25 mg/kg	Single SQ injection on Day 1	n = 4
5	AC912746	0.25 mg/kg	Single SQ injection on Day 1	n = 4
6	AC003791	0.5 mg/kg	Single SQ injection on Day 1	n = 4
7	AC003792	0.5 mg/kg	Single SQ injection on Day 1	n = 4
8	AC003782	0.5 mg/kg	Single SQ injection on Day 1	n = 4
9	AC003783	0.5 mg/kg	Single SQ injection on Day 1	n = 4

[00193] For purposes of evaluating the efficacy of the multimeric APOC3-PCSK9 RNAi agents, monomeric APOC3 RNAi agents were also evaluated in parallel. The monomeric APOC3 RNAi agents dosed were of the following structures:

AD09370
Sense strand: (NAG25)s(invAb)sacgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 251)
Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfcGfsu (SEQ ID NO: 252)
AC912744
Sense strand: (NAG37)s(invAb)sgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 253)
Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfsc (SEQ ID NO: 254)
AC912745
Sense strand: (NAG37)sgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 255)
Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfsc (SEQ ID NO: 256)
AC912746
Sense strand: (NAG37)sgsggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 257)
Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfsc (SEQ ID NO: 258)

[00194] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[00195] Each of the APOC3-PCSK9 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included N-acetyl-galactosamine groups having the modified sequences as set forth in the complex structures herein. (See Tables 3A, 3B, 4A, 4B, 4C, 5A, 5B, 5C, and 6 for specific modifications and structure information related to the APOC3-PCSK9 RNAi agents, including (NAG52)s ligand).

[00196] SEAP levels were determined pursuant to the procedure set forth in Example 5, above. Data from the experiment are shown in the following Table 8, with average SEAP reflecting the

normalized average value of SEAP. Inhibition of APOC3 expression by an APOC3-PCSK9 RNAi agent results in concomitant inhibition of SEAP expression, which is measured.

[00197] **Table 8.** Average SEAP normalized to pre-treatment and saline control in APOC3-SEAP mice of Example 6.

	Day 8		Day 15		Day 22	
Group ID	**Avg SEAP**	**Std Dev**	**Avg SEAP**	**Std Dev**	**Avg SEAP**	**Std Dev**
1. Saline	1.000	0.388	1.000	0.605	1.000	0.693
2. 0.25 mg/kg AD09370	0.537	0.091	0.592	0.253	0.811	0.350
3. 0.25 mg/kg AC912744	0.739	0.146	0.427	0.181	0.410	0.153
4. 0.25 mg/kg AC912745	0.825	0.310	0.563	0.081	0.454	0.133
5. 0.25 mg/kg AC912746	0.940	0.135	0.894	0.238	0.820	0.148
6. 0.5 mg/kg AC003791	0.874	0.516	0.583	0.176	0.652	0.243
7. 0.5 mg/kg AC003792	0.881	0.122	0.563	0.127	0.514	0.155
8. 0.5 mg/kg AC003782	0.760	0.199	0.529	0.164	0.438	0.118
9. 0.5 mg/kg AC003783	0.691	0.058	0.907	0.202	0.561	0.086

[00198] Groups 2-9 showed varying levels of reduction in APOC3-SEAP at Day 8, 15, and 22 compared to the saline control Group 1. Out to Day 22, multimeric APOC3-PCSK9 RNAi agent AC003782 at 0.5 mg/kg achieved approximately 56% (0.438) APOC3 inhibition, which is comparable to approximately 59% inhibition (0.410) by monomeric APOC3 RNAi agent AC912744 at 0.25 mg/kg.

Example 7. PCSK9-AAV Mouse Model.

[00199] To evaluate certain APOC3-PCSK9 RNAi agents *in vivo*, an AAV (Adeno-associated virus) mouse model was used. Human PCSK9 (hPCSK9) CDS and UTR were inserted into an Adeno-associated virus AAV serotype 8, to create AAV-PCSK9. Six- to eight-week-old male C57BL/6 mice were transduced with AAV-PCSK9, administered at least 14 days prior to administration of an APOC3-PCSK9 RNAi agent or control. 5E12 to 1E13 GC/kg of the respective virus in PBS in a total volume of 10 mL/kg animal's body weight was injected into mice via intravenous (IV) injection to create AAV-PCSK9 model mice. Inhibition of PCSK9 was then assessed via enzyme-linked immunosorbent assay (ELISA).

Example 8. ***APOC3-SEAP/PCSK9-AAV Mouse Model.***

[00200] To evaluate certain APOC3-PCSK9 RNAi agents *in vivo*, an APOC3-SEAP/AAV-PCSK9 (Adeno-associated virus) mouse model was used.

[00201] Six to eight week old female C57BL/6 albino mice were transiently transfected *in vivo* with plasmid by hydrodynamic tail vein injection, administered at least 15 days prior to administration of an APOC3 RNAi agent or control. The plasmid contains the APOC3 cDNA sequence (GenBank NM_000040.1 (SEQ ID NO:1)) inserted into the 3' UTR of the SEAP (secreted human placental alkaline phosphatase) reporter gene. 50 μg of the plasmid containing the APOC3 cDNA sequence in Ringer's Solution in a total volume of 10% of the animal's body weight was injected into mice via the tail vein to create APOC3-SEAP model mice. The solution was injected through a 27-gauge needle in 5-7 seconds as previously described (Zhang G et al., "High levels of foreign gene expression in hepatocytes after tail vein injection of naked plasmid DNA." Human Gene Therapy 1999 Vol. 10, p1735-1737.). Inhibition of expression of APOC3 by an APOC3 RNAi agent results in concomitant inhibition of SEAP expression, which is measured. At day -1, SEAP expression levels in serum were measured by the Phospha-Light™ SEAP Reporter Gene Assay System (Invitrogen), and the mice were grouped according to average SEAP levels. These procedures create the APOC3-SEAP mice.

[00202] The APOC3-SEAP mice were subsequently further transfected with an Adeno-associated virus (AAV). Human PCSK9 (hPCSK9) CDS and UTR were inserted into an Adeno-associated virus AAV serotype 8, to create AAV-PCSK9. The mice were transduced with AAV-PCSK9, administered at least 14 days prior to administration of an APOC3-PCSK9 RNAi agent or control. 5E12 to 1E13 GC/kg of the respective virus in PBS in a total volume of 10 mL/kg animal's body weight was injected into mice via intravenous (IV) injection.

[00203] The aforementioned procedures create the APOC3-SEAP/AAV-PCSK9 model mice.

[00204] *Analyses*: SEAP levels may be measured at various times, both before and after administration of APOC3 RNAi agents.

[00205] *i) Serum collection*: Mice were anesthetized with 2-3% isoflurane and blood samples were collected from the submandibular area into serum separation tubes (Sarstedt AG & Co., Nümbrecht, Germany). Blood was allowed to coagulate at ambient temperature for 20 min. The tubes were centrifuged at 8,000 ×g for 3 min to separate the serum and stored at 4°C.

[00206] *ii) Serum SEAP levels*: Serum was collected and measured by the Phospha-Light™ SEAP Reporter Gene Assay System (Invitrogen) according to the manufacturer's instructions. Serum SEAP levels for each animal was normalized to the control group of mice injected with

saline in order to account for the non-treatment related decline in APOC3 expression with this model. First, the SEAP level for each animal at a time point was divided by the pre-treatment level of expression in that animal (Day -1) in order to determine the ratio of expression "normalized to pre-treatment". Expression at a specific time point was then normalized to the control group by dividing the "normalized to pre-treatment" ratio for an individual animal by the average "normalized to pre-treatment" ratio of all mice in the normal saline control group. Alternatively, in some Examples set forth herein, the serum SEAP levels for each animal were assessed by normalizing to pre-treatment levels only.

[00207] Inhibition of PCSK9 was then assessed via enzyme-linked immunosorbent assay (ELISA).

Example 9. ***In Vivo Testing of APOC3-PCSK9 RNAi Agents in AAV-PCSK9 Mice.***

[00208] The APOC3-SEAP/AAV-PCSK9 mouse model as described in Example 8, above, was used.

[00209] On Day -21, female C57BL/6 albino mice were administered, via hydrodynamic tail vein injection (HTV), 20 ug of plasmid containing the APOC3 cDNA sequence (GenBank NM_000040.1 (SEQ ID NO:1)) inserted into the 3' UTR of the SEAP (secreted human placental alkaline phosphatase) reporter gene.

[00210] On Day -20, the mice were administered, via intravenous (IV) injection, 5x10^12 GC/kg (genome copies per kg weight) of plasmid containing the PCSK9 cDNA sequence (GenBank NM_174936.4 (SEQ ID NO:2)) inserted into an AAV8 vector.

[00211] On Day 1, eight (n=8) mice were dosed with saline, four (n=4) mice were dosed with monomeric APOC3 RNAi agents, monomeric PCSK9 RNAi agents, or multimeric APOC3-PCSK9 RNAi agents formulated in saline (at 1.0 mg/kg or 2.0 mg/kg), via subcutaneous (SQ) injection, at 250 µL per 25 g body weight injection volume. The dosing regimen was in accordance with Table 15 below.

[00212] **Table 15.** Dosing Groups of Example 9.

Group ID	RNAi Agent	Dose	Dosing Regimen	# Animals (n =)
1	Saline	N/A	Single SQ injection on Day 1	n = 8
2	AD08879 + AD09370	1.0 mg/kg, 1.0 mg/kg	Single SQ injection on Day 1	n = 4

3	AC004486 + AC004431	1.0 mg/kg, 1.0 mg/kg	Single SQ injection on Day 1	n = 4
4	AC004431	1.0 mg/kg	Single SQ injection on Day 1	n = 4
5	AC004486	1.0 mg/kg	Single SQ injection on Day 1	n = 4
6	AC004308 + AC004430	1.0 mg/kg, 1.0 mg/kg	Single SQ injection on Day 1	n = 4
7	AC004762 + AC004429	1.0 mg/kg, 1.0 mg/kg	Single SQ injection on Day 1	n = 4
8	AC003791	2.0 mg/kg	Single SQ injection on Day 1	n = 4
9	AC003792	2.0 mg/kg	Single SQ injection on Day 1	n = 4
10	AC004091	2.0 mg/kg	Single SQ injection on Day 1	n = 4

[00213] For purposes of evaluating the efficacy of the multimeric APOC3-PCSK9 RNAi agents, monomeric APOC3 RNAi agents and monomeric PCSK9 RNAi agents were also evaluated in parallel. The monomeric APOC3 and PCSK9 RNAi agents dosed were of the following structures, shown in Table 16 below:

[00214] **Table 16.** Complex structures of monomeric APOC3 and monomeric PCSK9 RNAi agents of dosing Groups of Example 9.

Complex ID	Target Gene	Antisense Strand	SEQ ID NO:	Sense Strand	SEQ ID NO:
AD08879	PCSK9	cPrpuAfguuacaaaaGfcAfaAfacasgsg	259	(NAG37)s(invAb)sccuguuuuGfcUfUfuuguaacuas(invAb)	266
AD09370	APOC3	usCfsasCfuGfagaauAfcUfgUfcCfcGfsu	252	(NAG25)s(invAb)sacgggacaGfUfAfuucucaguias(invAb)	254
AC004486	PCSK9	cPrpaCfaaaagcaaaAfcAfgGfuscsc	260	(NAG37)sggaccuGfuUfUfugcuuuugus(invAb)	267
AC004431	APOC3	cPrpusCfsaCfugagaauAfcUfgUfcscsc	261	(NAG37)sgggacaGfUfAfuucucaguias(invAb)	268
AC004308	PCSK9	cPrpusUfsguaa_2NaaaggCfaAfcAfgasg	262	(NAG37)scucuguUfGfCfcuuuuuacaas(invAb)	269
AC004430	APOC3	cPrpusCfsaCfuGfagaauAfcUfgUfcsCfsc	263	(NAG37)sgggacaGfuAfuUfcucaguias(invAb)	270
AC004762	PCSK9	cPrpusUfguaa_2NaaaggCfaAfcAfgasg	264	(NAG37)s(invAb)scucuguUfgCfcUfuuuuacaas(invAb)	271
AC004429	APOC3	cPrpusCfsaCfuGfagaauAfcUfgUfcsCfsc	265	(NAG37)sgggacaGfUfAfuucucaguias(invAb)	272

[00215] The injections were performed between the skin and muscle (i.e. subcutaneous injections) into the loose skin over the neck and shoulder area. Animals were weighed prior to dosing, and the dosing volume was individually adjusted based on the animal body weight. On Day -7, 1, 8, 15, and 22 post injection, serum was collected.

[00216] Each of the monomeric APOC3 or PCSK9 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included N-acetyl-galactosamine groups having the modified sequences as set forth in the complex structures herein. (See Table 16 and Table 6 for specific modifications and structure information related to the PCSK9 RNAi agents, including (NAG37)s or (NAG52)s ligands).

[00217] Each of the APOC3-PCSK9 RNAi agents included modified nucleotides that were conjugated at the 5' terminal end of the sense strand to a targeting ligand that included N-acetyl-galactosamine groups having the modified sequences as set forth in the complex structures herein. (See Tables 3A, 3B, 4A, 4B, 4C, 5A, 5B, 5C, and 6 for specific modifications and structure information related to the APOC3-PCSK9 RNAi agents, including (NAG52)s ligand).

[00218] SEAP levels were determined pursuant to the procedure set forth in Example 8, above. Data from the experiment are shown in the following Table 17, with average SEAP reflecting the normalized average value of SEAP. Inhibition of APOC3 expression by a monomeric APOC3 RNAi agent or a multimeric APOC3-PCSK9 RNAi agent results in concomitant inhibition of SEAP expression, which is measured.

[00219] PCSK9 levels were determined pursuant to the procedure set forth in Example 8, above. Mouse serum PCSK9 was quantified via ELISA (R&Q Systems, Cat. # SPC900). Data from the experiment are shown in the following Table 18, with average PCSK9 reflecting the normalized average value of PCSK9.

[00220] **Table 17.** Average SEAP normalized to pre-treatment and saline control in APOC3-SEAP/AAV-PCSK9 mice of Example 9.

	Day 8		Day 15		Day 22	
Group ID	**Avg SEAP**	**Std Dev**	**Avg SEAP**	**Std Dev**	**Avg SEAP**	**Std Dev**
1. Saline	1.000	0.248	1.000	0.438	1.000	0.462
2. 1.0 mg/kg AD08879 + 1.0 mg/kg AD09370	0.486	0.269	0.554	0.331	0.662	0.333
3. 1.0 mg/kg AC004486 + 1.0 mg/kg AC004431	0.483	0.115	0.457	0.095	0.561	0.046

4. 1.0 mg/kg AC004431	0.520	0.128	0.531	0.116	0.552	0.065
5. 1.0 mg/kg AC004486	1.371	0.141	0.849	0.256	1.006	0.338
6. 1.0 mg/kg AC004308 + 1.0 mg/kg AC004430	0.608	0.258	0.356	0.126	0.522	0.161
7. 1.0 mg/kg AC004762 + 1.0 mg/kg AC004429	0.759	0.077	0.399	0.054	0.601	0.164
8. 2.0 mg/kg AC003791	1.135	0.094	0.934	0.200	0.929	0.209
9. 2.0 mg/kg AC003792	0.677	0.144	0.778	0.140	0.883	0.299
10. 2.0 mg/kg AC004091	0.498	0.125	0.546	0.054	0.545	0.103

[00221] Groups 2-4 and 6, 7, 9, and 10 showed varying levels of reduction in APOC3-SEAP at Day 8, 15, and 22 compared to the saline control Group 1. Group 5 AC004486 showed negligible to no APOC3-SEAP inhibition at all time points, as AC004486 targets the PCSK9 gene. Group 8 AC003791 showed negligible inhibition at all time points. Out to Day 22, multimeric APOC3-PCSK9 RNAi agent AC004091 at 2.0 mg/kg achieved approximately 45% (0.545) APOC3 inhibition, which is comparable to approximately 47% inhibition (0.522) by monomeric APOC3 RNAi agent AC004430 at 1.0 mg/kg.

[00222] **Table 18.** Average PCSK9 normalized to pre-treatment and saline control in APOC3-SEAP/AAV-PCSK9 mice of Example 9.

	Day 8		**Day 15**		**Day 22**	
Group ID	**Avg PCSK9**	**Std Dev**	**Avg PCSK9**	**Std Dev**	**Avg PCSK9**	**Std Dev**
1. Saline	1.000	0.139	1.000	0.209	1.000	0.547
2. 1.0 mg/kg AD08879 + 1.0 mg/kg AD09370	0.274	0.172	0.203	0.137	0.278	0.280
3. 1.0 mg/kg AC004486 + 1.0 mg/kg AC004431	0.418	0.075	0.350	0.065	0.331	0.106
4. 1.0 mg/kg AC004431	0.987	0.086	0.803	0.188	1.377	0.590
5. 1.0 mg/kg AC004486	0.478	0.055	0.349	0.138	0.512	0.230
6. 1.0 mg/kg AC004308 + 1.0 mg/kg AC004430	0.503	0.132	0.445	0.101	0.517	0.346
7. 1.0 mg/kg AC004762 + 1.0 mg/kg AC004429	0.537	0.052	0.495	0.099	0.478	0.231
8. 2.0 mg/kg AC003791	0.641	0.076	0.484	0.050	0.398	0.187
9. 2.0 mg/kg AC003792	0.732	0.035	0.573	0.158	0.610	0.286

10. 2.0 mg/kg AC004091	0.907	0.138	0.660	0.136	0.521	0.088

[00223] Groups 2, 3, and 5-10 showed varying levels of reduction in PCSK9 at Day 8, 15, and 22 compared to the saline control Group 1. Group 4 (dosed with AC004431 targeting the APOC3 gene) showed negligible to no PCSK9 inhibition. Out to Day 22, multimeric APOC3-PCSK9 RNAi agent AC003791 at 2.0 mg/kg achieved approximately 60% (0.398) PCSK9 inhibition, which is comparable to approximately 70% inhibition (0.278) by monomeric PCSK9 RNAi agent AD08879 at 1.0 mg/kg.

Example 10. ***In Vivo Administration of APOC3-PCSK9 RNAi Agents in Cynomolgus Monkeys.***

[00224] APOC3-PCSK9 RNAi agents were tested in Cynomolgus monkeys for inhibition of APOC3 and PCSK9.

[00225] On Day 1, three (n=3) male Cynomolgus monkey test animals for each test group were dosed with APOC3-PCSK9 RNAi agents formulated in saline (at 6.0 mg/kg), via subcutaneous (SQ) injection with syringe and needle in the mid-scapular region, at 20.0 mL/kg dose volume.

[00226] Cynomolgus monkeys were acclimated for at least one (1) day. The animals were of 2 to 7 years. During the animals were not commingled for at least 24 hours after test article (RNAi agent) administration to allow for monitoring of any test article-related effects. The animals were fed with Certified Primate Diet $5048 (PMI, Inc.) and Greenfield city water provided *ad libitum*. Animals were maintained at a temperature of 20 to 26 degrees Centigrade, a relative humidity of 50 +/- 20%, and a 12-hour light/12-hour dark cycle.

[00227] The dosing regimen was in accordance with Table 19 below.

[00228] **Table 19.** Dosing for Cynomolgus animals of Example 10.

Group ID	**Dose (RNAi Agent)**	**Dose Concentration**	**Dosing Route**	**# of Animals (n =)**
1	6.0 mg/kg AC003791	20.0 mg/mL	Day 1 SQ Injection	n = 3
2	6.0 mg/kg AC005898	20.0 mg/mL	Day 1 SQ Injection	n = 3

[00229] Before each SQ injection, the test animals were first sedated. Sedation was accomplished using Ketamine HCl (10 mg/kg) or Telazol (5-8 mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (5 mg/kg) as needed).

[00230] The test animals were dosed via subcutaneous SQ dose via syringe and needle in the scapular region (upper left, upper right, lower left, or lower right scapular region). The dose site was clipped free of hair at least one day prior to each dose administration. Individual doses of APOC3-PCSK9 RNAi agents were calculated based on the body weights recorded on each day of dosing. On each day of dose administration, the APOC3-PCSK9 RNAi agents were allowed to warm to ambient temperature at approximately room temperature for at least 30 minutes prior to the dose administration. Animals were fasted overnight prior to dosing.

[00231] Serum blood (approximately 5.0 mL) was collected on Day -6, Day 8, Day 15, Day 22, Day 29, Day 36, Day 43, Day 50, Day 57, and Day 63, prior to liver biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. The collection site was the femoral vein, with a saphenous vein as an alternative collection site.

[00232] The liver biopsies and serum collected from the test animals were used for analysis for APOC3 and PCSK9 expression and additional biological parameters. Liver biopsies were collected on Day -6, Day 15, Day 36, Day 50, and Day 64 (post-mortem).

[00233] Liver biopsies were collected as a sedated procedure. Animals were fasted overnight (at least 12 hours but less than 18 hours) prior to each liver biopsy collection. For each animal, collected liver biopsy samples were of approximately 100 mg each (80 to 120 mg).

[00234] The collected liver biopsies were analyzed for APOC3 and PCSK9 expression and additional biological parameters. Liver APOC3 and PCSK9 mRNA expression levels were quantified via qPCR, using cARL1 as endogenous control gene, normalized to Day-6 (pre-dose). The qPCR APOC3 and PCSK9 expression data is shown in the following Table 20 and Table 21.

[00235] **Table 20.** Liver APOC3 expression of Cynomolgus animals of Example 10.

	Day -6			Day 15		
Group ID	**Rel. Exp. APOC3**	**Error Low**	**Error High**	**Rel. Exp. APOC3**	**Error Low**	**Error High**
1. 6.0 mg/kg AC003791	1.000	0.210	0.266	0.408	0.131	0.194
2. 6.0 mg/kg AC005898	1.000	0.229	0.298	0.545	0.107	0.133

	Day 36			Day 50		
Group ID	**Rel. Exp. APOC3**	**Error Low**	**Error High**	**Rel. Exp. APOC3**	**Error Low**	**Error High**
1. 6.0 mg/kg AC003791	0.611	0.111	0.136	0.336	0.074	0.095
2. 6.0 mg/kg AC005898	0.929	0.220	0.288	0.491	0.161	0.239
	Day 64					
Group ID	**Rel. Exp. APOC3**	**Error Low**	**Error High**			
1. 6.0 mg/kg AC003791	0.500	0.054	0.061			
2. 6.0 mg/kg AC005898	0.535	0.111	0.139			

[00236] APOC3-PCSK9 RNAi agents achieved knockdown of APOC3 transcripts for a duration of at least 64 days, with subcutaneous SQ injection at 6.0 mg/kg on Day 1. Groups 1 and 2 achieved APOC3 knockdown. More specifically, AC003791 achieved approximately 66% inhibition (0.336) on Day 50 at 6.0 mg/kg. At Day 64, AC003791 achieved approximately 50% inhibition (0.500) at a single 6.0 mg/kg dose.

[00237] **Table 21.** Liver PCSK9 expression of Cynomolgus animals of Example 10.

	Day -6			Day 15		
Group ID	**Rel. Exp. PCSK9**	**Error Low**	**Error High**	**Rel. Exp. PCSK9**	**Error Low**	**Error High**
1. 6.0 mg/kg AC003791	1.000	0.255	0.343	0.692	0.133	0.165
2. 6.0 mg/kg AC005898	1.000	0.269	0.367	0.614	0.193	0.282
	Day 36			**Day 50**		
Group ID	**Rel. Exp. PCSK9**	**Error Low**	**Error High**	**Rel. Exp. PCSK9**	**Error Low**	**Error High**
1. 6.0 mg/kg AC003791	0.908	0.233	0.313	1.271	0.345	0.474
2. 6.0 mg/kg AC005898	0.520	0.228	0.407	0.528	0.155	0.219
	Day 64					
Group ID	**Rel. Exp. PCSK9**	**Error Low**	**Error High**			
1. 6.0 mg/kg AC003791	0.351	0.109	0.158			
2. 6.0 mg/kg AC005898	0.457	0.085	0.104			

[00238] APOC3-PCSK9 RNAi agents achieved knockdown of PCSK9 transcripts for a duration of at least 64 days, with single subcutaneous SQ injection at 6.0 mg/kg on Day 1. Groups 1 and 2 achieved PCSK9 knockdown. More specifically, AC003791 achieved approximately 64% inhibition (0.351) on Day 64 at single 6.0 mg/kg dose.

[00239] Serum PCSK9 was quantified via ELISA (R&D Systems, Cat. #DPC900) in accordance with manufacturer's instructions. The relative PCSK9 levels were normalized to pre-dose Day -6. The data is shown in the following Table 22A.

[00240] **Table 22A.** Serum PCSK9 expression of Cynomolgus animals of Example 10.

	Day -6		**Day 1**		**Day 8**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 6.0 mg/kg AC003791	0.925	0.299	1.161	0.376	0.682	0.393
2. 6.0 mg/kg AC005898	1.140	0.514	1.004	0.434	0.724	0.220
	Day 15		**Day 22**		**Day 29**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 6.0 mg/kg AC003791	0.415	0.061	0.339	0.211	0.353	0.130
2. 6.0 mg/kg AC005898	0.561	0.458	0.560	0.488	0.617	0.608
	Day 36		**Day 43**		**Day 50**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 6.0 mg/kg AC003791	0.323	0.036	0.375	0.115	0.759	0.926
2. 6.0 mg/kg AC005898	0.426	0.221	0.790	0.826	0.728	0.615
	Day 57		**Day 64**			
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**		
1. 6.0 mg/kg AC003791	0.431	0.037	0.538	0.198		
2. 6.0 mg/kg AC005898	0.762	0.645	0.928	0.638		

[00241] APOC3-PCSK9 RNAi agents achieved knockdown of serum PCSK9 for a duration of at least 64 days, with single subcutaneous SQ injection at 6.0 mg/kg on Day 1. Groups 1 and 2 achieved PCSK9 knockdown. More specifically, AC003791 achieved approximately 67% inhibition (0.323) on Day 36 (nadir) at single 6.0 mg/kg dose. At Day 64, AC003791, with single 6.0 mg/kg dose, achieved approximately 46% inhibition (0.538).

[00242] Serum APOC3 was quantified via Roche Cobas® assay for APOC3 in accordance with manufacturer's instructions. The data is shown in the following Table 22B.

[00243] **Table 22B.** Serum APOC3 expression of Cynomolgus animals of Example 10.

	Day -6		Day 1		Day 8	
Group ID	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**
1. 6.0 mg/kg AC003791	6.15	1.04	6.48	0.32	4.57	1.13
2. 6.0 mg/kg AC005898	5.84	0.79	6.10	0.34	4.61	1.23
	Day 15		**Day 22**		**Day 29**	
Group ID	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**
1. 6.0 mg/kg AC003791	4.03	1.81	2.92	1.39	3.69	1.04
2. 6.0 mg/kg AC005898	3.59	0.45	3.50	0.81	4.16	0.85
	Day 36		**Day 43**		**Day 50**	
Group ID	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**
1. 6.0 mg/kg AC003791	2.96	0.87	3.18	1.39	3.32	1.51
2. 6.0 mg/kg AC005898	3.59	0.78	4.07	1.04	3.96	1.38
	Day 57		**Day 64**			
Group ID	**APOC3 (mg/dL)**	**Std. Dev**	**APOC3 (mg/dL)**	**Std. Dev**		
1. 6.0 mg/kg AC003791	4.56	1.37	4.65	0.99		
2. 6.0 mg/kg AC005898	4.94	0.99	4.47	1.14		

[00244] APOC3-PCSK9 RNAi agents achieved knockdown of serum APOC3 for a duration of at least 64 days, with single subcutaneous SQ injection at 6.0 mg/kg on Day 1. Groups 1 and 2 achieved APOC3 knockdown. More specifically, AC003791 achieved approximately 51% inhibition (2.96 mg/dL APOC3 on Day 36 relative to 6.15 mg/dL APOC3 on Day -6) on Day 36 (nadir) at single 6.0 mg/kg dose. Additionally, AC005898 achieved approximately 40% inhibition (3.50 mg/dL APOC3 on Day 22 relative to 5.84 mg/dL APOC3 on Day -6) on Day 22 (nadir) at single 6.0 mg/kg dose. At Day 64, AC003791, with single 6.0 mg/kg dose, achieved approximately 24% inhibition (4.65 mg/dL relative to 6.15 mg/dL on Day -6). At Day 64, AC005898, with single 6.0 mg/kg dose, achieved approximately 23% inhibition (4.47 mg/dL relative to 5.84 mg/dL on Day -6).

Example 11. ***In Vivo Administration of APOC3-PCSK9 RNAi Agents in Cynomolgus Monkeys.***

[00245] APOC3-PCSK9 RNAi agents were tested in Cynomolgus monkeys for inhibition of APOC3 and PCSK9. The APOC3-PCSK9 RNAi agents, which comprise a single RNAi molecule

targeting both hepatic APOC3 and PCSK9 mRNAs, was developed. The pharmacodynamic effects of APOC3-PCSK9 RNAi agents were evaluated in cynomolgus monkeys. Circulating APOC3 and PCSK9 were analyzed by ELISA. Hepatic APOC3 and PCSK9 expression was analyzed by RT-QPCR using the RNA from liver biopsies. Tissue concentration of ARO-AP was analyzed by LC-MS spectrometry.

[00246] Cynomolgus monkeys were acclimated for at least five (5) days prior to the start of the study. Food was withheld overnight (at least 12 but no more than 18 hours) for all groups. Harlan Teklad Monkey Chow, or its equivalent was provided daily in amounts appropriate for the size of the animal. The basic diet may be supplemented with fruit and/or novel treats. Tap water was provided *ad libitum* via automatic watering device. No contaminants were known to be present in the food or water which would interfere with the results of this study. Upon recovery from sedation, animals were fed.

[00247] The study animals were fasted overnight prior to dosing and blood collections. All groups of animals were weighted and dosed via subcutaneous (SQ) route of injection on Day 1 and Day 29. Blood samples were collected on Day -14, -7, 1, 8, 15, 22, 29, 36, 43, 57, 64, 71, and 85.

[00248] The dosing regimen was in accordance with Table 23 below.

[00249] **Table 23.** Dosing for Cynomolgus animals of Example 11.

Group ID	Dose (RNAi Agent)	Dose Concentration	Dosing Route	# of Animals (n =)
1	AC004021 + AC907467	3.0 mg/kg 3.0 mg/kg	Day 1 and Day 29 SQ Injection	n = 3
2	AC005432	6.0 mg/kg	Day 1 and Day 29 SQ Injection	n = 3
3	AC005433	6.0 mg/kg	Day 1 and Day 29 SQ Injection	n = 3
4	AC005495	6.0 mg/kg	Day 1 and Day 29 SQ Injection	n = 3
5	AC005496	6.0 mg/kg	Day 1 and Day 29 SQ Injection	n = 3

[00250] For purposes of evaluating the efficacy of the multimeric APOC3-PCSK9 RNAi agents, monomeric APOC3 RNAi agents and monomeric PCSK9 RNAi agents were also evaluated in parallel. The monomeric APOC3 RNAi agents dosed were of the following structures:

PCSK9 RNAi agent AC004021

Sense strand: (NAG25)s(invAb)sccuguuuuGfcUfuUfuguaacuus(invAb) (SEQ ID NO: 273)

Antisense strand: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 274)

APOC3 RNAi agent AC907467

Sense strand: (NAG25)s(invAb)sacgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 275)

Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfcGfsu (SEQ ID NO: 276)

[00251] Before each SQ injection, the test animals were first sedated. Sedation was accomplished using Ketamine HCl (10 mg/kg) or Telazol (5-8 mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (5 mg/kg) as needed).

[00252] The test animals were dosed via subcutaneous SQ dose via syringe and needle in the mid-scapular region. Individual doses of APOC3-PCSK9 RNAi agents were calculated based on the body weights recorded on each day of dosing.

[00253] Serum blood (approximately 5.0 mL) was collected from each test animal on Day -14 (pre-dose), Day -7 (pre-dose), Day 1 (pre-dose), 8, 15, 22, 29, 36, 43, 57, 64, 71, and 85, prior to liver biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. The collection site was the femoral vein, with a saphenous vein as an alternative collection site.

[00254] Liver biopsies were collected as sedated procedure by Menghini technique. Animals were sedated using Telazol (4-6 mg/kg) and supplemented with Ketamine (~5 mg/kg) if necessary to maintain an appropriate plane of sedation for biopsy sample collection. Animals were monitored post biopsy collection for at least an hour until recovered from sedation. For each animal, ~20 mg x2 of liver biopsy samples were collected with 2 passes of the needle. Liver biopsies were collected, for all test animal groups, on Day -7 (pre-dose), Day 29, Day 57, and Day 85.

[00255] This study did not have a pre-determined necropsy sacrifice date for the Cynomolgus test animals, and samples were collected (liver and/or blood serum) past the collection dates as described above. Should there be any additional samples collected in addition to the aforementioned dates, the samples' tested biological data were described in below.

[00256] The collected liver biopsies were analyzed for APOC3 and PCSK9 expression and additional biological parameters. Liver APOC3 and PCSK9 mRNA expression levels were quantified via qPCR, using cARL1 as endogenous control gene, normalized to Day-7 (pre-dose). The qPCR APOC3 and PCSK9 expression data is shown in the following Table 24 and Table 25.

[00257] **Table 24.** Liver APOC3 expression of Cynomolgus monkeys of Example 11.

	Day -7			Day 29		
Group ID	**Rel. Exp. APOC3**	**Error Low**	**Error High**	**Rel. Exp. APOC3**	**Error Low**	**Error High**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.000	0.308	0.446	0.369	0.049	0.057
2. 6.0 mg/kg AC005432	1.000	0.188	0.232	0.418	0.057	0.066
3. 6.0 mg/kg AC005433	1.000	0.235	0.306	0.317	0.083	0.113
4. 6.0 mg/kg AC005495	1.000	0.183	0.223	0.421	0.140	0.211
5. 6.0 mg/kg AC005496	1.000	0.155	0.184	0.414	0.101	0.133
	Day 57			**Day 85**		
Group ID	**Rel. Exp. APOC3**	**Error Low**	**Error High**	**Rel. Exp. APOC3**	**Error Low**	**Error High**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.701	0.116	0.138	0.347	0.088	0.118
2. 6.0 mg/kg AC005432	0.746	0.263	0.407	0.618	0.117	0.144
3. 6.0 mg/kg AC005433	0.443	0.292	0.856	0.296	0.163	0.363
4. 6.0 mg/kg AC005495	0.960	0.481	0.966	0.551	0.101	0.124
5. 6.0 mg/kg AC005496	0.289	0.131	0.240	0.322	0.135	0.232

[00258] APOC3-PCSK9 RNAi agents achieved knockdown of APOC3 transcripts for a duration of at least 85 days, with 2x subcutaneous SQ injection at 6.0 mg/kg on Day 1 and Day 29. Groups 1-5 achieved APOC3 knockdown. More specifically, AC005496 achieved approximately 71% inhibition (0.289) on Day 57 (nadir) at 6.0 mg/kg, surpassing the ~29% inhibition (0.701) by the monomeric AC907467 APOC3 RNAi agent at the same time point. At Day 85, AC005433 achieved approximately 70% inhibition (0.296) at 2x 6.0 mg/kg dose, surpassing the ~65% inhibition (0.347) by the monomeric AC00907467 APOC3 RNAi agent at the same time point.

[00259] **Table 25.** Liver PCSK9 expression of Cynomolgus animals of Example 11.

	Day -7			Day 29		
Group ID	**Rel. Exp. PCSK9**	**Error Low**	**Error High**	**Rel. Exp. PCSK9**	**Error Low**	**Error High**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.000	0.253	0.338	0.479	0.076	0.090
2. 6.0 mg/kg AC005432	1.000	0.171	0.206	0.815	0.126	0.149
3. 6.0 mg/kg AC005433	1.000	0.193	0.240	0.481	0.057	0.064

4. 6.0 mg/kg AC005495	1.000	0.266	0.363	0.483	0.232	0.446
5. 6.0 mg/kg AC005496	1.000	0.051	0.054	0.858	0.283	0.423
	Day 57			**Day 85**		
Group ID	**Rel. Exp. PCSK9**	**Error Low**	**Error High**	**Rel. Exp. PCSK9**	**Error Low**	**Error High**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.161	0.019	0.022	0.463	0.062	0.072
2. 6.0 mg/kg AC005432	0.398	0.145	0.227	0.412	0.131	0.191
3. 6.0 mg/kg AC005433	0.402	0.212	0.448	0.667	0.135	0.170
4. 6.0 mg/kg AC005495	0.305	0.087	0.121	0.371	0.095	0.127
5. 6.0 mg/kg AC005496	0.621	0.137	0.176	0.911	0.322	0.499

[00260] APOC3-PCSK9 RNAi agents achieved knockdown of PCSK9 transcripts for a duration of at least 85 days, with 2x subcutaneous SQ injection at 6.0 mg/kg on Day 1 and Day 29. Groups 1-5 achieved PCSK9 knockdown. More specifically, AC005496 achieved approximately 69% inhibition (0.305) on Day 57 (nadir) at 6.0 mg/kg. At Day 85, AC005495 achieved approximately 62% inhibition (0.371) at 2x 6.0 mg/kg dose, surpassing the ~53% inhibition (0.463) by the monomeric AC004021 PCSK9 RNAi agent at the same time point.

[00261] Serum PCSK9 was quantified via ELISA (R&D Systems, Cat. #DPC900) in accordance with manufacturer's instructions. The relative PCSK9 levels were normalized to pre-dose Day -7. The data is shown in the following Table 26A.

[00262] **Table 26A.** Serum PCSK9 expression of Cynomolgus animals of Example 11.

	Day -14		**Day -7**		**Day 1**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.159	0.307	1.213	0.145	0.761	0.247
2. 6.0 mg/kg AC005432	1.253	0.320	1.086	0.215	0.776	0.198
3. 6.0 mg/kg AC005433	0.871	0.221	1.093	0.035	1.100	0.300
4. 6.0 mg/kg AC005495	0.943	0.105	1.059	0.123	1.012	0.076
5. 6.0 mg/kg AC005496	1.146	0.109	1.184	0.343	0.771	0.164
	Day 8		**Day 15**		**Day 22**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**

1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.447	0.051	0.360	0.107	0.255	0.088
2. 6.0 mg/kg AC005432	0.376	0.201	0.473	0.096	0.333	0.261
3. 6.0 mg/kg AC005433	0.474	0.175	0.460	0.114	0.338	0.150
4. 6.0 mg/kg AC005495	0.745	0.339	0.659	0.296	0.591	0.339
5. 6.0 mg/kg AC005496	0.641	0.128	0.691	0.293	0.571	0.208
	Day 29		**Day 36**		**Day 43**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.265	0.063	0.165	0.054	0.146	0.087
2. 6.0 mg/kg AC005432	0.472	0.024	0.383	0.095	0.380	0.089
3. 6.0 mg/kg AC005433	0.331	0.077	0.292	0.090	0.215	0.067
4. 6.0 mg/kg AC005495	0.560	0.641	0.384	0.376	0.315	0.281
5. 6.0 mg/kg AC005496	0.437	0.355	0.469	0.274	0.517	0.257
	Day 50		**Day 57**		**Day 64**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.115	0.052	0.111	0.062	0.122	0.092
2. 6.0 mg/kg AC005432	0.317	0.041	0.495	0.192	0.354	0.076
3. 6.0 mg/kg AC005433	0.172	0.060	0.224	0.128	0.235	0.144
4. 6.0 mg/kg AC005495	0.304	0.223	0.353	0.322	0.305	0.196
5. 6.0 mg/kg AC005496	0.304	0.213	0.354	0.272	0.389	0.349
	Day 71		**Day 85**		**Day 99**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.160	0.079	0.182	0.131	0.228	0.124
2. 6.0 mg/kg AC005432	0.587	0.259	0.458	0.090	0.804	0.457
3. 6.0 mg/kg AC005433	0.229	0.087	0.341	0.193	0.456	0.309
4. 6.0 mg/kg AC005495	0.370	0.378	0.346	0.240	0.423	0.111
5. 6.0 mg/kg AC005496	0.342	0.362	0.388	0.284	0.374	0.263
	Day 106					
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**				
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.271	0.153				
2. 6.0 mg/kg AC005432	0.880	0.483				

3. 6.0 mg/kg AC005433	0.340	0.218
4. 6.0 mg/kg AC005495	0.529	0.137
5. 6.0 mg/kg AC005496	0.567	0.358

[00263] APOC3-PCSK9 RNAi agents achieved knockdown of serum PCSK9 for a duration of at least 106 days, with 2x subcutaneous SQ injections at 6.0 mg/kg on Day 1 and Day 29. Groups 1-5 achieved PCSK9 knockdown. More specifically, AC005433 achieved approximately 82% inhibition (0.172) on Day 50 (nadir) at 2x 6.0 mg/kg dose, comparable to the ~88% inhibition (0.115) by the monomeric AC004021 PCSK9 RNAi agent (Group 1) at the same time point. At Day 106, AC005433, with 2x 6.0 mg/kg dose, achieved approximately 66% inhibition (0.340), comparable to the ~72% inhibition (0.271) by the monomeric AC004021 PCSK9 RNAi agent (Group 1) at the same time point.

[00264] Serum APOC3 was quantified via Roche Cobas® assay for APOC3 in accordance with manufacturer's instructions. The relative APOC3 levels were normalized to pre-dose Day -7. The data is shown in the following Table 26B.

[00265] **Table 26B.** Serum APOC3 expression of Cynomolgus animals of Example 11.

	Day -14		**Day -7**		**Day 1**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.000	0.154	1.057	0.155	0.943	0.021
2. 6.0 mg/kg AC005432	1.053	0.240	0.997	0.217	0.953	0.031
3. 6.0 mg/kg AC005433	0.937	0.150	1.000	0.075	1.063	0.225
4. 6.0 mg/kg AC005495	0.960	0.182	1.007	0.211	1.030	0.052
5. 6.0 mg/kg AC005496	0.863	0.100	0.920	0.230	1.220	0.311
	Day 8		**Day 15**		**Day 22**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.760	0.111	0.523	0.083	0.470	0.132
2. 6.0 mg/kg AC005432	0.630	0.312	0.643	0.125	0.593	0.127
3. 6.0 mg/kg AC005433	0.640	0.209	0.637	0.152	0.623	0.178
4. 6.0 mg/kg AC005495	0.737	0.144	0.657	0.072	0.687	0.235
5. 6.0 mg/kg AC005496	0.673	0.127	0.593	0.214	0.557	0.197
	Day 29		**Day 36**		**Day 43**	

Group ID	Rel. Exp. APOC3	Std. Dev	Rel. Exp. APOC3	Std. Dev	Rel. Exp. APOC3	Std. Dev
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.470	0.225	0.293	0.140	0.190	0.085
2. 6.0 mg/kg AC005432	0.453	0.100	0.457	0.153	0.410	0.142
3. 6.0 mg/kg AC005433	0.443	0.287	0.420	0.207	0.290	0.223
4. 6.0 mg/kg AC005495	0.603	0.291	0.473	0.257	0.353	0.059
5. 6.0 mg/kg AC005496	0.320	0.212	0.320	0.248	0.185	0.163
	Day 50		**Day 57**		**Day 64**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.190	0.000	0.193	0.071	0.163	0.046
2. 6.0 mg/kg AC005432	0.437	0.172	0.400	0.187	0.410	0.170
3. 6.0 mg/kg AC005433	0.420	0.082	0.440	0.203	0.420	0.236
4. 6.0 mg/kg AC005495	0.430	0.137	0.470	0.270	0.423	0.180
5. 6.0 mg/kg AC005496	0.220	0.240	0.195	0.177	0.330	0.212
	Day 71		**Day 85**		**Day 99**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.223	0.106	0.347	0.071	0.447	0.170
2. 6.0 mg/kg AC005432	0.620	0.494	0.577	0.140	0.660	0.053
3. 6.0 mg/kg AC005433	0.487	0.278	0.560	0.200	0.737	0.274
4. 6.0 mg/kg AC005495	0.533	0.289	0.543	0.227	0.770	0.312
5. 6.0 mg/kg AC005496	0.255	0.276	0.405	0.361	0.555	0.417
	Day 106					
Group ID	**Rel. Exp. APOC3**	**Std. Dev**				
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.457	0.099				
2. 6.0 mg/kg AC005432	0.753	0.238				
3. 6.0 mg/kg AC005433	0.647	0.250				
4. 6.0 mg/kg AC005495	0.727	0.189				
5. 6.0 mg/kg AC005496	0.575	0.502				

[00266] APOC3-PCSK9 RNAi agents achieved knockdown of serum APOC3 for a duration of at least 106 days, with 2x subcutaneous SQ injections at 6.0 mg/kg on Day 1 and Day 29. Groups 1-5 achieved PCSK9 knockdown. More specifically, AC005496 achieved approximately

81% inhibition (0.185) on Day 43 (nadir) at 2x 6.0 mg/kg dose, comparable to the ~81% inhibition (0.190) by the monomeric AC004021 PCSK9 RNAi agent (Group 1) at the same time point. At Day 106, AC005496, with 2x 6.0 mg/kg dose, achieved approximately 42% inhibition (0.575), comparable to the ~54% inhibition (0.457) by the monomeric AC004021 PCSK9 RNAi agent (Group 1) at the same time point.

[00267] In cynomolgus monkeys, subcutaneous injection of APOC3-PCSK9 RNAi agents on Day 1 and Day 29 at 6 mg/kg caused >70% decreases in circulating APOC3 and PCSK9 proteins respectively. The extents of decreases in APOC3 and PCSK9 are comparable to those of the treatment by monomeric APOC3 and monomeric PCSK9 RNAi agents. The decreases in circulating APOC3 and PCSK9 proteins caused by the APOC3-PCSK9 RNAi agents exhibited a strong correlation with their antisense concentration in liver tissue. Furthermore, the decreases in circulating APOC3 and PCSK9 proteins were also corroborated by the decreases in hepatic APOC3 and PCSK9 transcripts.

Example 12. ***In Vivo Administration of APOC3-PCSK9 RNAi Agents in Cynomolgus Monkeys.***

[00268] APOC3-PCSK9 RNAi agents, which comprise a single RNAi molecule targeting both hepatic APOC3 and PCSK9 mRNAs, were developed and tested in Cynomolgus monkeys for inhibition of APOC3 and PCSK9. The pharmacodynamic effects of APOC3-PCSK9 RNAi agents were evaluated in cynomolgus monkeys. Circulating APOC3 and PCSK9 were analyzed by ELISA.

[00269] Cynomolgus monkeys (Cynomolgus macaque, male) were acclimated for at least five (5) days prior to the start of the study. Food was withheld overnight (at least 12 but no more than 18 hours) for all groups. Harlan Teklad Monkey Chow, or its equivalent was provided daily in amounts appropriate for the size of the animal. The basic diet may be supplemented with fruit and/or novel treats. Tap water was provided *ad libitum* via automatic watering device. No contaminants were known to be present in the food or water which would interfere with the results of this study. Upon recovery from sedation, animals were fed.

[00270] The study animals were fasted overnight prior to dosing and blood collections. All groups of animals receiving the APOC3-PCSK9 RNAi agents (i.e., groups 3-6) were weighed and dosed via subcutaneous (SQ) route of injection on Day 1 and Day 28. Animals receiving the PCSK9 RNAi agent and the APOC3 RNAi agent as monomers were dosed with the

monomeric PCSK9 RNAi agent on Day 1 and Day 28, while the monomeric APOC3 RNAi agent was dosed on Day 2 and Day 29.

[00271] The dosing regimen was in accordance with Table 27 below.

[00272] **Table 27.** Dosing for Cynomolgus animals of Example 12.

Group ID	Dose (RNAi Agent)	Dose Concentration	Dosing Route	# of Animals (n =)
1	Saline	N/A	Day 1 and Day 28 SQ Injection	n = 4
2	AC004021	4.5 mg/kg	Day 1 and Day 28 SQ Injection	n = 4
	AC907467	4.5 mg/kg	Day 2 and Day 29 SQ Injection	n = 4
3	AC005495	3.0 mg/kg	Day 1 and Day 28 SQ Injection	n = 4
4	AC005495	9.0 mg/kg	Day 1 and Day 28 SQ Injection	n = 4
5	AC007746	3.0 mg/kg	Day 1 and Day 28 SQ Injection	n = 3
6	AC007746	9.0 mg/kg	Day 1 and Day 28 SQ Injection	n = 3

[00273] For purposes of evaluating the efficacy of the multimeric APOC3-PCSK9 RNAi agents, monomeric APOC3 RNAi agents and monomeric PCSK9 RNAi agents were also evaluated in parallel. The monomeric APOC3 and monomeric PCSK9 RNAi agents dosed were of the following structures:

PCSK9 RNAi agent AC004021

Sense strand: (NAG25)s(invAb)sccuguuuuGfcUfuUfuguaacuus(invAb) (SEQ ID NO: 273)

Antisense strand: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 274)

APOC3 RNAi agent AC907467

Sense strand: (NAG25)s(invAb)sacgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 275)

Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfcGfsu (SEQ ID NO: 276)

[00274] Before each SQ injection, the test animals were first sedated. Sedation was accomplished using Ketamine HCl (10 mg/kg) or Telazol (5-8 mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (5 mg/kg) as needed).

[00275] The test animals were dosed via subcutaneous SQ dose via syringe and needle in the mid-scapular region. Individual doses of APOC3-PCSK9 RNAi agents were calculated based on the body weights recorded on each day of dosing. Animals were placed in an animal restraint

chair. Individual doses were calculated based on body weights recorded on each day of dosing. RNAi agents were administered, via subcutaneous administration, on according to the dosing shown in Table 27 above.

[00276] Serum blood (approximately 5.0 mL) was collected from each test animal on Day -14 (pre-dose), Day -7 (pre-dose), Day 1 (pre-dose), 2 (Group 2) 8, 15, 22, 28, 29 (Group 2), 36, 43, 57, 64, 71, and 85, prior to liver biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. The collection site was the femoral vein, with a saphenous vein as an alternative collection site.

[00277] No necropsy was planned. However, should an animal become deceased, it will be necropsied immediately or as soon as possible after death, with tissue samples for histological evaluation taken at veterinarian's discretion.

[00278] Serum PCSK9 was quantified via ELISA (R&D Systems, Cat. #DPC900) in accordance with manufacturer's instructions. The relative PCSK9 levels were normalized to pre-dose Day -14. The data is shown in the following Table 28A.

[00279] **Table 28A.** Serum PCSK9 expression of Cynomolgus animals of Example 12.

	Day -14		**Day 1**		**Day 2**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. Saline	1.059	0.157	0.941	0.157		
2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.940	0.064	1.060	0.064	0.926	0.198
3. 3.0 mg/kg AC005495	0.968	0.076	1.032	0.076		
4. 9.0 mg/kg AC005495	1.064	0.211	0.936	0.211		
5. 3.0 mg/kg AC007746	1.055	0.134	0.945	0.134		
6. 9.0 mg/kg AC007746	1.107	0.062	0.893	0.062		
	Day 15		**Day 28**		**Day 29**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. Saline	1.305	0.280	0.966	0.286		
2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.358	0.139	0.289	0.030	0.334	0.075
3. 3.0 mg/kg AC005495	1.071	0.099	0.953	0.173		
4. 9.0 mg/kg AC005495	0.387	0.120	0.347	0.189		
5. 3.0 mg/kg AC007746	0.768	0.173	0.533	0.093		

6. 9.0 mg/kg AC007746	0.377	0.060	0.268	0.047		
	Day 43		**Day 57**			
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**		
1. Saline	1.034	0.212	0.931	0.131		
2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.236	0.060	0.247	0.036		
3. 3.0 mg/kg AC005495	0.773	0.124	0.767	0.126		
4. 9.0 mg/kg AC005495	0.274	0.132	0.278	0.135		
5. 3.0 mg/kg AC007746	0.557	0.110	0.524	0.125		
6. 9.0 mg/kg AC007746	0.191	0.084	0.154	0.045		

[00280] APOC3-PCSK9 RNAi agents achieved knockdown of serum PCSK9 out to at least 57 days, with 2x subcutaneous SQ injections at 3.0 mg/kg or 9.0 mg/kg on Day 1 and Day 28. Group 3 (3.0 mg/kg AC005495) showed negligible knockdown on Day 15 and 28. At Day 57, Group 6 cynos administered with 2 doses of 9.0 mg/kg AC007746, achieved ~85 inhibition (0.154) of PCSK9. This PCSK9 inhibition is superior to Group 2 cynos dosed with monomeric PCSK9 RNAi agent of ~75% inhibition (0.247) at the same time point.

[00281] Serum APOC3 was quantified via Roche Cobas® assay for APOC3 in accordance with manufacturer's instructions. The relative APOC3 levels were normalized to pre-dose Day 1. The data is shown in the following Table 28B.

[00282] **Table 28B.** Serum APOC3 expression of Cynomolgus animals of Example 12.

	Day -14		**Day 1**		**Day 2**	
Group ID	**Rel Exp APOC3**	**Std. Dev**	**Rel Exp APOC3**	**Std. Dev**	**Rel Exp APOC3**	**Std. Dev**
1. Saline	0.987	0.159	1.000	0.116		
2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.998	0.124	1.000	0.200	1.049	0.135
3. 3.0 mg/kg AC005495	0.874	0.209	1.000	0.244		
4. 9.0 mg/kg AC005495	0.935	0.341	1.000	0.132		
5. 3.0 mg/kg AC007746	1.002	0.109	1.000	0.105		
6. 9.0 mg/kg AC007746	1.170	0.254	1.000	0.132		
	Day 15		**Day 28**		**Day 29**	
Group ID	**Rel Exp APOC3**	**Std. Dev**	**Rel Exp APOC3**	**Std. Dev**	**Rel Exp APOC3**	**Std. Dev**
1. Saline	1.155	0.152	1.028	0.125		

2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.601	0.124	0.542	0.087	0.512	0.041
3. 3.0 mg/kg AC005495	0.745	0.177	0.920	0.379		
4. 9.0 mg/kg AC005495	0.459	0.106	0.453	0.096		
5. 3.0 mg/kg AC007746	0.810	0.079	0.814	0.093		
6. 9.0 mg/kg AC007746	0.533	0.278	0.521	0.213		

	Day 43		**Day 57**	
Group ID	**Rel Exp APOC3**	**Std. Dev**	**Rel Exp APOC3**	**Std. Dev**
1. Saline	1.285	0.092	1.316	0.054
2. 4.5 mg/kg AC004021 + 4.5 mg/kg AC907467	0.528	0.122	0.552	0.170
3. 3.0 mg/kg AC005495	0.949	0.513	1.034	0.490
4. 9.0 mg/kg AC005495	0.425	0.215	0.439	0.268
5. 3.0 mg/kg AC007746	0.917	0.174	1.018	0.138
6. 9.0 mg/kg AC007746	0.456	0.337	0.491	0.294

[00283] APOC3-PCSK9 RNAi agents achieved knockdown of serum APOC3 for a duration of at least 57 days, with 2x subcutaneous SQ injections at 9.0 mg/kg on Day 1 and Day 29. Groups 2, 4, and 6 achieved APOC3 knockdown. More specifically, AC005495 achieved ~56% inhibition (0.439) on Day 57 after two 9.0 mg/kg doses, superior to the ~45% inhibition (0.552) by the monomeric AC907467 APOC3 RNAi agent (Group 2) at the same time point (Day 57). AC005495 achieved ~57% inhibition (0.425) on Day 43 (nadir) after two 9.0 mg/kg doses, superior to the ~47% inhibition (0.528) by the monomeric AC907467 APOC3 RNAi agent (Group 2) at the same time point (Day 43).

Example 13. ***In Vivo Administration of APOC3-PCSK9 RNAi Agents in Cynomolgus Monkeys.***

[00284] APOC3-PCSK9 RNAi agents were tested in Cynomolgus monkeys for inhibition of APOC3 and PCSK9. The APOC3-PCSK9 RNAi agents, which comprise a single RNAi molecule targeting both hepatic APOC3 and PCSK9 mRNAs, was developed. The pharmacodynamic effects of APOC3-PCSK9 RNAi agents were evaluated in cynomolgus monkeys. Circulating APOC3 and PCSK9 were analyzed by ELISA. Hepatic APOC3 and PCSK9 expression was analyzed by RT-QPCR using the RNA from liver biopsies.

[00285] The study animals (Cynomolgus monkeys) were fasted overnight prior to dosing and blood collections. All groups of animals were weighted and dosed via subcutaneous (SQ) route of injection on Day 1, Group 2 was also dosed on Day 2.

[00286] The dosing regimen was in accordance with Table 29 below.

[00287] **Table 29.** Dosing for Cynomolgus animals of Example 13.

Group ID	Dose (RNAi Agent)	Dose Concentration	Dosing Route	# of Animals (n =)
1	AC004021 + AC907467	3.0 mg/kg 3.0 mg/kg	Day 1 and Day 2 SQ Injection	n = 4
2	AC008307	6.0 mg/kg	Day 1 SQ Injection	n = 4
3	AC007746	6.0 mg/kg	Day 1 SQ Injection	n = 4

[00288] For purposes of evaluating the efficacy of the multimeric APOC3-PCSK9 RNAi agents, monomeric APOC3 RNAi agents and monomeric PCSK9 RNAi agents were also evaluated in parallel. The monomeric APOC3 and monomeric PCSK9 RNAi agents dosed were of the following structures:

PCSK9 RNAi agent AC004021

Sense strand: (NAG25)s(invAb)sccuguuuuGfcUfuUfuguaacuus(invAb) (SEQ ID NO: 273)

Antisense strand: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 274)

APOC3 RNAi agent AC907467

Sense strand: (NAG25)s(invAb)sacgggacaGfUfAfuucucaguias(invAb) (SEQ ID NO: 275)

Antisense strand: usCfsasCfuGfagaauAfcUfgUfcCfcGfsu (SEQ ID NO: 276)

[00289] Before each SQ injection, the test animals were first sedated. Sedation was accomplished using Ketamine HCl (10 mg/kg) or Telazol (5-8 mg/kg), administered as an intramuscular (IM) injection and supplemented with Ketamine (5 mg/kg) as needed).

[00290] The test animals were dosed via subcutaneous SQ dose via syringe and needle in the mid-scapular region. Individual doses of APOC3-PCSK9 RNAi agents were calculated based on the body weights recorded on each day of dosing. Animals were placed in an animal restraint chair. Individual doses were calculated based on body weights recorded on each day of dosing.

RNAi agents were administered, via subcutaneous administration, on Day 1 for all test groups. Group 1 animals were administered with AC004021 on Day 1 and AC907467 on Day 2.

[00291] Serum blood (approximately 5.0 mL) was collected from each test animal on Day -14 (pre-dose), Day -7 (pre-dose), Day 1 (pre-dose), 8, 15, 22, 29, 36, 43, 57, 64, 71, and 85, prior to liver biopsy sample collections or dose administration (when applicable), and from any animals found in moribund condition or sacrificed at an unscheduled interval. The collection site was the femoral vein, with a saphenous vein as an alternative collection site.

[00292] Serum PCSK9 was quantified via ELISA (R&D Systems, Cat. #DPC900) in accordance with manufacturer's instructions. The relative PCSK9 levels were normalized to pre-dose (Day -14, Day -7, and Day 1). The data is shown in the following Table 30A.

[00293] **Table 30A.** Serum PCSK9 expression of Cynos of Example 13.

	Day -14		**Day -7**		**Day 1**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.169	0.166	0.810	0.221	1.021	0.055
2. 6.0 mg/kg AC008307	1.385	0.163	0.820	0.035	0.795	0.140
3. 6.0 mg/kg AC007746	1.210	0.025	0.828	0.015	0.961	0.025
	Day 8		**Day 15**		**Day 22**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.365	0.270	0.227	0.164	0.189	0.134
2. 6.0 mg/kg AC008307	0.575	0.121	0.276	0.068	0.323	0.189
3. 6.0 mg/kg AC007746	0.363	0.133	0.197	0.103	0.164	0.115
	Day 29		**Day 36**		**Day 43**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.191	0.148	0.276	0.211	0.210	0.162
2. 6.0 mg/kg AC008307	0.319	0.069	0.560	0.121	0.403	0.086
3. 6.0 mg/kg AC007746	0.161	0.097	0.234	0.107	0.170	0.089
	Day 50		**Day 57**		**Day 64**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.219	0.164	0.235	0.140	0.369	0.340

2. 6.0 mg/kg AC008307	0.354	0.031	0.506	0.145	0.627	0.290
3. 6.0 mg/kg AC007746	0.176	0.074	0.193	0.124	0.318	0.186
	Day 78		**Day 85**		**Day 92**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.290	0.250	0.321	0.182	0.470	0.306
2. 6.0 mg/kg AC008307	0.640	0.107	0.557	0.208	0.683	0.172
3. 6.0 mg/kg AC007746	0.338	0.160	0.420	0.266	0.517	0.247
	Day 99		**Day 106**		**Day 113**	
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**	**Rel. Exp. PCSK9**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.536	0.463	0.513	0.307	0.626	0.543
2. 6.0 mg/kg AC008307	0.661	0.190	0.632	0.031	0.857	0.254
3. 6.0 mg/kg AC007746	0.421	0.166	0.606	0.286	0.675	0.173
	Day 120					
Group ID	**Rel. Exp. PCSK9**	**Std. Dev**				
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.634	0.446				
2. 6.0 mg/kg AC008307	0.745	0.102				
3. 6.0 mg/kg AC007746	0.705	0.235				

[00294] APOC3-PCSK9 RNAi agents achieved knockdown of serum PCSK9 out to at least 120 days, with single subcutaneous SQ injection at 6.0 mg/kg on Day 1. At Day 120, Group 3 cynos administered with 6.0 mg/kg AC007746 achieved ~29% inhibition (0.705) of PCSK9. This PCSK9 inhibition is comparable to Group 1 cynos dosed with monomeric PCSK9 RNAi agent of ~27% inhibition (0.634) at the same time point. At nadir on Day 29, Group 3 cynos administered with 6.0 mg/kg AC007746 achieved ~84% inhibition (0.161) of PCSK9, comparable to Group 1 cynos dosed with monomeric PCSK9 RNAi agent of ~81% inhibition (0.191) at the same time point (Day 29).

[00295] Serum APOC3 was quantified via Roche Cobas® assay for APOC3 in accordance with manufacturer's instructions. The relative APOC3 levels were normalized to pre-dose Day 1. The data is shown in the following Table 30B.

[00296] **Table 30B.** Serum APOC3 expression of Cynos of Example 13.

	Day -14		Day -7		Day 1	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	1.177	0.246	0.992	0.102	1.000	0.132
2. 6.0 mg/kg AC008307	0.985	0.279	1.020	0.397	1.000	0.470
3. 6.0 mg/kg AC007746	1.135	0.005	1.080	0.262	1.000	0.219
	Day 2		**Day 8**		**Day 15**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.126	0.126	0.252	0.159	0.171	0.096
2. 6.0 mg/kg AC008307			0.427	0.364	0.189	0.090
3. 6.0 mg/kg AC007746			0.393	0.114	0.277	0.043
	Day 22		**Day 29**		**Day 36**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.301	0.158	0.251	0.101	0.278	0.143
2. 6.0 mg/kg AC008307	0.380	0.102	0.409	0.213	0.660	0.287
3. 6.0 mg/kg AC007746	0.341	0.063	0.392	0.055	0.428	0.078
	Day 43		**Day 50**		**Day 57**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.315	0.166	0.265	0.135	0.249	0.160
2. 6.0 mg/kg AC008307	0.559	0.248	0.678	0.323	0.675	0.347
3. 6.0 mg/kg AC007746	0.426	0.060	0.564	0.218	0.507	0.232
	Day 64		**Day 71**		**Day 78**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.360	0.293	0.390	0.247	0.337	0.234
2. 6.0 mg/kg AC008307	0.867	0.373	0.775	0.393	0.798	0.491
3. 6.0 mg/kg AC007746	0.689	0.149	0.687	0.166	0.671	0.281
	Day 85		**Day 92**		**Day 99**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.409	0.204	0.541	0.182	0.580	0.373
2. 6.0 mg/kg AC008307	0.953	0.460	0.961	0.387	1.147	0.471

3. 6.0 mg/kg AC007746	0.813	0.268	0.806	0.293	1.224	0.234
	Day 106		**Day 113**		**Day 120**	
Group ID	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**	**Rel. Exp. APOC3**	**Std. Dev**
1. 3.0 mg/kg AC004021 + 3.0 mg/kg AC907467	0.674	0.266	0.614	0.356	0.858	0.238
2. 6.0 mg/kg AC008307	1.168	0.445	1.143	0.398	1.191	0.387
3. 6.0 mg/kg AC007746	1.116	0.204	1.172	0.207	1.135	0.214

[00297] APOC3-PCSK9 RNAi agents achieved knockdown of serum APOC3 for a duration of 78 days, with single subcutaneous SQ injection at 6.0 mg/kg on Day 1. At nadir on Day 15, Group 3 cynos dosed with a single dose 6.0 mg/kg AC007746 achieved ~72% APOC3 inhibition (0.277). This is comparable to Group 1 cynos dosed with monomeric APOC3 RNAi agent AC907467 (single dose 3.0 mg/kg AC907467), of ~83% inhibition (0.171).

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[00302] Peterson. A.. et al., PCSK9 Function and Physiology, 49(7) *J. Lipid Res.* 1595-99 (2008).

[00303] Horton, J., et al., Molecular Biology of PCSK9: Its Role in LDL Metabolism, 32(2) *Trends Biochem Sci.* 71-77 (2007).

[00304] Horton, J., et al., PCKS9: A Convertase That Coordinates LDL Catabolism, 50(Suppl) *J. Lipid Res.* S172-77 (2009)

[00305] Klein-Szanto & Bassi, Keep Recycling Going: New Approaches to Reduce LDL-C, 164 *Biochem. Pharmacol.* 336-341 (2019).

OTHER EMBODIMENTS

[00306] 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 the expression of an Apolipoprotein C-III (APOC3) gene and a Proprotein Convertase Subtilisin Kexin 9 (PCSK9) gene, comprising:

(i) a first antisense strand that comprises at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any of the sequences of Table 2A, or Table 2B, Table 3A, Table 3B, or Table 5C, and

(ii) a second antisense strand that comprises at least 17 contiguous nucleotides differing by 0 or 1 nucleotides from any of the sequences of Table 2A, Table 2B, Table 3A, Table 3B, or Table 5C, and

(iii) one or more sense strands,

wherein the one or more sense strands comprise a nucleotide sequence that is at least partially complementary to the first antisense strand and the second antisense strand.

2. The RNAi agent of claim 1, wherein the one or more sense strands comprise a nucleotide sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C.

3. The RNAi agent of claim 1 or claim 2, wherein the RNAi agent comprises a first sense strand sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C and a second sense strand sequence of any one of the sequences of Table 4A, Table 4B, or Table 4C.

4. The RNAi agent of any one of claims 1-3, wherein the RNAi agent comprises a first antisense strand sequence of any one of the sequences of Table 2A, Table 3A, or Table 5C, and a second sense strand sequence of any one of the sequences of Table 2B, Table 3B, or Table 5C.

5. The RNAi agent of any one of claims 1-3, wherein the RNAi agent comprises a first antisense strand sequence of any one of the sequences of Table 2B, Table 3B, or Table 5C, and a second sense strand sequence of any one of the sequences of Table 2A, Table 3A, or Table 5C.

6. The RNAi agent of any one of claims 1-5, wherein at least one nucleotide of the RNAi agent is a modified nucleotide or includes a modified internucleoside linkage.

7. The RNAi agent of any one of claims 1-6, wherein all or substantially all of the nucleotides of the sense and/or antisense strand of the RNAi agent are modified nucleotides.

8. The RNAi agent of any one of claims 1-7, wherein the modified nucleotide is selected from the group consisting of: 2′-O-methyl nucleotide, 2′-fluoro nucleotide, 2′-deoxy nucleotide, 2′,3′-seco nucleotide mimic, locked nucleotide, 2′-F-arabino nucleotide, 2′-methoxyethyl nucleotide, abasic nucleotide, ribitol, inverted nucleotide, inverted 2′-O-methyl nucleotide, inverted 2′-deoxy nucleotide, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholine nucleotide, vinyl phosphonate containing nucleotide, cyclopropyl phosphonate containing nucleotide, and 3′-O-methyl nucleotide.

9. The RNAi agent of claim 8, wherein all or substantially all of the modified nucleotides are 2′-O-methyl nucleotides, 2′-fluoro nucleotides, or combinations thereof.

10. The RNAi agent of any one of claims 1-9, wherein the first antisense strand sequence is selected from any one of the sequences of Table 3A, and the second antisense strand sequence is selected from any one of the sequences of Table 3B.

11. The RNAi agent of any one of claims 1-9, wherein the first antisense strand sequence is selected from any one of the sequences of Table 3B, and the second antisense strand sequence is selected from any one of the sequences of Table 3A.

12. The RNAi agent of any one of claims 1-11, wherein:
(a) the one or more sense strands comprise between 30 and 60 nucleotides, or
(b) the first antisense strand is between 18 and 30 nucleotides in length, or
(c) the second antisense strand is between 18 and 30 nucleotides in length, or
(d) any combination of (a) through (c) above.

13. The RNAi agent of claim 12, wherein:
(a) the one or more sense strands comprise between 36 and 54 nucleotides, or
(b) the first antisense strand is between 18 and 27 nucleotides in length, or

(c) the second antisense strand is between 18 and 27 nucleotides in length, or

(d) any combination of (a) through (c) above.

14. The RNAi agent of claim 13, wherein:

(a) the one or more sense strands comprise between 36 and 48 nucleotides, or

(b) the first antisense strand is between 18 and 24 nucleotides in length, or

(c) the second antisense strand is between 18 and 24 nucleotides in length, or

(d) any combination of (a) through (c) above.

15. The RNAi agent of claim 14, wherein:

(a) the one or more sense strands comprise between 38 and 42 nucleotides, or

(b) the first antisense strand is between 19 and 21 nucleotides in length, or

(c) the second antisense strand is between 19 and 21 nucleotides in length, or

(d) any combination of (a) through (c) above.

16. The RNAi agent of claim 15, wherein:

(a) the one or more sense strands comprises 38 or 42 nucleotides, or

(b) the second antisense strand is 19 nucleotides in length, or

(c) the second antisense strand is 21 nucleotides in length, or

(d) any combination of (a) through (c) above.

17. The RNAi agent of claim 1, wherein the one or more sense strands comprises the structure:

SS_1——L——SS_2

wherein SS_1 comprises a first sense strand sequence;

SS_2 comprises a second sense strand sequence; and

L is a linker, or a bond.

18. The RNAi agent of claim 17, wherein L is a nucleotide linker.

19. The RNAi agent of claim 17, wherein L is a non-nucleotide linker.

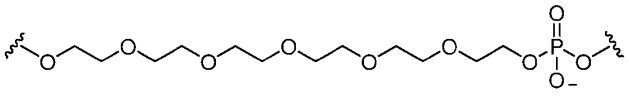
20. The RNAi agent of claim 19, wherein L is polyethylene glycol (PEG).

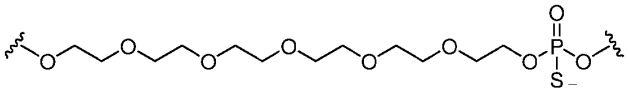
21. The RNAi agent of any one of claims 17-20, wherein SS_1 comprises any one of the sense strand sequences listed in Table 4A or Table 4B.

22. The RNAi agent of any one of claims 17-21, wherein SS_2 comprises any one of the sense strand sequences listed in Table 4A or Table 4B.

23. The RNAi agent of claim 17, wherein SS_1 and SS_2 each independently comprise any one of the sense strand sequences listed in Table 4A or Table 4B, wherein at least one sense strand sequence is selected from Table 4A and the other sense strand sequence is selected from Table 4B.

24. The RNAi agent of any one of claims 17-23, wherein L comprises the structure:


(Sp18), or


(Sp18s).

25. The RNAi agent of claim 17, wherein SS_1——L——SS_2 is selected from any one of the sense strands listed in Table 4C.

26. The RNAi agent of any one of claims 17-25, wherein the first antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3A, and the second antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3B.

27. The RNAi agent of any one of claims 17-26, wherein the first antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3B, and the second antisense strand consists of, consists essentially of, or comprises the nucleotide sequence of any one of the modified antisense strand sequences of Table 3A.

28. The RNAi agent of any one of claims 1-27, wherein the one or more sense strands consists of, consists essentially of, or comprises the nucleotide sequence of any of the modified sense strand sequences of Table 4A, Table 4B, Table 4C, or Table 5C.

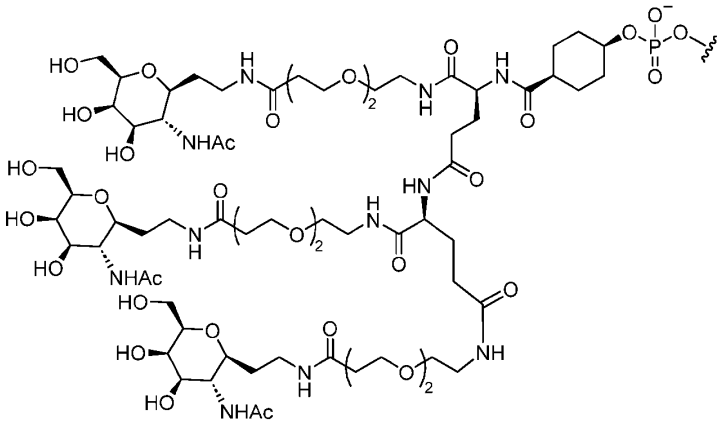
29. The RNAi agent of any one of claims 1-28, wherein the RNAi agent is linked to a targeting ligand.

30. The RNAi agent of claim 29, wherein the targeting ligand comprises N-acetylgalactosamine.

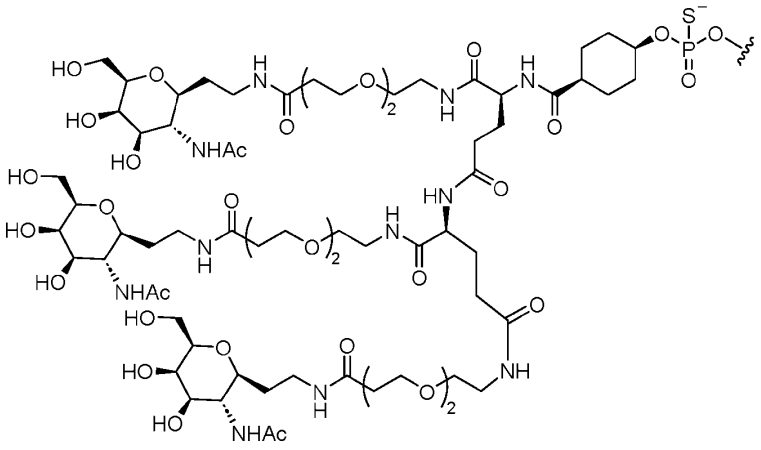
31. The RNAi agent of claim 29, wherein the targeting ligand comprises a metabolically stabilized carbohydrate ligand.

32. The RNAi agent of claim 31, wherein the targeting ligand comprises a metabolically stabilized N-acetylgalactosamine ligand.

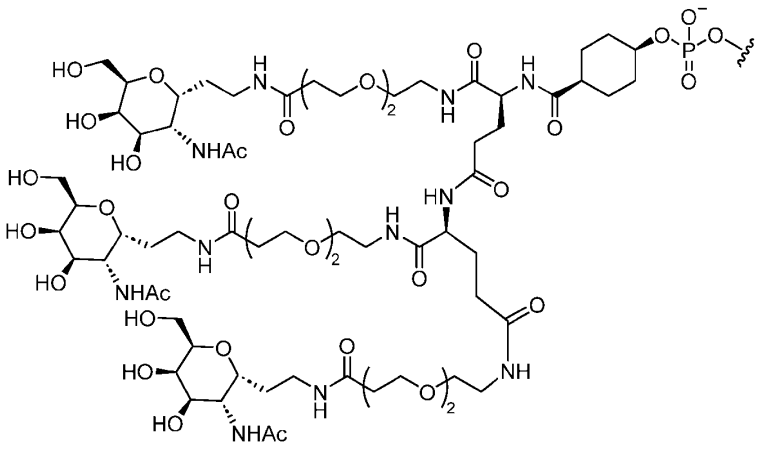
33. The RNAi agent of any one of claims 1-32, comprising



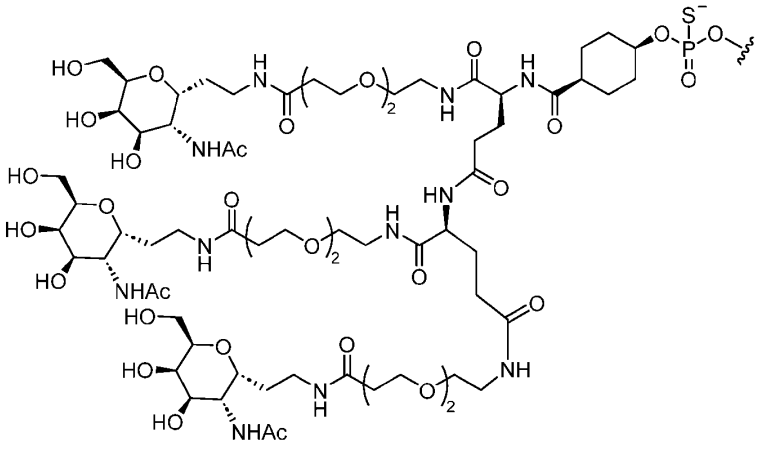
(NAG42),



(NAG42)s,



(NAG52),



(NAG52)s, or a pharmaceutically acceptable salt thereof.

34. The RNAi agent of any one of claims 29-33, wherein the targeting ligand is linked to the one or more sense strands.

35. The RNAi agent of claim 34, wherein the targeting ligand is linked to the 5' terminal end of the one or more sense strands.

36. The RNAi agent of any of claims 1-35, wherein the RNAi agent has two blunt ends.

37. The RNAi agent of any of claims 1-36, wherein the one or more sense strands comprise one or two terminal caps.

38. The RNAi agent of any of claims 1-37, wherein the one or more sense strands comprise one or two inverted abasic residues.

39. The RNAi agent of claim 1, wherein the RNAi agent forms a complex of any of the complexes set forth in Table 5A, Table 5B, or Table 5C.

40. The RNAi agent of any one of claims 1-39, wherein the one or more sense strands comprises inverted abasic residues at the 3' terminal end of the nucleotide sequence, at the 5' end of the nucleotide sequence, or at both.

41. The RNAi agent of claim 1, wherein:

the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

AAGUUACAAAAGCAAAACA (SEQ ID NO: 279); and

the second antisense strand that consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

UCACUGAGAAUACUGUCCC (SEQ ID NO: 277).

42. The RNAi agent of claim 41, wherein the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

AAGUUACAAAAGCAAAACAGG (SEQ ID NO: 295).

43. The RNAi agent of claim 41 or 42, wherein:

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

UGUUUUGCUUUUGUAACUU (SEQ ID NO: 311); and

the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

GGGACAGUAUUCUCAGUIA (SEQ ID NO: 299).

44. The RNAi agent of claim 43, wherein the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence: CCUGUUUUGCUUUUGUAACUU (SEQ ID NO: 313).

45. The RNAi agent of any one of claims 41-44, wherein all or substantially all of the nucleotides are modified nucleotides.

46. The RNAi agent of claim 1, wherein:

the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

cPrpasAfsguuacaaaaGfcAfaAfaca (SEQ ID NO: 342); and

the second antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:

cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO: 71);

wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5’-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5’-cyclopropyl phosphonate-2′-O-methyluridine; s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the first sense strand or second sense strand are modified nucleotides.

47. The RNAi agent of claim 46, wherein the first antisense strand consists of, consists essentially of, or comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:
cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 101).

48. The RNAi agent of claim 46 or 47, wherein:
the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:
uguuuuGfcUfuUfuguaacuu (SEQ ID NO: 178); and
the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:
gsggacaGfuAfuUfcucaguia (SEQ ID NO: 134)
wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; s represents a phosphorothioate linkage; and wherein all or substantially all of the nucleotides on the first sense strand or second sense strand are modified nucleotides.

49. The RNAi agent of claim 48, wherein the one or more sense strands comprises a nucleotide sequence that differs by 0 or 1 nucleotides from the nucleotide sequence:
ccuguuuuGfcUfuUfuguaacuu (SEQ ID NO: 194).

50. The RNAi agent of any one of claims 41-49, wherein the one or more sense strands further comprises inverted abasic residues at the 3’ terminal end of the nucleotide sequence, at the 5’ end of the nucleotide sequence, or at both.

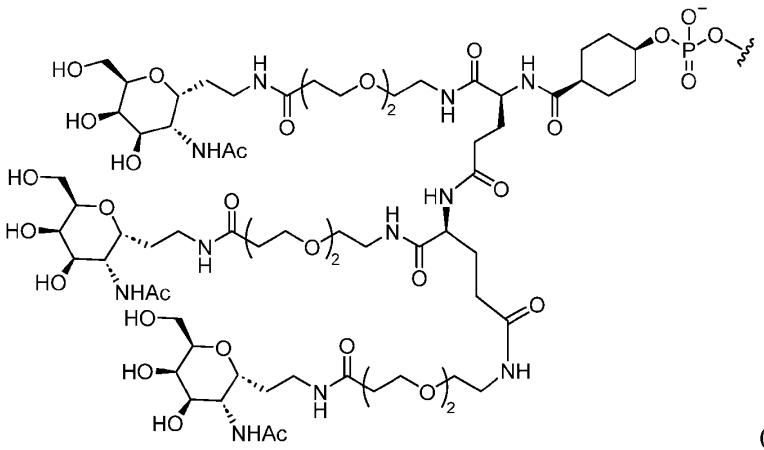
51. The RNAi agent of claim 50, wherein the first sense strand further includes an inverted abasic residue at the 3’ terminal end, and wherein the second sense strand further comprises an inverted abasic residue at the 3’ terminal end.

52. The RNAi agent of any one of claims 41-51, wherein the one or more sense strands comprises a linker.

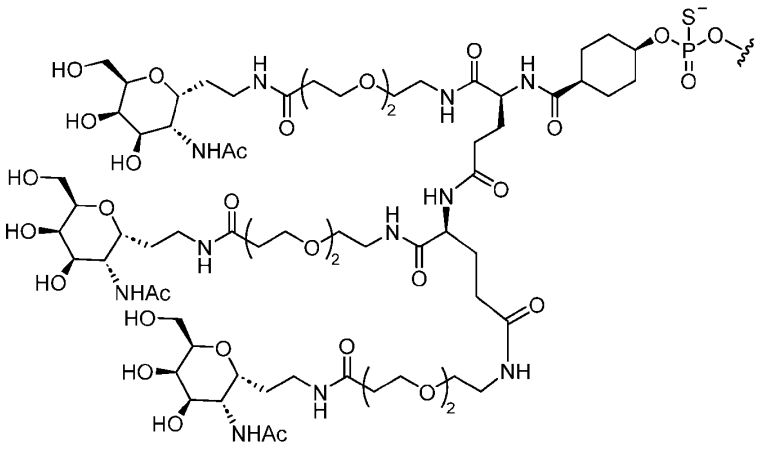
53. The RNAi agent of claim 52, wherein the linker comprises the structure: (Sp18).

54. The RNAi agent of any one of claims 41-53, wherein the RNAi agent comprises a targeting ligand.

55. The RNAi agent of any one of claims 41-54 comprising:

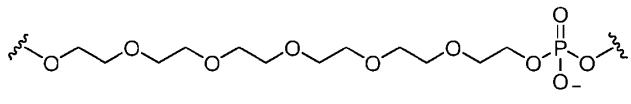


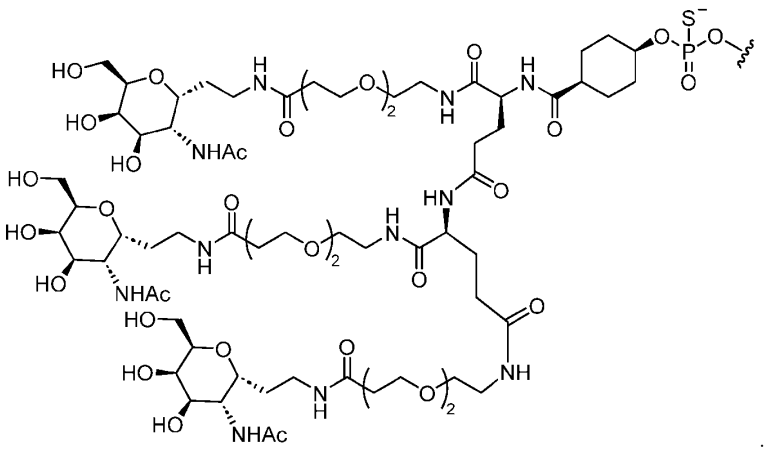
(NAG52),



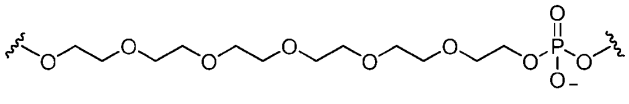
(NAG52)s, or a pharmaceutically acceptable salt of either (NAG52) or (NAG52)s.

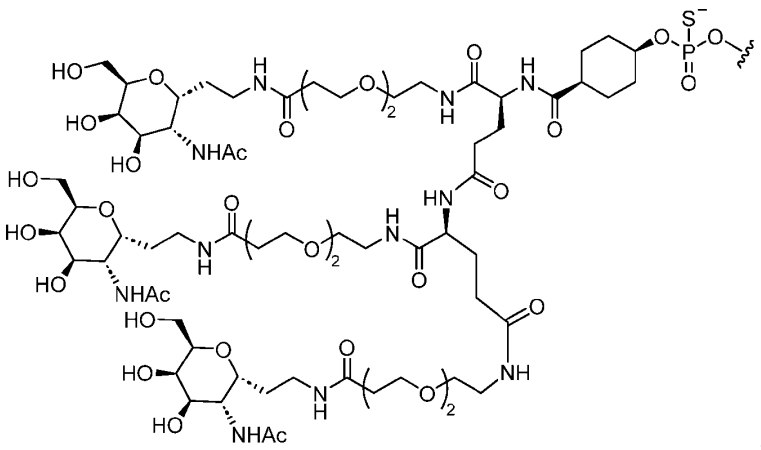
56. The RNAi agent of any one of claims 41-55, wherein the sense strand has the structure: (NAG52)sccuguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb) (SEQ ID NO: 243), and wherein the first antisense strand has the structure: cPrpasAfsguuacaaaaGfcAfaAfacasgsg (SEQ ID NO: 101), and the second antisense strand has the structure: cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO:

71), wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5’-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5’-cyclopropyl phosphonate-2′-O-methyluridine; s represents a phosphorothioate linkage; (invAb) is an inverted abasic residue, Sp18 represents the structure: , and (NAG52)s represents the structure:



57. The RNAi agent of any one of claims 41-55, wherein the sense strand has the structure: (NAG52)suguuuuGfcUfuUfuguaacuus(invAb)-Sp18-gsggacaGfuAfuUfcucaguias(invAb) (SEQ ID NO: 244), and wherein the first antisense strand has the structure: cPrpasAfsguuacaaaaGfcAfaAfacsa (SEQ ID NO: 103), and the second antisense strand has the structure: cPrpusCfsaCfugagaauAfcUfgUfcscsc (SEQ ID NO: 71), wherein a represents 2′-O-methyl adenosine, c represents 2′-O-methyl cytidine, g represents 2′-O-methyl guanosine, and u represents 2′-O-methyl uridine; Af, represents 2′-fluoro adenosine, Cf represents 2′-fluoro cytidine, Gf represents 2′-fluoro guanosine, and Uf represents 2′-fluoro uridine; cPrpa represents 5’-cyclopropyl phosphonate-2′-O-methyladenosine, cPrpu represents 5’-cyclopropyl phosphonate-2′-O-methyluridine; s represents a phosphorothioate linkage; (invAb) is an inverted abasic residue, Sp18 represents

the structure:  , and (NAG52)s represents the structure:



58. The RNAi agent of any one of claims 1-57, wherein the RNAi agent is a pharmaceutically acceptable salt.

59. A composition comprising the RNAi agent of any one of claims 1-58, wherein the composition comprises a pharmaceutically acceptable excipient.

60. The composition of claim 59, wherein the pharmaceutically acceptable excipient is sodium phosphate buffer.

61. The composition of claim 59, wherein the pharmaceutically acceptable excipient is isotonic saline or water for injection.

62. A method for inhibiting expression of an APOC3 and a PCSK9 gene in a cell, the method comprising introducing into a cell an effective amount of an RNAi agent of any one of claims 1-57 or the composition of any one of claims 59-61.

63. The method of claim 62, wherein the cell is within a subject.

64. The method of claim 63, wherein the subject is a human subject.

65. The method of any one of claims 62-64, wherein APOC3 gene expression is inhibited by at least about 30% and PCSK9 gene expression of the subject is inhibited by at least about 30%, as measured by reductions in mRNA and/or protein levels.

66. The method of any one of claims 62-65, wherein APOC3 protein levels are reduced by about 50% and PCSK9 protein levels are reduced by about 50%.

67. A method of treating an APOC3-related and/or PCSK9-related disease, disorder, or symptom, the method comprising administering to a human subject in need thereof a therapeutically effective amount of the composition of any one of claims 59-61.

68. The method of claim 67, wherein the disease is Familial Combined Hyperlipidemia (FCH) hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome, familial partial lipodystrophy, hypercholesterolemia, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, or cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD).

69. The method of any one of claims 62-68, wherein the RNAi agent is administered at a dose of about 0.05 mg/kg to about 5.0 mg/kg of body weight of the human subject.

70. The method of any one of claims 62-69, wherein the RNAi agent is administered in two or more doses.

71. The RNAi agent of any one of claims 1-58 or the composition of any one of claims 59-61, for use in the treatment of a disease, disorder, or symptom that is mediated at least in part by a reduction in APOC3 gene expression and/or PCSK9 gene expression.

72. The RNAi agent or composition of claim 71, wherein the disease is Familial Combined Hyperlipidemia (FCH), hypertriglyceridemia, hypercholesterolemia, hyperlipoproteinemia, hypertriglyceridemia, obesity, hyperlipidemia, abnormal lipid and/or cholesterol metabolism, atherosclerosis, cardiovascular disease, coronary artery disease, hypertriglyceridemia induced pancreatitis, metabolic syndrome, type II diabetes mellitus, familial chylomicronemia syndrome, familial partial lipodystrophy, hypercholesterolemia, familial hypercholesterolemia including heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH), familial hypobetalipoproteinemia, polygenic dyslipidemia, heart disease, cardiovascular disease (CVD) including clinical atherosclerotic cardiovascular disease (ASCVD).

73. The RNAi agent or composition of claim 71 or claim 72, wherein the RNAi agent is administered at a dose of about 0.05 mg/kg to about 10.0 mg/kg of body weight of the human subject.

73. Use of the RNAi agent of any one of claims 1-58 or the composition of any one of claims 59-61, for the preparation of a pharmaceutical composition for treating a disease, disorder, or symptom that is mediated at least in part by a reduction in APOC3 mRNA and/or protein levels and/or a reduction in PCSK9 mRNA and/or protein levels.