Alpha-1 AntiTrypsin (AAT) RNAi Agents, Compositions Including AAT RNAi Agents, And Methods Of Use

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from United States Provisional Patent Application Serial No. 62/444,452, filed on January 10, 2017, United States Provisional Patent Application Serial No. 62/486,720, filed on April 18, 2017, and United States Provisional Patent Application Serial No. 62/596,232, filed on December 8, 2017, the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

Disclosed herein are RNA interference (RNAi) agents for inhibition of alpha-1 antitrypsin gene expression, compositions that include alpha-1 antitrypsin RNAi agents, and methods of use thereof.

BACKGROUND

Alpha-1 antitrypsin (AAT, α1-antitrypsin, or A1AT) deficiency is an inherited, autosomal codominant genetic disorder that causes misfolding of the AAT protein and poor secretion of the misfolded protein leading to lung and liver diseases. AAT deficiency (AATD) occurs with a frequency of about 1 in every 1,500 to 3,500 individuals and most often affects persons with European ancestry.

Alpha-1 Antitrypsin is a protease inhibitor belonging to the serpin superfamily. Normal AAT protein is a circulating glycoprotein protease inhibitor primarily synthesized in the liver by hepatocytes and secreted into the blood. The known physiologic function of AAT is to inhibit neutrophil proteases, which serves to protect host tissues from non-specific injury during periods of inflammation.

The most clinically significant form of AATD, a genetic disorder associated with liver disease in children and adults, and pulmonary disease in adults, is caused by the Z mutation. The Z mutant allele (PiZ), through a single point mutation, renders the mutant Z form AAT protein (the "Z-AAT protein") prone to abnormal folding causing intracellular retention. The mutant Z-AAT protein monomers are able to form chains of polymers that amass into aggregates,

which are sometimes referred to as "globules." The misfolded Z-AAT protein is ineffective in traversing the secretory pathway, and instead polymerizes and accumulates in the endoplasmic reticulum (ER) of hepatocytes. The polymeric globule masses stress the ER and trigger continuous hepatocyte injury, leading to fibrosis, cirrhosis, and increased risk of hepatocellular carcinoma. Further, the absence of circulating anti-protease activity leaves the lung vulnerable to injury by neutrophil elastase, resulting in the development of respiratory complications such as emphysema.

Individuals with the homozygous PiZZ genotype have severe deficiency of functional AAT, which leads to pulmonary disease. Weekly use of AAT augmentation therapy, using purified human AAT, results in near normal plasma levels of AAT in subjects with AATD, and helps prevent lung damage in affected individuals. However, while the administration of purified AAT can ameliorate or help prevent lung damage caused by the absence of endogenously secreted AAT, AATD patients remain vulnerable to endoplasmic reticulum liver storage disease caused by the deposition and accumulation of excessive abnormally folded AAT protein. Accumulated Z-AAT protein in the globule conformation in hepatocytes is a well-known characteristic of AATD liver disease and is believed to lead to proteotoxic effects that are responsible for inducing liver injury, including liver cell damage and death and chronic liver injury, in individuals with AATD. (*see, e.g.*, D. Lindblad et al., Hepatology 2007, 46: 1228-1235). Patients with AATD often develop liver disease, which can be severe or fatal, even in infancy. Clinical presentations of injury in the liver include chronic hepatitis, cirrhosis, hepatocellular carcinoma, transaminitis, cholestasis, fibrosis, and even fulminant hepatic failure.

There is currently no clinically approved treatment to prevent the onset or slow the progression of liver disease due to AATD. Further, while U.S. Patent Application Publication No. 2015/0361427 discloses certain RNAi agents capable of inhibiting the expression of an AAT gene, there remains a need for novel and effective AAT RNAi agents having improved potency that can selectively, efficiently, and safely inhibit the expression of an AAT gene, thereby preventing and potentially reversing Z-AAT accumulation-related liver injury and fibrosis. Similarly, while U.S. Patent Application Publication No. 2015/0011607 to Brown et al. ("Brown '607") discloses various sequences for inhibiting expression of an AAT gene, Brown teaches the use of longer double-stranded constructs (referred to in Brown as DsiRNAs), which according to Brown have been found to give "unexpected effective results in terms of potency

and duration of action" as compared to 19-23mer siRNA agents. (*See, e.g.*, Brown '607 at paragraph [0376]). Moreover, many of the sequences disclosed in Brown '607 are designed to be used in DsiRNA constructs that are designed to target different locations of an AAT mRNA as compared to the sequences disclosed in the present invention. Such differences lead to different binding affinity to the AAT mRNA and produces a different cleavage site, which can impact the inhibitory effect of the compound, while also potentially leading to additional off-target issues (*see, e.g.*, Piotr J. Kamola et al., *PLoS Comput Biol*, 2015, 11(12):e1004656 at Fig. 1 (illustrating the mechanism of siRNA-Mediated Gene Silencing)). For example, nothing in Brown '607 teaches or suggests the design of an RNAi agent (of any length) wherein the 5′ terminal nucleobase or nucleotide of the antisense strand would be aligned with the position that is 19 nucleotides downstream (towards the 3′ end) from position 1000 on an AAT gene (SEQ ID NO: 1). Put different, and again solely as an example involving one such potential AAT RNAi agent sequence, nothing in Brown '607 teaches or suggests the design of an RNAi agent wherein the 5′ terminal nucleobase of the antisense strand of an RNAi agent corresponds to position 1018 on an AAT gene (SEQ ID NO: 1). Further, nothing in Brown '607 teaches or suggests the modified AAT RNAi agent constructs disclosed herein.

SUMMARY

There exists a need for novel AAT-specific RNA interference (RNAi) agents (also herein termed RNAi agent, RNAi trigger, or trigger) that are able to selectively and efficiently inhibit the expression of an AAT gene. Further, there exists a need for compositions of novel AAT-specific RNAi agents for the treatment of diseases associated with AAT deficiency.

Because liver damage resulting from AATD occurs through a gain-of-function mechanism, inhibition of AAT gene expression is useful in preventing accumulation of the Z-AAT protein in the liver. Further, the reduction or removal of the Z-AAT polymer aggregates reduces the ER stress in hepatocytes, and offers additional advantages in reducing the likelihood of occurrence of liver cell damage and assisting in the treatment of liver cell damage and chronic liver injury such as fibrosis, cirrhosis, hepatocellular carcinoma, and other conditions and diseases caused by AATD. Reduction of inflammatory Z-AAT protein, which has been clearly defined as the cause of progressive liver disease in AATD patients, is important as it can slow or halt the progression of liver disease and allow fibrotic tissue repair.

In general, the disclosure features novel AAT RNAi agents, compositions comprising the AAT RNAi agents, and methods for inhibiting the expression of an AAT gene *in vivo* and/or *in vitro* using AAT RNAi agents and compositions that include AAT RNAi agents. Further described herein are methods of treatment of AATD-related diseases using the AAT RNAi agents described herein and compositions that include AAT RNAi agents.

The AAT RNAi agents and methods disclosed herein can provide for the treatment of AATD, including the treatment of conditions and diseases caused by AATD, such as chronic hepatitis, cirrhosis, hepatocellular carcinoma, and fulminant hepatic failure. The AAT RNAi agents disclosed herein, when administered to a subject, can prevent and/or reverse Z-AAT accumulation-related liver injury and fibrosis. The AAT RNAi agents described herein may be administered to a subject, e.g., a human or animal subject, by any suitable methods known in the art, such as subcutaneous injection or intravenous administration.

In one aspect, the disclosure features RNAi agents for inhibiting the expression of an alpha-1 antitrypsin (AAT) gene, wherein the RNAi agent comprises a sense strand and an antisense strand. Also described herein are compositions comprising an RNAi agent capable of inhibiting the expression of an alpha-1 antitrypsin gene, wherein the RNAi agent comprises a sense strand and an antisense strand, and at least one pharmaceutically acceptable excipient.

Each AAT RNAi agent described herein includes a sense strand and an antisense strand. The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the RNAi agent sense and antisense strands described herein each can be 16 to 30 nucleotides in length. In some embodiments, the sense and antisense strands are independently 17 to 26 nucleotides in length. In some embodiments, the sense and antisense strands are independently 21 to 26 nucleotides in length. In some embodiments, the sense and antisense strands are independently 21 to 24 nucleotides in length. In some embodiments, the sense and/or antisense strands are independently 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. The RNAi agents described herein, upon delivery to a cell expressing AAT, inhibit the expression of one or more AAT genes *in vivo* or *in vitro*.

An AAT RNAi agent includes a sense strand (also referred to as a passenger strand), and an antisense strand (also referred to as a guide strand). A sense strand of the AAT RNAi agents

described herein includes a nucleotide sequence having at least 85% identity to a core stretch of at least 16 consecutive nucleotides to a sequence in an AAT mRNA. In some embodiments, the sense strand core stretch having at least 85% identity to a sequence in an AAT mRNA is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. An antisense strand of an AAT RNAi agent includes a nucleotide sequence having at least 85% complementarity over a core stretch of at least 16 consecutive nucleotides to a sequence in an AAT mRNA and the corresponding sense strand. In some embodiments, the antisense strand core stretch having at least 85% complementarity to a sequence in an AAT mRNA or the corresponding sense strand is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

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

Examples of AAT RNAi agent sense strands and antisense strands that can be used in AAT RNAi agents are provided in Tables 2, 3, 4 and 5. Examples of duplexes that include AAT RNAi agent are provided in Table 6. Examples of 19-nucleotide core stretch sequences that may consist of or may be included in the sense strands and antisense strands of certain AAT RNAi agents disclosed herein, are provided in Table 2.

In another aspect, the disclosure features methods for delivering AAT RNAi agents to liver cells in a subject, such as a mammal, *in vivo*. In some embodiments, one or more AAT RNAi agents are delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. Nucleic acid delivery methods include, but are not limited to, encapsulation in liposomes, iontophoresis, or incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, proteinaceous vectors, or Dynamic Polyconjugates™ (DPCs) (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, each of which is incorporated herein by reference). In some embodiments, a delivery vehicle, such as a polymer, an amphipathic polymer, a membrane active polymer, a peptide, such as a melittin or melittin-like peptide, a reversibly modified polymer or peptide, or a lipid, can be used with the AAT RNAi agents disclosed herein.

In some embodiments, an AAT RNAi agent is delivered to target cells or tissues by covalently linking or conjugating the RNAi agent to a targeting group such as an asialoglycoprotein

receptor ligand. In some embodiments, an asialoglycoprotein receptor ligand includes, consists of, or consists essentially of, a galactose or galactose-derivative cluster. In some embodiments, an AAT RNAi agent is linked to a targeting ligand comprising the galactose-derivative N-acetyl-galactosamine. In some embodiments, a galactose-derivative cluster includes an N-acetyl-galactosamine trimer or an N-acetyl-galactosamine tetramer. In some embodiments, a galactose derivative cluster is an N-acetyl-galactosamine trimer or an N-acetyl-galactosamine tetramer. Example targeting groups useful for delivering RNAi agents are disclosed, for example, in U.S. Patent Application No. 15/452,324 and WO 2017/156012, which are incorporated by reference herein in their entirety.

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

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

In another aspect, the disclosure features compositions that include one or more AAT RNAi agents that have the duplex structures disclosed in Table 6.

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

of targeting ligands that include one or more N-acetyl-galactosamines. In some embodiments, when two or more RNAi agents are included in a composition, each RNAi agent is independently linked to the same targeting group. In some embodiments, when two or more RNAi agents are included in a composition, each RNAi agent is independently linked to a different targeting group, such as targeting groups having different chemical structures.

In some embodiments, targeting groups are linked to the AAT 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 AAT RNAi agent. In some embodiments, when two or more RNAi agents are included in a composition, the two or more RNAi agents may 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.

In another aspect, the disclosure features methods for inhibiting alpha-1 antitrypsin gene expression in a subject, the methods comprising administering to the subject an amount of an AAT RNAi agent capable of inhibiting the expression of an AAT gene, wherein the AAT RNAi agent comprises a sense strand and an antisense strand.

Also described herein are methods for the treatment of a condition or disease caused by AATD, comprising administering to a subject a therapeutically effective amount of an RNAi agent described herein. Further described are methods for inhibiting expression of an AAT gene, wherein the methods include administering to a cell an AAT RNAi agent described herein.

In some embodiments, disclosed herein are methods for the treatment of AATD (including the treatment of a condition or disease caused by AATD), the methods comprising administering to a subject a therapeutically effective amount of an RNAi agent having an antisense strand comprising the sequence of any of the sequences in Tables 2, 3, or 4.

In some embodiments, disclosed herein are methods for inhibiting expression of an AAT gene, the methods comprising administering to a cell an AAT RNAi agent that includes an antisense strand comprising the sequence of any of the sequences in Tables 2, 3, or 4.

In some embodiments, disclosed herein methods for the treatment of AATD (including the treatment of a condition or disease caused by AATD), the methods comprising administering to a subject a therapeutically effective amount of an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Tables 2, 3, or 5.

In some embodiments, disclosed herein are methods for inhibiting expression of an AAT gene, wherein the methods include administering to a cell an AAT RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Tables 2, 3, or 5.

In some embodiments, disclosed herein are methods for the treatment of AATD (including the treatment of a condition or disease caused by AATD), wherein the methods include administering to a subject a therapeutically effective amount of an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 5, and an antisense strand comprising any of the sequences in Table 4.

In some embodiments, disclosed herein are methods for inhibiting expression of an AAT gene, wherein the methods include administering to a subject a therapeutically effective amount of an RNAi agent that includes a sense strand comprising the sequence of any of the sequences in Table 5, and an antisense strand comprising any of the sequences in Table 4.

In some embodiments, disclosed herein are methods of inhibiting expression of an AAT gene, wherein the methods include administering to a subject an AAT RNAi agent that includes a sense strand consisting of the nucleobase sequence of any of the sequences in Table 5, and the antisense strand consisting of the nucleobase sequence of any of the sequences in Table 4. In other embodiments, disclosed herein are methods of inhibiting expression of an AAT gene, wherein the methods include administering to a subject an AAT RNAi agent that includes a sense strand consisting of the modified sequence of any of the modified sequences in Table 5, and the antisense strand consisting of the modified sequence of any of the modified sequences in Table 4.

In some embodiments, disclosed herein are methods for inhibiting expression of an AAT gene in a cell, wherein the methods include administering one or more AAT RNAi agents having the duplex structure set forth in Table 6.

In some embodiments, the AAT RNAi agents disclosed herein have structures that include, consist of, or consist essentially of, the structure shown in any one of Figure 1, Figure 2, Figure 3, Figure 4, Figure 5, Figure 6, Figure 7, or Figure 8.

The AAT RNAi agents disclosed herein are designed to target specific positions on an AAT gene (SEQ ID NO:1). As defined herein, an antisense strand sequence is designed to target an AAT gene at a given position on the gene when the 5′ terminal nucleobase of the antisense strand would be aligned with the position that is 19 nucleotides downstream (towards the 3′ end) from the position on the gene when base pairing to the gene. For example, as illustrated in Tables 1, 2, and 3 herein, an antisense strand sequence designed to target an AAT gene at position 1000 requires that when base pairing to the gene, the 5′ terminal nucleobase of the antisense strand is aligned with position 1018 of the AAT gene. As provided herein, an AAT 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 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 AAT RNAi agent disclosed herein that is designed to target position 1000 of an AAT gene, the 5′ terminal nucleobase of the antisense strand of the of the AAT RNAi agent must be aligned with position 1018 of the gene; however, the 5′ terminal nucleobase of the antisense strand may be, but is not required to be, complementary to position 1018 of an AAT gene, provided that there is at least 85% complementarity (e.g., at least 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 AAT RNAi agent (e.g., whether the AAT RNAi agent is designed to target an AAT gene at position 1000, at position 1142, or at some other position) is highly important to the level of inhibition achieved by the AAT RNAi agent.

In some embodiments, the antisense strand sequence is designed to have a sequence target position 1000 of an AAT gene (SEQ ID NO: 1).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein at least one or more nucleotides is a modified nucleotide. In some embodiments, the antisense

strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein at least one or more nucleotides is a modified nucleotide. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein at least one or more nucleotides is a modified nucleotide. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein at least one or more nucleotides is a modified nucleotide. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACG (SEQ ID NO: 80), wherein one or more nucleotides is a modified nucleotide. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACG (SEQ ID NO: 80), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGUUAAACAUGCCUAAACG (SEQ ID NO: 81), wherein one

or more nucleotides is a modified nucleotide. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGUUAAACAUGCCUAAACG (SEQ ID NO: 81), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACA (SEQ ID NO: 429), wherein one or more nucleotides is a modified nucleotide. In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACA (SEQ ID NO: 429), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACU (SEQ ID NO: 430), wherein one or more nucleotides is a modified nucleotide. In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACU (SEQ ID NO: 430), wherein all or substantially all of the nucleotides are modified nucleotides.

In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACA (SEQ ID NO: 429), wherein one or more nucleotides is a modified nucleotide, and the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACG (SEQ ID NO: 80), wherein one or more nucleotides is a modified nucleotide.

In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACU (SEQ ID NO: 430), wherein one or more nucleotides is a modified nucleotide, and the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGUUAAACAUGCCUAAACG (SEQ ID NO: 81), wherein one or more nucleotides is a modified nucleotide.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi

agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 857).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of GCGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 885).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 864).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 866).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 857) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of GCGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 885) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 864) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 866) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04824.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04825.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04826.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04827.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04828.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04829.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04830.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04831.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex AD04832.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04833.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04834.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04835.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04836.

In some embodiments, the AAT RNAi agent comprises, consists of, or consists essentially of the duplex structure of AD04837.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACG (SEQ ID NO: 80), wherein one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 80 is located at positions 1 to 19 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACG (SEQ ID NO: 80), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 80 is located at positions 1 to 19 (5′ → 3′) of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGUUAAACAUGCCUAAACG (SEQ ID NO: 81), wherein one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 81 is located at positions 1 to 19 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGUUAAACAUGCCUAAACG (SEQ ID NO: 81), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 81 is located at positions 1 to 19 (5′ → 3′) of the antisense strand.

In some embodiments, the sense strand of an AAT RNAi agent comprises the nucleobase sequence of CGUUUAGGCAUGUUUAACA (SEQ ID NO: 429), wherein one or more nucleotides is a modified nucleotide, and wherein position 19 of SEQ ID NO: 429 forms a base pair with the nucleotide located at the 5′ terminal end of the antisense strand. In some embodiments, the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACA (SEQ ID NO: 429), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein position 19 of SEQ ID NO: 429 forms a base pair with the nucleotide located at the 5′ terminal end of the antisense strand.

In some embodiments, the sense strand of an AAT RNAi agent comprises the nucleobase sequence of CGUUUAGGCAUGUUUAACU (SEQ ID NO: 430), wherein one or more nucleotides is a modified nucleotide, and wherein position 19 of SEQ ID NO: 430 forms a base pair with the nucleotide located at the 5′ terminal end of the antisense strand. In some embodiments, the sense strand of AN AAT RNAI agent comprises the nucleobase sequence of CGUUUAGGCAUGUUUAACU (SEQ ID NO: 430), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein position 19 of SEQ ID NO: 430 forms a base pair with the nucleotide located at the 5′ terminal end of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 794 is located at positions 1 to 21 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein all or substantially all of the

nucleotides are modified nucleotides, and wherein SEQ ID NO: 794 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 839 is located at positions 1 to 22 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 839 is located at positions 1 to 22 (5′ → 3′) of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 800 is located at positions 1 to 21 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 800 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 801 is located at positions 1 to 21 (5′ → 3′) of the antisense strand. In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 801 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 794 is located at the 5′ the terminal end of the antisense strand, and wherein sense strand of an AAT

RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 857).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 839 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of GCGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 885).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 800 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 864).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 801 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 866).

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 794 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 857) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified

nucleotide, and wherein SEQ ID NO: 839 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of GCGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 885) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 800 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of CGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 864) differing by 0, 1, 2, or 3 nucleotides.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801) differing by 0, 1, 2, or 3 nucleotides, wherein at least one or more nucleotides is a modified nucleotide, and wherein SEQ ID NO: 801 is located at the 5′ the terminal end of the antisense strand, and the sense strand of an AAT RNAi agent comprises or consists of the nucleobase sequence of AGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 866) differing by 0, 1, 2, or 3 nucleotides.

The AAT RNAi agents described herein can include one or more modified nucleotides. The AAT RNAi agents described herein can also include one or more phosphorothioate internucleoside linkages.

The AAT RNAi agents described herein can also include one or more targeting groups or linking groups. In some embodiments, the AAT RNAi agents disclosed herein include one or more targeting groups. In some embodiments, the targeting groups are comprised of an asialoglycoprotein receptor ligand. In some embodiments, the asialoglycoprotein receptor ligand comprises a galactose or galactose-derivative cluster. In some embodiments, the galactose-derivative cluster comprises N-acetyl-galactosamine. In some embodiments, the targeting ligand comprises an N-acetyl-galactosamine trimer. In some embodiments, a targeting group is conjugated to the sense strand of the AAT RNAi agents disclosed herein.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu (SEQ ID NO: 913), wherein

a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage, and the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcusu (SEQ ID NO: 958), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage, and the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfaCfaUfgCfcUfaAfaCfgsCfsg (SEQ ID NO: 959), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage, and the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfacaugCfcUfaAfaCfgCfsu (SEQ ID NO: 960), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage, and the sense strand is at least substantially complementary to the antisense strand.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu (SEQ ID NO: 913) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) cguuuaGfGfCfauguuuaacausu (SEQ ID NO: 1276), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one, two, or more inverted abasic deoxyribose

(invAb); and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcusu (SEQ ID NO: 958) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) gcguuuaGfGfCfauguuuaacausu (SEQ ID NO: 1277), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one, two, or more inverted abasic deoxyribose (invAb); and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfaCfaUfgCfcUfaAfaCfgsCfsg (SEQ ID NO: 959) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) cgcguuuaGfGfCfauguuuaaca (SEQ ID NO: 1278), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one, two, or more inverted abasic deoxyribose (invAb); and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfacaugCfcUfaAfaCfgCfsu (SEQ ID NO: 960) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) agcguuuaGfGfCfauguuuaaca (SEQ ID NO: 1279), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one, two, or more inverted abasic deoxyribose (invAb); and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu (SEQ ID NO: 913) and the sense strand of an AAT RNAi agent comprises or consists of (5′ → 3′) (NAG37)s(invAb)scguuuaGfGfCfauguuuaacausu(invAb) (SEQ ID NO: 1028), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; (invAb) is inverted abasic deoxyribose (invAb); and (NAG37) is the targeting ligand that includes N-acetyl-galactosamine having the structure shown in Table 7 herein.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcusu (SEQ ID NO: 958) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) (NAG37)s(invAb)sgcguuuaGfGfCfauguuuaacausu(invAb) (SEQ ID NO: 1030), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; (invAb) is inverted abasic deoxyribose (invAb); and (NAG37) is the targeting ligand that includes N-acetyl-galactosamine having the structure shown in Table 7 herein.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfaCfaUfgCfcUfaAfaCfgsCfsg (SEQ ID NO: 959) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) (NAG37)s(invAb)scgcguuuaGfGfCfauguuuaacas(invAb) (SEQ ID NO: 1024), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; (invAb) is inverted abasic deoxyribose (invAb); and (NAG37) is the targeting ligand that includes N-acetyl-galactosamine having the structure shown in Table 7 herein.

In some embodiments, the antisense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) usGfsuUfaAfacaugCfcUfaAfaCfgCfsu (SEQ ID NO: 960) and the sense strand of an AAT RNAi agent comprises or consists of the sequence (5′ → 3′) (NAG37)s(invAb)sagcguuuaGfGfCfauguuuaacas(invAb) (SEQ ID NO: 1033), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and

Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; (invAb) is inverted abasic deoxyribose (invAb); and (NAG37) is the targeting ligand that includes N-acetyl-galactosamine having the structure shown in Table 7 herein.

In some embodiments, the AAT RNAi agents described herein can include one or more targeting groups having the structure of (PAZ), (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s, as defined herein in Table 7.

In some embodiments, the AAT RNAi agents described herein include one targeting group at the 5′ end of the sense strand having the structure of (PAZ), (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s, as defined herein in Table 7.

The AAT RNAi agents disclosed herein can be incorporated into a composition comprising one or more disclosed AAT RNAi agent and at least one pharmaceutically acceptable excipient. In some embodiments, the compositions disclosed herein comprising one or more of the disclosed AAT RNAi agents and at least one pharmaceutically acceptable excipient is a pharmaceutical composition.

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

In some embodiments, the compositions comprising one or more disclosed AAT RNAi agents and at least one pharmaceutically acceptable excipient can further comprise one or more additional therapeutics or treatments.

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

The AAT RNAi agents and compositions comprising same that are disclosed herein can be administered to a subject to inhibit the expression of the alpha-1 antitrypsin gene in the subject. In some embodiments, the subject is a human. In some embodiments, the subject is a human that has been diagnosed with having AATD.

In some embodiments, disclosed herein are methods for inhibiting expression of an AAT gene in a cell, the methods comprising administering an AAT RNAi agent that has an antisense strand that is at least partially complementary to the portion of an AAT mRNA having any one of the sequences listed in Table 1.

The AAT RNAi agents and compositions comprising same disclosed herein may be administered to a subject for the treatment of AATD (including a condition or disease caused by alpha-1 antitrypsin deficiency). The condition or disease that may be treated, prevented, and/or managed by administration of the AAT RNAi agents and compositions comprising same disclosed herein include chronic hepatitis, cirrhosis, hepatocellular carcinoma, transaminitis, cholestasis, fibrosis, or fulminant hepatic failure.

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

As used herein, an "RNAi agent" or "RNAi trigger" means a composition that contains an RNA or RNA-like (e.g., chemically modified RNA) oligonucleotide molecule that is capable of degrading or inhibiting 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 interfering RNAs (siRNAs), double-strand 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 (e.g. AAT mRNA). RNAi agents can include one or more modified nucleotides and/or one or more non-phosphodiester linkages.

As used herein, the terms "silence," "reduce," "inhibit," "down-regulate," or "knockdown" when referring to expression of a given gene, mean that the expression of the gene, as measured by the level of RNA transcribed from the gene or the level of polypeptide, protein or protein subunit translated from the mRNA in a cell, group of cells, tissue, organ, or subject in which the gene is transcribed, is reduced when the cell, group of cells, tissue, organ, or subject is treated with the RNAi agents described herein as compared to a second cell, group of cells, tissue, organ, or subject that has not or have not been so treated.

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

As used herein, a "base", "nucleotide base," or "nucleobase," is a heterocyclic pyrimidine or purine compound, which is a standard constituent of all nucleic acids, and includes the bases that form the nucleotides adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). A nucleobase may further be modified to include, without limitation, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. As used herein, the term "nucleotide" can include a modified nucleotide (such as, for example, a nucleotide mimic, abasic residue (Ab), or a surrogate replacement moiety).

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleobase or nucleotide sequence (e.g., RNAi agent sense strand or targeted mRNA) in relation to a second nucleobase or nucleotide sequence (e.g., RNAi agent antisense strand or a single-stranded antisense oligonucleotide), means the ability of an oligonucleotide or polynucleotide including the first nucleotide sequence to hybridize (form base pair hydrogen bonds under mammalian physiological conditions (or similar conditions in vitro)) and form a duplex or double helical structure under certain standard conditions with an oligonucleotide or

polynucleotide including the second nucleotide sequence. Complementary sequences include Watson-Crick base pairs or non-Watson-Crick base pairs and include natural or modified nucleotides or nucleotide mimics, at least to the extent that the above hybridization requirements are fulfilled. Sequence identity or complementarity is independent of modification. For example, a and Af, as defined herein, are complementary to U (or T) and identical to A for the purposes of determining identity or complementarity.

As used herein, "perfectly complementary" or "fully complementary" means that all (100%) of the nucleobases or nucleotides in a contiguous sequence of a first polynucleotide will hybridize with the same number of nucleobases or nucleotides in a contiguous sequence of a second polynucleotide. The contiguous sequence may comprise all or a part of a first or second nucleotide sequence.

As used herein, "partially complementary" means that in a hybridized pair of nucleobase sequences, at least 70%, but not all, of the bases in a contiguous sequence of a first polynucleotide will hybridize with the same number of bases in a contiguous sequence of a second polynucleotide.

As used herein, "substantially complementary" means that in a hybridized pair of nucleobase sequences, at least 85%, but not all, of the bases in a contiguous sequence of a first polynucleotide will hybridize with the same number of bases in a contiguous sequence of a second polynucleotide. The terms "complementary," "fully complementary," "partially complementary," and "substantially complementary" herein are used with respect to the nucleobase or nucleotide matching between the sense strand and the antisense strand of an RNAi agent, or between the antisense strand of an RNAi agent and a sequence of an AAT mRNA.

As used herein, the term "substantially identical" or "substantially identity" as applied to nucleic acid sequence means that a nucleic acid sequence comprises a sequence that 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 identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions

by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The inventions disclosed herein encompass nucleotide sequences substantially identical to those disclosed herein.

As used herein, the terms "treat," "treatment," and the like, mean the methods or steps taken to provide relief from or alleviation of the number, severity, and/or frequency of one or more symptoms of a disease in a subject. As used herein, "treat" and treatment" may include the prevention, management, prophylactic treatment, and/or inhibition of the number, severity, and/or frequency of one or more symptoms of a disease in a subject.

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

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

As used herein, the term "isomers" refers to compounds that have identical molecular formulae, but that differ in the nature or the sequence of bonding of their atoms or in the arrangement of their atoms in space. Isomers that differ in the arrangement of their atoms in space are termed "stereoisomers." Stereoisomers that are not mirror images of one another are termed "diastereoisomers," and stereoisomers that are non-superimposable mirror images are termed "enantiomers," or sometimes optical isomers. A carbon atom bonded to four non-identical substituents is termed a "chiral center."

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

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

The person of ordinary skill in the art would readily understand and appreciate that the compounds and compositions disclosed herein may have certain atoms (e.g., N, O, or S atoms) in a protonated or deprotonated state, depending upon the environment in which the compound or composition is placed. Accordingly, as used herein, the structures disclosed herein envisage that certain functional groups, such as, for example, OH, SH, or NH, may be protonated or deprotonated. The disclosure herein is intended to cover the disclosed compounds and compositions regardless of their state of protonation based on the environment (such as pH), as would be readily understood by the person of ordinary skill in the art.

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

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIGs. 1A to 1E represent the chemical duplex structure of AD04828 shown as a sodium salt.

FIGs. 2A to 2E represent the chemical duplex structure of AD04828 shown as a free acid.

FIGs. 3A to 3E represent the chemical duplex structure of AD04831 shown as a sodium salt.

FIG. 4A to 4E represent the chemical duplex structure of AD04831 shown as a free acid.

FIG. 5A to 5E represent the chemical duplex structure of AD04836 shown as a sodium salt.

FIG. 6A to 6E represent the chemical duplex structure of AD04836 shown as a free acid.

FIG. 7A to 7E represent the chemical duplex structure of AD04837 shown as a sodium salt.

FIG. 8A to 8E represent the chemical duplex structure of AD04837 shown as a free acid.

FIG. 9 is a bar graph showing average normalized cynomolgus monkey (cyno) AAT (cAAT) serum levels in cynos (n=3) following a single subcutaneous administration of 3 mg/kg of either AD04824, AD04825, AD04826, or AD04827, according to Example 4. AAT serum levels were normalized to average pre-treatment values. Experimental error is shown as standard deviation.

FIG. 10 is a bar graph showing average normalized cAAT serum levels in cynos (n=2 or n=3) following a single subcutaneous administration of 3 mg/kg of either AD04828, AD04836, AD04831, or AD04837, according to Example 5. AAT serum levels were normalized to average pre-treatment values. Experimental error is shown as standard deviation.

FIG. 11. is a bar graph showing the results of a western blot analysis of the soluble fractions (Z-AAT monomer) from livers of PiZ mice dosed with either saline or NAG-conjugated AAT RNAi agent having the duplex structure AD04837, dosed for 8 weeks q2w, normalized to baseline control, according to Example 7. Individual mouse measurements are shown grouped by treatment group, and experimental error is shown as standard deviation.

FIG. 12. is a bar graph showing the results of a western blot analysis of the insoluble fractions (Z-AAT polymer) from livers of PiZ mice dosed with either saline or NAG-conjugated AAT RNAi agent having the duplex structure AD04837, according to Example 7. Individual mouse measurements are shown grouped by treatment group, and experimental error is shown as standard deviation.

DETAILED DESCRIPTION

RNAi Agents

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

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

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

strand core stretch sequence is 100% (perfectly) complementary or at least about 85% (substantially) complementary to a core stretch sequence in the antisense strand, and thus the sense strand core stretch sequence is perfectly identical or at least about 85% identical to a nucleotide sequence (target sequence) present in the AAT mRNA target. A sense strand core stretch sequence can be the same length as a corresponding antisense core sequence or it can be a different length. In some embodiments, the antisense strand core stretch sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. In some embodiments, the sense strand core stretch sequence is 16, 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

Examples of nucleotide sequences used in forming AAT RNAi agents are provided in Tables 2, 3, 4 and 5. Examples of AAT RNAi agent duplexes, that include the sense strand and antisense strand sequences in Tables 2, 3, 4, and 5, are shown in Table 6.

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

In some embodiments, the antisense strand of an AAT RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 2, Table 3, or Table 4. In some embodiments, the sense strand of an AAT RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 2, Table 3, or Table 5.

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

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

As used herein, an extension comprises 1, 2, 3, 4, 5, or 6 nucleotides at the 5' and/or 3' end of the sense strand core stretch sequence and/or antisense strand core stretch sequence. The extension nucleotides on a sense strand may or may not be complementary to nucleotides, either core stretch sequence nucleotides or extension nucleotides, in the corresponding antisense strand. Conversely, the extension nucleotides on an antisense strand may or may not be complementary to nucleotides, either core stretch nucleotides or extension nucleotides, in the corresponding sense strand. In some embodiments, both the sense strand and the antisense strand of an RNAi agent contain 3′ and 5′ extensions. In some embodiments, one or more of the 3′ extension nucleotides of one strand base pairs with one or more 5′ extension nucleotides of the other strand. In other embodiments, one or more of 3′ extension nucleotides of one strand do not base pair with one or more 5′ extension nucleotides of the other strand. In some embodiments, an AAT RNAi agent has an antisense strand having a 3′ extension and a sense strand having a 5′ extension.

In some embodiments, an AAT RNAi agent comprises an antisense strand having a 3′ extension of 1, 2, 3, 4, 5, or 6 nucleotides in length. In other embodiments, an AAT RNAi agent comprises an antisense strand having a 3′ extension of 1, 2, or 3 nucleotides in length. In some embodiments, one or more of the antisense strand extension nucleotides comprise uracil or thymidine nucleotides or nucleotides that are complementary to the corresponding AAT mRNA sequence. In some embodiments, a 3′ antisense strand extension includes or consists of one of the following sequences, but is not limited to: AUA, UGCUU, CUG, UG, UGCC, CUGCC, CGU, CUU, UGCCUA, CUGCCU, UGCCU, UGAUU, GCCUAU, T, TT, U, UU (each listed 5′ → 3′).

In some embodiments, the 3′ end of the antisense strand can include additional abasic residues (Ab). An "abasic residue" or "abasic site" is a nucleotide or nucleoside that lacks a nucleobase at the 1′ position of the sugar. In some embodiments, Ab or AbAb can be added to the 3′ end of the antisense strand. In some embodiments, the abasic residue(s) can be added as inverted abasic residues (invAb) (see Table 7). (*See, e.g.*, F. Czauderna, Nucleic Acids Res., 2003,

31(11), 2705-16).

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

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

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

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

(abasic ribose) residue.

In some embodiments, the 3′ end of the antisense strand core stretch sequence, or the 3′ end of the antisense strand sequence, may include an inverted abasic residue (invAb (see Table 7)).

Examples of sequences used in forming AAT RNAi agents are provided in Tables 2, 3, 4, and 5. In some embodiments, an AAT RNAi agent antisense strand includes a sequence of any of the sequences in Tables 2, 3, or 4. In some embodiments, an AAT RNAi agent antisense strand includes the sequence of nucleotides (from 5′ end → 3′ end) 1-17, 2-15, 2-17, 1-18, 2-18, 1-19, 2-19, 1-20, 2-20, 1-21, 2-21, 1-22, 2-22, 1-23, 2-23, 1-24, or 2-24, of any of the sequences in Table 2, Table 3, or Table 4. In certain embodiments, an AAT RNAi agent antisense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 4. In some embodiments, an AAT RNAi agent sense strand includes the sequence of any of the sequences in Tables 2, 3, or 5. In some embodiments, an AAT RNAi agent sense strand includes the sequence of nucleotides (from 5′ end → 3′ end) 1-18, 1-19, 1-20, 1-21, 1-22, 1-23, 1-24, 2-19, 2-20, 2-21, 2-22, 2-23, 2-24, 3-20, 3-21, 3-22, 3-23, 3-24, 4-21, 4-22, 4-23, 4-24, 5-22, 5-23, 5-24, 6-23, 6-24, 7-24, of any of the sequences in Tables 2, 3, or 5. In certain embodiments, an AAT RNAi agent sense strand comprises or consists of a modified sequence of any one of the modified sequences in Table 5.

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

In some embodiments, the sense strand 5′ end and the antisense strand 3′ end of an RNAi agent form a frayed end. In some embodiments, the sense strand 3′ end and the antisense strand 5′ end of an RNAi agent form a frayed end. In some embodiments, both ends of an RNAi agent form a frayed end. In some embodiments, neither end of an RNAi agent is a frayed end. As

used herein a frayed end refers to an end of a double stranded RNAi agent in which the terminal nucleotides of the two annealed strands from a pair (i.e., do not form an overhang) but are not complementary (i.e. form a non-complementary pair). As used herein, an overhang is a stretch of one or more unpaired nucleotides at the end of one strand of a double stranded RNAi agent. The unpaired nucleotides may be on the sense strand or the antisense strand, creating either 3' or 5' overhangs. In some embodiments, the RNAi agent contains: a blunt end and a frayed end, a blunt end and 5′ overhang end, a blunt end and a 3′ overhang end, a frayed end and a 5′ overhang end, a frayed end and a 3′ overhang end, two 5′ overhang ends, two 3′ overhang ends, a 5′ overhang end and a 3′ overhang end, two frayed ends, or two blunt ends.

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

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

Modified Nucleotides

In some embodiments, an AAT 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 include, but are not limited to, deoxyribonucleotides, nucleotide mimics, abasic nucleotides (represented herein as Ab), 2′-modified nucleotides, 3′ to 3′ linkages (inverted) nucleotides (represented herein as invdN, invN, invn), modified nucleobase-comprising nucleotides, bridged nucleotides, peptide nucleic acids (PNAs), 2′,3′-seco nucleotide mimics (unlocked nucleobase analogues, represented herein as N_{UNA} or NUNA), locked nucleotides (represented herein as N_{LNA} or NLNA), 3′-O-methoxy (2′ internucleoside linked) nucleotides (represented herein as 3′-OMen), 2'-F-Arabino nucleotides (represented herein as NfANA or Nf_{ANA}), 5'-Me, 2'-fluoro nucleotide (represented herein as 5Me-Nf), morpholino nucleotides, vinyl phosphonate deoxyribonucleotides (represented herein as vpdN), vinyl phosphonate containing nucleotides, and cyclopropyl

phosphonate containing nucleotides (cPrpN). 2′-modified nucleotides (i.e. a nucleotide with a group other than a hydroxyl group at the 2′ position of the five-membered sugar ring) include, but are not limited to, 2′-O-methyl nucleotides (represented herein as a lower case letter 'n' in a nucleotide sequence), 2′-deoxy-2′-fluoro nucleotides (represented herein as Nf, also represented herein as 2′-fluoro nucleotide), 2′-deoxy nucleotides (represented herein as dN), 2′-methoxyethyl (2′-O-2-methoxylethyl) nucleotides (represented herein as NM or 2′-MOE), 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 AAT RNAi agent or even in a single nucleotide thereof. The AAT RNAi agent sense strands and antisense strands can be synthesized and/or modified by methods known in the art. Modification at one nucleotide is independent of modification at another nucleotide.

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

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

Modified Internucleoside Linkages

In some embodiments, one or more nucleotides of an AAT 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, 5'-phosphorothioate groups (represented herein as a lower case "s"), chiral phosphorothioates, thiophosphates, phosphorodithioates, phosphotriesters, aminoalkyl-phosphotriesters, alkyl phosphonates (e.g., methyl phosphonates or 3′-alkylene phosphonates), chiral phosphonates, phosphinates, phosphoramidates (e.g., 3′-amino phosphoramidate, aminoalkylphosphoramidates, or thionophosphoramidates), thionoalkyl-phosphonates, thionoalkylphosphotriesters, morpholino linkages, boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of boranophosphates, or boranophosphates having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′. In some embodiments, a modified internucleoside linkage or backbone lacks a phosphorus atom. Modified internucleoside linkages lacking a phosphorus atom include, but are not limited to, short chain alkyl or cycloalkyl inter-sugar linkages, mixed heteroatom and alkyl or cycloalkyl inter-sugar linkages, or one or more short chain heteroatomic or heterocyclic inter-sugar linkages. In some embodiments, modified internucleoside backbones include, but are not limited to, siloxane backbones, sulfide backbones, sulfoxide backbones, sulfone backbones, formacetyl and thioformacetyl backbones, methylene formacetyl and thioformacetyl backbones, alkene-containing backbones, sulfamate backbones, methyleneimino and methylenehydrazino backbones, sulfonate and sulfonamide backbones, amide backbones, and other backbones having mixed N, O, S, and CH_2 components.

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

In some embodiments, an AAT 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, the at least two phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3, 2-4, 3-5, 4-6, 4-5, or 6-8 from the 5' end of the sense strand. In some embodiments, an AAT RNAi agent antisense strand contains four phosphorothioate internucleoside linkages. In some embodiments, the four phosphorothioate internucleoside linkages are between the nucleotides at positions 1-3 from the 5′ end of the antisense strand and between the nucleotides at positions 19-21, 20-22, 21-23, 22-24, 23-25, or 24-26 from the 5′ end. In some embodiments, an AAT RNAi agent contains at least two phosphorothioate internucleoside linkages in the sense strand and three or four phosphorothioate internucleoside linkages in the antisense strand.

In some embodiments, an AAT 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.

AAT RNAi Agents

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

Table 1. AAT 19-mer mRNA Target Sequences (taken from human AAT cDNA, GenBank NM_000295.4 (SEQ ID NO:1))

SEQ ID NO:	AAT 19-mer Target Sequences (5' → 3')	Corresponding Genome Position (taken from SEQ ID NO: 1)
2	CGUUUAGGCAUGUUUAACA	1000-1018
3	AACAGCACCAAUAUCUUCU	469-487
4	AUAUCAUCACCAAGUUCCU	1142-1160
5	AGAUGCUGCCCAGAAGACA	348-366
6	CUGGCACACCAGUCCAACA	454-472

SEQ ID NO:	AAT 19-mer Target Sequences (5' → 3')	Corresponding Genome Position (taken from SEQ ID NO: 1)
7	UGGCACACCAGUCCAACAG	455-473
8	GCACACCAGUCCAACAGCA	457-475
9	CAGUCCAACAGCACCAAUA	463-481
10	AGUCCAACAGCACCAAUAU	464-482
11	GUCCAACAGCACCAAUAUC	465-483
12	CCAACAGCACCAAUAUCUU	467-485
13	CCCCAGUGAGCAUCGCUAC	491-509
14	GAGCAUCGCUACAGCCUUU	498-516
15	GCAUCGCUACAGCCUUUGC	500-518
16	CAUCGCUACAGCCUUUGCA	501-519
17	UCGCUACAGCCUUUGCAAU	503-521
18	CUACAGCCUUUGCAAUGCU	506-524
19	ACAGCCUUUGCAAUGCUCU	508-526
20	GAAGGCUUCCAGGAACUCC	613-631
21	UAGUGGAUAAGUUUUUGGA	710-728
22	UGUACCACUCAGAAGCCUU	743-761
23	GUACCACUCAGAAGCCUUC	744-762
24	ACACCGAAGAGGCCAAGAA	779-797
25	ACCGAAGAGGCCAAGAAAC	781-799
26	AGGCCAAGAAACAGAUCAA	788-806
27	GGCCAAGAAACAGAUCAAC	789-807
28	GCCAAGAAACAGAUCAACG	790-808
29	UACUCAAGGGAAAAUUGUG	825-843
30	CUCAAGGGAAAAUUGUGGA	827-845
31	UCAAGGGAAAAUUGUGGAU	828-846
32	UUGGUCAAGGAGCUUGACA	847-865
33	AGGAGCUUGACAGAGACAC	854-872
34	AGCUUGACAGAGACACAGU	857-875
35	UUUGCUCUGGUGAAUUACA	877-895
36	AGCGUUUAGGCAUGUUUAA	998-1016
37	GCGUUUAGGCAUGUUUAAC	999-1017
38	UUAGGCAUGUUUAACAUCC	1003-1021
39	UGGGUGCUGCUGAUGAAAU	1045-1063
40	UGCCACCGCCAUCUUCUUC	1074-1092
41	CCUGGAAAAUGAACUCACC	1119-1137
42	CGAUAUCAUCACCAAGUUC	1140-1158
43	ACCAAGUUCCUGGAAAAUG	1150-1168
44	UCCAUUACUGGAACCUAUG	1207-1225
45	CCAUUACUGGAACCUAUGA	1208-1226
46	ACUGGAACCUAUGAUCUGA	1213-1231
47	GGAACCUAUGAUCUGAAGA	1216-1234

SEQ ID NO:	AAT 19-mer Target Sequences (5' → 3')	Corresponding Genome Position (taken from SEQ ID NO: 1)
48	GAACCUAUGAUCUGAAGAG	1217-1235
49	CAGCAAUGGGGCUGACCUC	1269-1287
50	GCAAUGGGGCUGACCUCUC	1271-1289
51	AGAGGAGGCACCCCUGAAG	1299-1317
52	AGGCACCCCUGAAGCUCUC	1304-1322
53	UCUCCAAGGCCGUGCAUAA	1319-1337
54	UCCAAGGCCGUGCAUAAGG	1321-1339
55	CCAAGGCCGUGCAUAAGGC	1322-1340
56	CAAGGCCGUGCAUAAGGCU	1323-1341
57	AAGGCUGUGCUGACCAUCG	1336-1354
58	GGCUGUGCUGACCAUCGAC	1338-1356
59	CUGCUGGGGCCAUGUUUUU	1373-1391
60	GCUGGGGCCAUGUUUUUAG	1375-1393
61	CUGGGGCCAUGUUUUUAGA	1376-1394
62	GGGGCCAUGUUUUUAGAGG	1378-1396
63	GGGCCAUGUUUUUAGAGGC	1379-1397
64	GAGGCCAUACCCAUGUCUA	1393-1411
65	GGCCAUACCCAUGUCUAUC	1395-1413
66	CCCGAGGUCAAGUUCAACA	1417-1435
67	AGGUCAAGUUCAACAAACC	1421-1439
68	CAAGUUCAACAAACCCUUU	1425-1443
69	AGUUCAACAAACCCUUUGU	1427-1445
70	GUUCAACAAACCCUUUGUC	1428-1446
71	UCAACAAACCCUUUGUCUU	1430-1448
72	ACCCUUUGUCUUCUUAAUG	1437-1455
73	CCUUUGUCUUCUUAAUGAU	1439-1457
74	UACCAAGUCUCCCCUCUUC	1467-1485
75	AAGUCUCCCCUCUUCAUGG	1471-1489
76	AGUCUCCCCUCUUCAUGGG	1472-1490
77	UCUCCCCUCUUCAUGGGAA	1474-1492
78	CUCCCCUCUUCAUGGGAAA	1475-1493
79	AUGACAUUAAAGAAGGGUU	1569-1587

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

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

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

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

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

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

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

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
80	UGUUAAACAUGCCUAAACG	429	CGUUUAGGCAUGUUUAACA	1000-1018
81	AGUUAAACAUGCCUAAACG	430	CGUUUAGGCAUGUUUAACU	1000-1018
82	NGUUAAACAUGCCUAAACG	431	CGUUUAGGCAUGUUUAACN	1000-1018
83	NGUUAAACAUGCCUAAACN	432	NGUUUAGGCAUGUUUAACN	1000-1018
84	AGAAGAUAUUGGUGCUGUU	433	AACAGCACCAAUAUCUUCU	469-487
85	UGAAGAUAUUGGUGCUGUU	434	AACAGCACCAAUAUCUUCA	469-487
86	NGAAGAUAUUGGUGCUGUU	435	AACAGCACCAAUAUCUUCN	469-487
87	NGAAGAUAUUGGUGCUGUN	436	NACAGCACCAAUAUCUUCN	469-487
88	AGGAACUUGGUGAUGAUAU	437	AUAUCAUCACCAAGUUCCU	1142-1160
89	UGGAACUUGGUGAUGAUAU	438	AUAUCAUCACCAAGUUCCA	1142-1160
90	NGGAACUUGGUGAUGAUAU	439	AUAUCAUCACCAAGUUCCN	1142-1160
91	NGGAACUUGGUGAUGAUAN	440	NUAUCAUCACCAAGUUCCN	1142-1160
92	UGUCUUCUGGGCAGCAUCU	441	AGAUGCUGCCCAGAAGACA	348-366
93	AGUCUUCUGGGCAGCAUCU	442	AGAUGCUGCCCAGAAGACU	348-366
94	NGUCUUCUGGGCAGCAUCU	443	AGAUGCUGCCCAGAAGACN	348-366
95	NGUCUUCUGGGCAGCAUCN	444	NGAUGCUGCCCAGAAGACN	348-366
96	UGUUGGACUGGUGUGCCAG	445	CUGGCACACCAGUCCAACA	454-472
97	AGUUGGACUGGUGUGCCAG	446	CUGGCACACCAGUCCAACU	454-472
98	NGUUGGACUGGUGUGCCAG	447	CUGGCACACCAGUCCAACN	454-472
99	NGUUGGACUGGUGUGCCAN	448	NUGGCACACCAGUCCAACN	454-472
100	CUGUUGGACUGGUGUGCCA	449	UGGCACACCAGUCCAACAG	455-473
101	UUGUUGGACUGGUGUGCCA	450	UGGCACACCAGUCCAACAA	455-473
102	AUGUUGGACUGGUGUGCCA	451	UGGCACACCAGUCCAACAU	455-473
103	NUGUUGGACUGGUGUGCCA	452	UGGCACACCAGUCCAACAN	455-473
104	NUGUUGGACUGGUGUGCCN	453	NGGCACACCAGUCCAACAN	455-473
105	UGCUGUUGGACUGGUGUGC	454	GCACACCAGUCCAACAGCA	457-475

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
106	AGCUGUUGGACUGGUGUGC	455	GCACACCAGUCCAACAGCU	457-475
107	NGCUGUUGGACUGGUGUGC	456	GCACACCAGUCCAACAGCN	457-475
108	NGCUGUUGGACUGGUGUGN	457	NCACACCAGUCCAACAGCN	457-475
109	UAUUGGUGCUGUUGGACUG	458	CAGUCCAACAGCACCAAUA	463-481
110	AAUUGGUGCUGUUGGACUG	459	CAGUCCAACAGCACCAAUU	463-481
111	NAUUGGUGCUGUUGGACUG	460	CAGUCCAACAGCACCAAUN	463-481
112	NAUUGGUGCUGUUGGACUN	461	NAGUCCAACAGCACCAAUN	463-481
113	AUAUUGGUGCUGUUGGACU	462	AGUCCAACAGCACCAAUAU	464-482
114	UUAUUGGUGCUGUUGGACU	463	AGUCCAACAGCACCAAUAA	464-482
115	NUAUUGGUGCUGUUGGACU	464	AGUCCAACAGCACCAAUAN	464-482
116	NUAUUGGUGCUGUUGGACN	465	NGUCCAACAGCACCAAUAN	464-482
117	GAUAUUGGUGCUGUUGGAC	466	GUCCAACAGCACCAAUAUC	465-483
118	UAUAUUGGUGCUGUUGGAC	467	GUCCAACAGCACCAAUAUA	465-483
119	AAUAUUGGUGCUGUUGGAC	468	GUCCAACAGCACCAAUAUU	465-483
120	NAUAUUGGUGCUGUUGGAC	469	GUCCAACAGCACCAAUAUN	465-483
121	NAUAUUGGUGCUGUUGGAN	470	NUCCAACAGCACCAAUAUN	465-483
122	AAGAUAUUGGUGCUGUUGG	471	CCAACAGCACCAAUAUCUU	467-485
123	UAGAUAUUGGUGCUGUUGG	472	CCAACAGCACCAAUAUCUA	467-485
124	NAGAUAUUGGUGCUGUUGG	473	CCAACAGCACCAAUAUCUN	467-485
125	NAGAUAUUGGUGCUGUUGN	474	NCAACAGCACCAAUAUCUN	467-485
126	GUAGCGAUGCUCACUGGGG	475	CCCCAGUGAGCAUCGCUAC	491-509
127	UUAGCGAUGCUCACUGGGG	476	CCCCAGUGAGCAUCGCUAA	491-509
128	AUAGCGAUGCUCACUGGGG	477	CCCCAGUGAGCAUCGCUAU	491-509
129	NUAGCGAUGCUCACUGGGG	478	CCCCAGUGAGCAUCGCUAN	491-509
130	NUAGCGAUGCUCACUGGGN	479	NCCCAGUGAGCAUCGCUAN	491-509
131	AAAGGCUGUAGCGAUGCUC	480	GAGCAUCGCUACAGCCUUU	498-516
132	UAAGGCUGUAGCGAUGCUC	481	GAGCAUCGCUACAGCCUUA	498-516
133	NAAGGCUGUAGCGAUGCUC	482	GAGCAUCGCUACAGCCUUN	498-516

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
134	NAAGGCUGUAGCGAUGCUN	483	NAGCAUCGCUACAGCCUUN	498-516
135	GCAAAGGCUGUAGCGAUGC	484	GCAUCGCUACAGCCUUUGC	500-518
136	UCAAAGGCUGUAGCGAUGC	485	GCAUCGCUACAGCCUUUGA	500-518
137	ACAAAGGCUGUAGCGAUGC	486	GCAUCGCUACAGCCUUUGU	500-518
138	NCAAAGGCUGUAGCGAUGC	487	GCAUCGCUACAGCCUUUGN	500-518
139	NCAAAGGCUGUAGCGAUGN	488	NCAUCGCUACAGCCUUUGN	500-518
140	UGCAAAGGCUGUAGCGAUG	489	CAUCGCUACAGCCUUUGCA	501-519
141	AGCAAAGGCUGUAGCGAUG	490	CAUCGCUACAGCCUUUGCU	501-519
142	NGCAAAGGCUGUAGCGAUG	491	NAUCGCUACAGCCUUUGCN	501-519
143	NGCAAAGGCUGUAGCGAUN	492	NAUCGCUACAGCCUUUGCN	501-519
144	AUUGCAAAGGCUGUAGCGA	493	UCGCUACAGCCUUUGCAAU	503-521
145	UUUGCAAAGGCUGUAGCGA	494	UCGCUACAGCCUUUGCAAA	503-521
146	NUUGCAAAGGCUGUAGCGA	495	UCGCUACAGCCUUUGCAAN	503-521
147	NUUGCAAAGGCUGUAGCGN	496	NCGCUACAGCCUUUGCAAN	503-521
148	AGCAUUGCAAAGGCUGUAG	497	CUACAGCCUUUGCAAUGCU	506-524
149	UGCAUUGCAAAGGCUGUAG	498	CUACAGCCUUUGCAAUGCA	506-524
150	NGCAUUGCAAAGGCUGUAG	499	CUACAGCCUUUGCAAUGCN	506-524
151	NGCAUUGCAAAGGCUGUAN	500	NUACAGCCUUUGCAAUGCN	506-524
152	AGAGCAUUGCAAAGGCUGU	501	ACAGCCUUUGCAAUGCUCU	508-526
153	UGAGCAUUGCAAAGGCUGU	502	ACAGCCUUUGCAAUGCUCA	508-526
154	NGAGCAUUGCAAAGGCUGU	503	ACAGCCUUUGCAAUGCUCN	508-526
155	NGAGCAUUGCAAAGGCUGU	504	NCAGCCUUUGCAAUGCUCN	508-526
156	GGAGUUCCUGGAAGCCUUC	505	GAAGGCUUCCAGGAACUCC	613-631
157	UGAGUUCCUGGAAGCCUUC	506	GAAGGCUUCCAGGAACUCA	613-631
158	AGAGUUCCUGGAAGCCUUC	507	GAAGGCUUCCAGGAACUCU	613-631
159	NGAGUUCCUGGAAGCCUUC	508	GAAGGCUUCCAGGAACUCN	613-631
160	NGAGUUCCUGGAAGCCUUN	509	NAAGGCUUCCAGGAACUCN	613-631
161	UCCAAAAACUUAUCCACUA	510	UAGUGGAUAAGUUUUUGGA	710-728

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
162	ACCAAAAACUUAUCCACUA	511	UAGUGGAUAAGUUUUUGGU	710-728
163	NCCAAAAACUUAUCCACUA	512	UAGUGGAUAAGUUUUUGGN	710-728
164	NCCAAAAACUUAUCCACUN	513	NAGUGGAUAAGUUUUUGGN	710-728
165	AAGGCUUCUGAGUGGUACA	514	UGUACCACUCAGAAGCCUU	743-761
166	UAGGCUUCUGAGUGGUACA	515	UGUACCACUCAGAAGCCUA	743-761
167	NAGGCUUCUGAGUGGUACA	516	UGUACCACUCAGAAGCCUN	743-761
168	NAGGCUUCUGAGUGGUACN	517	NGUACCACUCAGAAGCCUN	743-761
169	GAAGGCUUCUGAGUGGUAC	518	GUACCACUCAGAAGCCUUC	744-762
170	UAAGGCUUCUGAGUGGUAC	519	GUACCACUCAGAAGCCUUA	744-762
171	AAAGGCUUCUGAGUGGUAC	520	GUACCACUCAGAAGCCUUU	744-762
172	NAAGGCUUCUGAGUGGUAC	521	GUACCACUCAGAAGCCUUN	744-762
173	NAAGGCUUCUGAGUGGUAN	522	NUACCACUCAGAAGCCUUN	744-762
174	UUCUUGGCCUCUUCGGUGU	523	ACACCGAAGAGGCCAAGAA	779-797
175	AUCUUGGCCUCUUCGGUGU	524	ACACCGAAGAGGCCAAGAU	779-797
176	NUCUUGGCCUCUUCGGUGU	525	ACACCGAAGAGGCCAAGAN	779-797
177	NUCUUGGCCUCUUCGGUGN	526	NCACCGAAGAGGCCAAGAN	779-797
178	GUUUCUUGGCCUCUUCGGU	527	ACCGAAGAGGCCAAGAAAC	781-799
179	UUUUCUUGGCCUCUUCGGU	528	ACCGAAGAGGCCAAGAAAA	781-799
180	AUUUCUUGGCCUCUUCGGU	529	ACCGAAGAGGCCAAGAAAU	781-799
181	NUUUCUUGGCCUCUUCGGU	530	ACCGAAGAGGCCAAGAAAN	781-799
182	NUUUCUUGGCCUCUUCGGN	531	NCCGAAGAGGCCAAGAAAN	781-799
183	UUGAUCUGUUUCUUGGCCU	532	AGGCCAAGAAACAGAUCAA	788-806
184	AUGAUCUGUUUCUUGGCCU	533	AGGCCAAGAAACAGAUCAU	788-806
185	NUGAUCUGUUUCUUGGCCU	534	AGGCCAAGAAACAGAUCAN	788-806
186	NUGAUCUGUUUCUUGGCCN	535	NGGCCAAGAAACAGAUCAN	788-806
187	GUUGAUCUGUUUCUUGGCC	536	GGCCAAGAAACAGAUCAAC	789-807
188	UUUGAUCUGUUUCUUGGCC	537	GGCCAAGAAACAGAUCAAA	789-807
189	AUUGAUCUGUUUCUUGGCC	538	GGCCAAGAAACAGAUCAAU	789-807

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
190	NUUGAUCUGUUUCUUGGCC	539	GGCCAAGAAACAGAUCAAN	789-807
191	NUUGAUCUGUUUCUUGGCN	540	NGCCAAGAAACAGAUCAAN	789-807
192	CGUUGAUCUGUUUCUUGGC	541	GCCAAGAAACAGAUCAACG	790-808
193	UGUUGAUCUGUUUCUUGGC	542	GCCAAGAAACAGAUCAACA	790-808
194	AGUUGAUCUGUUUCUUGGC	543	GCCAAGAAACAGAUCAACU	790-808
195	NGUUGAUCUGUUUCUUGGC	544	GCCAAGAAACAGAUCAACN	790-808
196	NGUUGAUCUGUUUCUUGGN	545	NCCAAGAAACAGAUCAACN	790-808
197	CACAAUUUUCCCUUGAGUA	546	UACUCAAGGGAAAAUUGUG	825-843
198	UACAAUUUUCCCUUGAGUA	547	UACUCAAGGGAAAAUUGUA	825-843
199	AACAAUUUUCCCUUGAGUA	548	UACUCAAGGGAAAAUUGUU	825-843
200	NACAAUUUUCCCUUGAGUA	549	UACUCAAGGGAAAAUUGUN	825-843
201	NACAAUUUUCCCUUGAGUN	550	NACUCAAGGGAAAAUUGUN	825-843
202	UCCACAAUUUUCCCUUGAG	551	CUCAAGGGAAAAUUGUGGA	827-845
203	ACCACAAUUUUCCCUUGAG	552	CUCAAGGGAAAAUUGUGGU	827-845
204	NCCACAAUUUUCCCUUGAG	553	CUCAAGGGAAAAUUGUGGN	827-845
205	NCCACAAUUUUCCCUUGAN	554	NUCAAGGGAAAAUUGUGGN	827-845
206	AUCCACAAUUUUCCCUUGA	555	UCAAGGGAAAAUUGUGGAU	828-846
207	UUCCACAAUUUUCCCUUGA	556	UCAAGGGAAAAUUGUGGAA	828-846
208	NUCCACAAUUUUCCCUUGA	557	UCAAGGGAAAAUUGUGGAN	828-846
209	NUCCACAAUUUUCCCUUGN	558	NCAAGGGAAAAUUGUGGAN	828-846
210	UGUCAAGCUCCUUGACCAA	559	UUGGUCAAGGAGCUUGACA	847-865
211	AGUCAAGCUCCUUGACCAA	560	UUGGUCAAGGAGCUUGACU	847-865
212	NGUCAAGCUCCUUGACCAA	561	NUGGUCAAGGAGCUUGACA	847-865
213	NGUCAAGCUCCUUGACCAA	562	NUGGUCAAGGAGCUUGACN	847-865
214	GUGUCUCUGUCAAGCUCCU	563	AGGAGCUUGACAGAGACAC	854-872
215	UUGUCUCUGUCAAGCUCCU	564	AGGAGCUUGACAGAGACAA	854-872
216	AUGUCUCUGUCAAGCUCCU	565	AGGAGCUUGACAGAGACAU	854-872
217	NUGUCUCUGUCAAGCUCCU	566	AGGAGCUUGACAGAGACAN	854-872

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
218	NUGUCUCUGUCAAGCUCCN	567	NGGAGCUUGACAGAGACAN	854-872
219	ACUGUGUCUCUGUCAAGCU	568	AGCUUGACAGAGACACAGU	857-875
220	UCUGUGUCUCUGUCAAGCU	569	AGCUUGACAGAGACACAGA	857-875
221	NCUGUGUCUCUGUCAAGCU	570	AGCUUGACAGAGACACAGN	857-875
222	NCUGUGUCUCUGUCAAGCN	571	NGCUUGACAGAGACACAGN	857-875
223	UGUAAUUCACCAGAGCAAA	572	UUUGCUCUGGUGAAUUACA	877-895
224	AGUAAUUCACCAGAGCAAA	573	UUUGCUCUGGUGAAUUACU	877-895
225	NGUAAUUCACCAGAGCAAA	574	UUUGCUCUGGUGAAUUACN	877-895
226	NGUAAUUCACCAGAGCAAN	575	NUUGCUCUGGUGAAUUACN	877-895
227	UUAAACAUGCCUAAACGCU	576	AGCGUUUAGGCAUGUUUAA	998-1016
228	AUAAACAUGCCUAAACGCU	577	AGCGUUUAGGCAUGUUUAU	998-1016
229	NUAAACAUGCCUAAACGCU	578	AGCGUUUAGGCAUGUUUAN	998-1016
230	NUAAACAUGCCUAAACGCN	579	NGCGUUUAGGCAUGUUUAN	998-1016
231	GUUAAACAUGCCUAAACGC	580	GCGUUUAGGCAUGUUUAAC	999-1017
232	UUUAAACAUGCCUAAACGC	581	GCGUUUAGGCAUGUUUAAA	999-1017
233	AUUAAACAUGCCUAAACGC	582	GCGUUUAGGCAUGUUUAAU	999-1017
234	NUUAAACAUGCCUAAACGC	583	GCGUUUAGGCAUGUUUAAN	999-1017
235	NUUAAACAUGCCUAAACGN	584	NCGUUUAGGCAUGUUUAAN	999-1017
236	GGAUGUUAAACAUGCCUAA	585	UUAGGCAUGUUUAACAUCC	1003-1021
237	UGAUGUUAAACAUGCCUAA	586	UUAGGCAUGUUUAACAUCA	1003-1021
238	AGAUGUUAAACAUGCCUAA	587	UUAGGCAUGUUUAACAUCU	1003-1021
239	NGAUGUUAAACAUGCCUAA	588	UUAGGCAUGUUUAACAUCN	1003-1021
240	NGAUGUUAAACAUGCCUAN	589	NUAGGCAUGUUUAACAUCN	1003-1021
241	AUUUCAUCAGCAGCACCCA	590	UGGGUGCUGCUGAUGAAAU	1045-1063
242	UUUUCAUCAGCAGCACCCA	591	UGGGUGCUGCUGAUGAAAA	1045-1063
243	NUUUCAUCAGCAGCACCCA	592	UGGGUGCUGCUGAUGAAAN	1045-1063
244	NUUUCAUCAGCAGCACCCN	593	NGGGUGCUGCUGAUGAAAN	1045-1063
245	GAAGAAGAUGGCGGUGGCA	594	UGCCACCGCCAUCUUCUUC	1074-1092

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
246	UAAGAAGAUGGCGGUGGCA	595	UGCCACCGCCAUCUUCUUA	1074-1092
247	AAAGAAGAUGGCGGUGGCA	596	UGCCACCGCCAUCUUCUUU	1074-1092
248	NAAGAAGAUGGCGGUGGCA	597	UGCCACCGCCAUCUUCUUN	1074-1092
249	NAAGAAGAUGGCGGUGGCN	598	NGCCACCGCCAUCUUCUUN	1074-1092
250	GGUGAGUUCAUUUUCCAGG	599	CCUGGAAAAUGAACUCACC	1119-1137
251	UGUGAGUUCAUUUUCCAGG	600	CCUGGAAAAUGAACUCACA	1119-1137
252	AGUGAGUUCAUUUUCCAGG	601	CCUGGAAAAUGAACUCACU	1119-1137
253	NGUGAGUUCAUUUUCCAGG	602	CCUGGAAAAUGAACUCACN	1119-1137
254	NGUGAGUUCAUUUUCCAGN	603	NCUGGAAAAUGAACUCACN	1119-1137
255	GAACUUGGUGAUGAUAUCG	604	CGAUAUCAUCACCAAGUUC	1140-1158
256	UAACUUGGUGAUGAUAUCG	605	CGAUAUCAUCACCAAGUUA	1140-1158
257	AAACUUGGUGAUGAUAUCG	606	CGAUAUCAUCACCAAGUUU	1140-1158
258	NAACUUGGUGAUGAUAUCG	607	CGAUAUCAUCACCAAGUUN	1140-1158
259	NAACUUGGUGAUGAUAUCN	608	NGAUAUCAUCACCAAGUUN	1140-1158
260	CAUUUUCCAGGAACUUGGU	609	ACCAAGUUCCUGGAAAAUG	1150-1168
261	UAUUUUCCAGGAACUUGGU	610	ACCAAGUUCCUGGAAAAUA	1150-1168
262	AAUUUUCCAGGAACUUGGU	611	ACCAAGUUCCUGGAAAAUU	1150-1168
263	NAUUUUCCAGGAACUUGGU	612	ACCAAGUUCCUGGAAAAUN	1150-1168
264	NAUUUUCCAGGAACUUGGN	613	NCCAAGUUCCUGGAAAAUN	1150-1168
265	CAUAGGUUCCAGUAAUGGA	614	UCCAUUACUGGAACCUAUG	1207-1225
266	UAUAGGUUCCAGUAAUGGA	615	UCCAUUACUGGAACCUAUA	1207-1225
267	AAUAGGUUCCAGUAAUGGA	616	UCCAUUACUGGAACCUAUU	1207-1225
268	NAUAGGUUCCAGUAAUGGA	617	UCCAUUACUGGAACCUAUN	1207-1225
269	NAUAGGUUCCAGUAAUGGN	618	NCCAUUACUGGAACCUAUN	1207-1225
270	UCAUAGGUUCCAGUAAUGG	619	CCAUUACUGGAACCUAUGA	1208-1226
271	ACAUAGGUUCCAGUAAUGG	620	CCAUUACUGGAACCUAUGU	1208-1226
272	NCAUAGGUUCCAGUAAUGG	621	CCAUUACUGGAACCUAUGN	1208-1226
273	NCAUAGGUUCCAGUAAUGN	622	NCAUUACUGGAACCUAUGN	1208-1226

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
274	UCAGAUCAUAGGUUCCAGU	623	ACUGGAACCUAUGAUCUGA	1213-1231
275	ACAGAUCAUAGGUUCCAGU	624	ACUGGAACCUAUGAUCUGU	1213-1231
276	NCAGAUCAUAGGUUCCAGU	625	ACUGGAACCUAUGAUCUGN	1213-1231
277	NCAGAUCAUAGGUUCCAGN	626	NCUGGAACCUAUGAUCUGN	1213-1231
278	UCUUCAGAUCAUAGGUUCC	627	GGAACCUAUGAUCUGAAGA	1216-1234
279	ACUUCAGAUCAUAGGUUCC	628	GGAACCUAUGAUCUGAAGU	1216-1234
280	NCUUCAGAUCAUAGGUUCC	629	GGAACCUAUGAUCUGAAGN	1216-1234
281	NCUUCAGAUCAUAGGUUCN	630	NGAACCUAUGAUCUGAAGN	1216-1234
282	CUCUUCAGAUCAUAGGUUC	631	GAACCUAUGAUCUGAAGAG	1217-1235
283	UUCUUCAGAUCAUAGGUUC	632	GAACCUAUGAUCUGAAGAA	1217-1235
284	AUCUUCAGAUCAUAGGUUC	633	GAACCUAUGAUCUGAAGAU	1217-1235
285	NUCUUCAGAUCAUAGGUUC	634	GAACCUAUGAUCUGAAGAG	1217-1235
286	NUCUUCAGAUCAUAGGUUN	635	NAACCUAUGAUCUGAAGAN	1217-1235
287	GAGGUCAGCCCCAUUGCUG	636	CAGCAAUGGGGCUGACCUC	1269-1287
288	UAGGUCAGCCCCAUUGCUG	637	CAGCAAUGGGGCUGACCUA	1269-1287
289	AAGGUCAGCCCCAUUGCUG	638	CAGCAAUGGGGCUGACCUU	1269-1287
290	NAGGUCAGCCCCAUUGCUG	639	CAGCAAUGGGGCUGACCUN	1269-1287
291	NAGGUCAGCCCCAUUGCUN	640	NAGCAAUGGGGCUGACCUN	1269-1287
292	GAGAGGUCAGCCCCAUUGC	641	GCAAUGGGGCUGACCUCUC	1271-1289
293	UAGAGGUCAGCCCCAUUGC	642	GCAAUGGGGCUGACCUCUA	1271-1289
294	AAGAGGUCAGCCCCAUUGC	643	GCAAUGGGGCUGACCUCUU	1271-1289
295	NAGAGGUCAGCCCCAUUGC	644	GCAAUGGGGCUGACCUCUN	1271-1289
296	NAGAGGUCAGCCCCAUUGN	645	NCAAUGGGGCUGACCUCUN	1271-1289
297	CUUCAGGGGUGCCUCCUCU	646	AGAGGAGGCACCCCUGAAG	1299-1317
298	UUUCAGGGGUGCCUCCUCU	647	AGAGGAGGCACCCCUGAAA	1299-1317
299	AUUCAGGGGUGCCUCCUCU	648	AGAGGAGGCACCCCUGAAU	1299-1317
300	NUUCAGGGGUGCCUCCUCU	649	AGAGGAGGCACCCCUGAAN	1299-1317
301	NUUCAGGGGUGCCUCCUCN	650	NGAGGAGGCACCCCUGAAN	1299-1317

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
302	GAGAGCUUCAGGGGUGCCU	651	AGGCACCCCUGAAGCUCUC	1304-1322
303	UAGAGCUUCAGGGGUGCCU	652	AGGCACCCCUGAAGCUCUA	1304-1322
304	AAGAGCUUCAGGGGUGCCU	653	AGGCACCCCUGAAGCUCUU	1304-1322
305	NAGAGCUUCAGGGGUGCCU	654	AGGCACCCCUGAAGCUCUN	1304-1322
306	NAGAGCUUCAGGGGUGCCN	655	NGGCACCCCUGAAGCUCUN	1304-1322
307	UUAUGCACGGCCUUGGAGA	656	UCUCCAAGGCCGUGCAUAA	1319-1337
308	AUAUGCACGGCCUUGGAGA	657	UCUCCAAGGCCGUGCAUAU	1319-1337
309	NUAUGCACGGCCUUGGAGA	658	UCUCCAAGGCCGUGCAUAN	1319-1337
310	NUAUGCACGGCCUUGGAGN	659	NCUCCAAGGCCGUGCAUAN	1319-1337
311	CCUUAUGCACGGCCUUGGA	660	UCCAAGGCCGUGCAUAAGG	1321-1339
312	UCUUAUGCACGGCCUUGGA	661	UCCAAGGCCGUGCAUAAGA	1321-1339
313	ACUUAUGCACGGCCUUGGA	662	UCCAAGGCCGUGCAUAAGU	1321-1339
314	NCUUAUGCACGGCCUUGGA	663	UCCAAGGCCGUGCAUAAGN	1321-1339
315	NCUUAUGCACGGCCUUGGN	664	NCCAAGGCCGUGCAUAAGN	1321-1339
316	GCCUUAUGCACGGCCUUGG	665	CCAAGGCCGUGCAUAAGGC	1322-1340
317	UCCUUAUGCACGGCCUUGG	666	CCAAGGCCGUGCAUAAGGA	1322-1340
318	ACCUUAUGCACGGCCUUGG	667	CCAAGGCCGUGCAUAAGGU	1322-1340
319	NCCUUAUGCACGGCCUUGG	668	CCAAGGCCGUGCAUAAGGN	1322-1340
320	NCCUUAUGCACGGCCUUGN	669	NCAAGGCCGUGCAUAAGGN	1322-1340
321	AGCCUUAUGCACGGCCUUG	670	CAAGGCCGUGCAUAAGGCU	1323-1341
322	UGCCUUAUGCACGGCCUUG	671	CAAGGCCGUGCAUAAGGCA	1323-1341
323	NGCCUUAUGCACGGCCUUG	672	CAAGGCCGUGCAUAAGGCN	1323-1341
324	NGCCUUAUGCACGGCCUUN	673	NAAGGCCGUGCAUAAGGCN	1323-1341
325	CGAUGGUCAGCACAGCCUU	674	AAGGCUGUGCUGACCAUCG	1336-1354
326	UGAUGGUCAGCACAGCCUU	675	AAGGCUGUGCUGACCAUCA	1336-1354
327	AGAUGGUCAGCACAGCCUU	676	AAGGCUGUGCUGACCAUCU	1336-1354
328	NGAUGGUCAGCACAGCCUU	677	AAGGCUGUGCUGACCAUCN	1336-1354
329	NGAUGGUCAGCACAGCCUN	678	NAGGCUGUGCUGACCAUCN	1336-1354

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
330	GUCGAUGGUCAGCACAGCC	679	GGCUGUGCUGACCAUCGAC	1338-1356
331	UUCGAUGGUCAGCACAGCC	680	GGCUGUGCUGACCAUCGAA	1338-1356
332	AUCGAUGGUCAGCACAGCC	681	GGCUGUGCUGACCAUCGAU	1338-1356
333	NUCGAUGGUCAGCACAGCC	682	GGCUGUGCUGACCAUCGAN	1338-1356
334	NUCGAUGGUCAGCACAGCN	683	NGCUGUGCUGACCAUCGAN	1338-1356
335	AAAAACAUGGCCCCAGCAG	684	CUGCUGGGGCCAUGUUUUU	1373-1391
336	UAAAACAUGGCCCCAGCAG	685	CUGCUGGGGCCAUGUUUUA	1373-1391
337	NAAAACAUGGCCCCAGCAG	686	CUGCUGGGGCCAUGUUUUN	1373-1391
338	NAAAACAUGGCCCCAGCAN	687	NUGCUGGGGCCAUGUUUUN	1373-1391
339	CUAAAAACAUGGCCCCAGC	688	GCUGGGGCCAUGUUUUUAG	1375-1393
340	UUAAAAACAUGGCCCCAGC	689	GCUGGGGCCAUGUUUUUAA	1375-1393
341	AUAAAAACAUGGCCCCAGC	690	GCUGGGGCCAUGUUUUUAU	1375-1393
342	NUAAAAACAUGGCCCCAGC	691	GCUGGGGCCAUGUUUUUAN	1375-1393
343	NUAAAAACAUGGCCCCAGN	692	NCUGGGGCCAUGUUUUUAN	1375-1393
344	UCUAAAAACAUGGCCCCAG	693	CUGGGGCCAUGUUUUUAGA	1376-1394
345	ACUAAAAACAUGGCCCCAG	694	CUGGGGCCAUGUUUUUAGU	1376-1394
346	NCUAAAAACAUGGCCCCAG	695	CUGGGGCCAUGUUUUUAGN	1376-1394
347	NCUAAAAACAUGGCCCCAN	696	NUGGGGCCAUGUUUUUAGN	1376-1394
348	CCUCUAAAAACAUGGCCCC	697	GGGGCCAUGUUUUUAGAGG	1378-1396
349	UCUCUAAAAACAUGGCCCC	698	GGGGCCAUGUUUUUAGAGA	1378-1396
350	ACUCUAAAAACAUGGCCCC	699	GGGGCCAUGUUUUUAGAGU	1378-1396
351	NCUCUAAAAACAUGGCCCC	700	GGGGCCAUGUUUUUAGAGN	1378-1396
352	NCUCUAAAAACAUGGCCCN	701	NGGGCCAUGUUUUUAGAGN	1378-1396
353	GCCUCUAAAAACAUGGCCC	702	GGGCCAUGUUUUUAGAGGC	1379-1397
354	UCCUCUAAAAACAUGGCCC	703	GGGCCAUGUUUUUAGAGGA	1379-1397
355	ACCUCUAAAAACAUGGCCC	704	GGGCCAUGUUUUUAGAGGU	1379-1397
356	NCCUCUAAAAACAUGGCCC	705	GGGCCAUGUUUUUAGAGGN	1379-1397
357	NCCUCUAAAAACAUGGCCN	706	NGGCCAUGUUUUUAGAGGN	1379-1397

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
358	UAGACAUGGGUAUGGCCUC	707	GAGGCCAUACCCAUGUCUA	1393-1411
359	AAGACAUGGGUAUGGCCUC	708	GAGGCCAUACCCAUGUCUU	1393-1411
360	NAGACAUGGGUAUGGCCUC	709	GAGGCCAUACCCAUGUCUN	1393-1411
361	NAGACAUGGGUAUGGCCUN	710	NAGGCCAUACCCAUGUCUN	1393-1411
362	GAUAGACAUGGGUAUGGCC	711	GGCCAUACCCAUGUCUAUC	1395-1413
363	UAUAGACAUGGGUAUGGCC	712	GGCCAUACCCAUGUCUAUA	1395-1413
364	AAUAGACAUGGGUAUGGCC	713	GGCCAUACCCAUGUCUAUU	1395-1413
365	NAUAGACAUGGGUAUGGCC	714	GGCCAUACCCAUGUCUAUN	1395-1413
366	NAUAGACAUGGGUAUGGCN	715	NGCCAUACCCAUGUCUAUN	1395-1413
367	UGUUGAACUUGACCUCGGG	716	CCCGAGGUCAAGUUCAACA	1417-1435
368	AGUUGAACUUGACCUCGGG	717	CCCGAGGUCAAGUUCAACU	1417-1435
369	NGUUGAACUUGACCUCGGG	718	CCCGAGGUCAAGUUCAACN	1417-1435
370	NGUUGAACUUGACCUCGGN	719	NCCGAGGUCAAGUUCAACN	1417-1435
371	GGUUUGUUGAACUUGACCU	720	AGGUCAAGUUCAACAAACC	1421-1439
372	UGUUUGUUGAACUUGACCU	721	AGGUCAAGUUCAACAAACA	1421-1439
373	AGUUUGUUGAACUUGACCU	722	AGGUCAAGUUCAACAAACU	1421-1439
374	NGUUUGUUGAACUUGACCU	723	AGGUCAAGUUCAACAAACN	1421-1439
375	NGUUUGUUGAACUUGACCN	724	NGGUCAAGUUCAACAAACN	1421-1439
376	AAAGGGUUUGUUGAACUUG	725	CAAGUUCAACAAACCCUUU	1425-1443
377	UAAGGGUUUGUUGAACUUG	726	CAAGUUCAACAAACCCUUA	1425-1443
378	NAAGGGUUUGUUGAACUUG	727	CAAGUUCAACAAACCCUUN	1425-1443
379	NAAGGGUUUGUUGAACUUN	728	NAAGUUCAACAAACCCUUN	1425-1443
380	ACAAAGGGUUUGUUGAACU	729	AGUUCAACAAACCCUUUGU	1427-1445
381	UCAAAGGGUUUGUUGAACU	730	AGUUCAACAAACCCUUUGA	1427-1445
382	NCAAAGGGUUUGUUGAACU	731	AGUUCAACAAACCCUUUGN	1427-1445
383	NCAAAGGGUUUGUUGAACN	732	NGUUCAACAAACCCUUUGN	1427-1445
384	GACAAAGGGUUUGUUGAAC	733	GUUCAACAAACCCUUUGUC	1428-1446
385	UACAAAGGGUUUGUUGAAC	734	GUUCAACAAACCCUUUGUA	1428-1446

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
386	AACAAAGGGUUUGUUGAAC	735	GUUCAACAAACCCUUUGUU	1428-1446
387	NACAAAGGGUUUGUUGAAC	736	GUUCAACAAACCCUUUGUN	1428-1446
388	NACAAAGGGUUUGUUGAAN	737	NUUCAACAAACCCUUUGUN	1428-1446
389	AAGACAAAGGGUUUGUUGA	738	UCAACAAACCCUUUGUCUU	1430-1448
390	UAGACAAAGGGUUUGUUGA	739	UCAACAAACCCUUUGUCUA	1430-1448
391	NAGACAAAGGGUUUGUUGA	740	UCAACAAACCCUUUGUCUN	1430-1448
392	NAGACAAAGGGUUUGUUGN	741	NCAACAAACCCUUUGUCUN	1430-1448
393	CAUUAAGAAGACAAAGGGU	742	ACCCUUUGUCUUCUUAAUG	1437-1455
394	UAUUAAGAAGACAAAGGGU	743	ACCCUUUGUCUUCUUAAUA	1437-1455
395	AAUUAAGAAGACAAAGGGU	744	ACCCUUUGUCUUCUUAAUU	1437-1455
396	NAUUAAGAAGACAAAGGGU	745	ACCCUUUGUCUUCUUAAUN	1437-1455
397	NAUUAAGAAGACAAAGGGN	746	NCCCUUUGUCUUCUUAAUN	1437-1455
398	AUCAUUAAGAAGACAAAGG	747	CCUUUGUCUUCUUAAUGAU	1439-1457
399	UUCAUUAAGAAGACAAAGG	748	CCUUUGUCUUCUUAAUGAA	1439-1457
400	NUCAUUAAGAAGACAAAGG	749	CCUUUGUCUUCUUAAUGAN	1439-1457
401	NUCAUUAAGAAGACAAAGN	750	NCUUUGUCUUCUUAAUGAN	1439-1457
402	GAAGAGGGGAGACUUGGUA	751	UACCAAGUCUCCCCUCUUC	1467-1485
403	UAAGAGGGGAGACUUGGUA	752	UACCAAGUCUCCCCUCUUA	1467-1485
404	AAAGAGGGGAGACUUGGUA	753	UACCAAGUCUCCCCUCUUU	1467-1485
405	NAAGAGGGGAGACUUGGUA	754	UACCAAGUCUCCCCUCUUN	1467-1485
406	NAAGAGGGGAGACUUGGUN	755	NACCAAGUCUCCCCUCUUN	1467-1485
407	CCAUGAAGAGGGGAGACUU	756	AAGUCUCCCCUCUUCAUGG	1471-1489
408	UCAUGAAGAGGGGAGACUU	757	AAGUCUCCCCUCUUCAUGA	1471-1489
409	ACAUGAAGAGGGGAGACUU	758	AAGUCUCCCCUCUUCAUGU	1471-1489
410	NCAUGAAGAGGGGAGACUU	759	AAGUCUCCCCUCUUCAUGN	1471-1489
411	NCAUGAAGAGGGGAGACUN	760	NAGUCUCCCCUCUUCAUGN	1471-1489
412	CCCAUGAAGAGGGGAGACU	761	AGUCUCCCCUCUUCAUGGG	1472-1490
413	UCCAUGAAGAGGGGAGACU	762	AGUCUCCCCUCUUCAUGGA	1472-1490

SEQ ID NO:	Antisense Base Sequence (5' → 3') (19-mers)	SEQ ID NO:	Sense Base Sequence (5' → 3') (19-mers)	Genome Position of SEQ ID NO: 1
414	ACCAUGAAGAGGGGAGACU	763	AGUCUCCCCUCUUCAUGGU	1472-1490
415	NCCAUGAAGAGGGGAGACU	764	AGUCUCCCCUCUUCAUGGN	1472-1490
416	NCCAUGAAGAGGGGAGACN	765	NGUCUCCCCUCUUCAUGGN	1472-1490
417	UUCCCAUGAAGAGGGGAGA	766	UCUCCCCUCUUCAUGGGAA	1474-1492
418	AUCCCAUGAAGAGGGGAGA	767	UCUCCCCUCUUCAUGGGAU	1474-1492
419	NUCCCAUGAAGAGGGGAGA	768	UCUCCCCUCUUCAUGGGAN	1474-1492
420	NUCCCAUGAAGAGGGGAGN	769	NCUCCCCUCUUCAUGGGAN	1474-1492
421	UUUCCCAUGAAGAGGGGAG	770	CUCCCCUCUUCAUGGGAAA	1475-1493
422	AUUCCCAUGAAGAGGGGAG	771	CUCCCCUCUUCAUGGGAAU	1475-1493
423	NUUCCCAUGAAGAGGGGAG	772	CUCCCCUCUUCAUGGGAAN	1475-1493
424	NUUCCCAUGAAGAGGGGAN	773	NUCCCCUCUUCAUGGGAAN	1475-1493
425	AACCCUUCUUUAAUGUCAU	774	AUGACAUUAAAGAAGGGUU	1569-1587
426	UACCCUUCUUUAAUGUCAU	775	AUGACAUUAAAGAAGGGUA	1569-1587
427	NACCCUUCUUUAAUGUCAU	776	AUGACAUUAAAGAAGGGUN	1569-1587
428	NACCCUUCUUUAAUGUCAN	777	NUGACAUUAAAGAAGGGUN	1569-1587

Table 3. Example AAT RNAi Agent Antisense Strand and Sense Strand Base Sequences

SEQ ID NO:	Antisense Base Sequence (5' → 3')	SEQ ID NO:	Sense Base Sequence (5' → 3')
778	GGAACUUGGUGAUGAUAU	840	AUAUCAUCACCAAGUUCC
779	GAUCAUAGGUUCCAGUAA	841	UUACUGGAACCUAUGAUC
780	ACAGCCUUAUGCACGGCC	842	GGCCGUGCAUAAGGCUGU
781	UCGAUGGUCAGCACAGCC	843	GGCUGUGCUGACCAUCGA
782	CAAAGGGUUUGUUGAACU	844	AGUUCAACAAACCCUUUG
783	TGGAACUUGGUGAUGAUAUTT	845	UAUAUAUCAUCACCAAGUUCCAT
783	TGGAACUUGGUGAUGAUAUTT	846	AUAUCAUCACCAAGUUCCAT
784	TGGAACUUGGUGAUGAUAUCGUG	847	CGAUAUCAUCACCAAGUUCCA
785	ACUUGGUGAUGAUAUTT	848	UAUCAUCACCAAGUUCCAT
786	TGGAACTTGGTGATGATATTT	849	TATATATCATCACCAAGTTCCAT
787	UUUAAACAUGCCUAAACGCUU	850	GCGUUUAGGCAUGUUUAAAUU
788	UGCAUUGCCCAGGUAUUUCUU	851	GAAAUACCUGGGCAAUGCAUU
789	UGGAACUUGGUGAUGAUAUUU	852	AUAUCAUCACCAAGUUCCAUU
790	UGAUCAUAGGUUCCAGUAAUU	853	UUACUGGAACCUAUGAUCAUU
791	UACAGCCUUAUGCACGGCCUU	854	GGCCGUGCAUAAGGCUGUAUU
792	UUCGAUGGUCAGCACAGCCUU	855	GGCUGUGCUGACCAUCGAAUU
793	UCAAAGGGUUUGUUGAACUUU	856	AGUUCAACAAACCCUUUGAUU
794	UGUUAAACAUGCCUAAACGUU	857	CGUUUAGGCAUGUUUAACAUU
795	UUUAAACGUGCCUAAACGCUG	858	CAGCGUUUAGGCAUGUUUAAA
796	UGCAUUGCCCAGGUAUUUCAG	859	CUGAAAUACCUGGGCAAUGCA
797	UGGAACUUGGUGAUGAUAUCG	847	CGAUAUCAUCACCAAGUUCCA
798	UGAUCAUAGGUUCCAGUAAUG	860	CAUUACUGGAACCUAUGAUCA
791	UACAGCCUUAUGCACGGCCUU	861	AAGGCCGUGCAUAAGGCUGUA
792	UUCGAUGGUCAGCACAGCCUU	862	AAGGCUGUGCUGACCAUCGAA
799	UCAAAGGGUUUGUUGAACUUG	863	CAAGUUCAACAAACCCUUUGA
800	UGUUAAACAUGCCUAAACGCG	864	CGCGUUUAGGCAUGUUUAACA
801	UGUUAAACAUGCCUAAACGCU	857	CGUUUAGGCAUGUUUAACAUU

SEQ ID NO:	Antisense Base Sequence (5' → 3')	SEQ ID NO:	Sense Base Sequence (5' → 3')
794	UGUUAAACAUGCCUAAACGUU	1265	CGUUUAGGCAUGUUUAACA
801	UGUUAAACAUGCCUAAACGCU	1265	CGUUUAGGCAUGUUUAACA
794	UGUUAAACAUGCCUAAACGUU	865	AACGUUUAGGCAUGUUUAACA
801	UGUUAAACAUGCCUAAACGCU	866	AGCGUUUAGGCAUGUUUAACA
802	UGUUAAACAUGCCUAAACGCUUC	866	AGCGUUUAGGCAUGUUUAACA
803	UGCUGUUGGACUGGUGUGCUU	1266	GCACACCAGUCCAACAGCA
804	UGCUGUUGGACUGGUGUGCCA	1266	GCACACCAGUCCAACAGCA
804	UGCUGUUGGACUGGUGUGCCA	867	UGGCACACCAGUCCAACAGCA
803	UGCUGUUGGACUGGUGUGCUU	868	AAGCACACCAGUCCAACAGCA
805	UGCUGUUGGACUGGUGUGCCAUU	867	UGGCACACCAGUCCAACAGCA
806	UGCUGUUGGACUGGUGUGCCAGC	867	UGGCACACCAGUCCAACAGCA
807	UAAGGCUUCUGAGUGGUACUU	1267	GUACCACUCAGAAGCCUUA
808	UAAGGCUUCUGAGUGGUACAA	1267	GUACCACUCAGAAGCCUUA
808	UAAGGCUUCUGAGUGGUACAA	869	UUGUACCACUCAGAAGCCUUA
809	UAAGGCUUCUGAGUGGUACAACU	869	UUGUACCACUCAGAAGCCUUA
810	GAAGGCUUCUGAGUGGUACUU	1268	GUACCACUCAGAAGCCUUC
811	AAGACAAAGGGUUUGUUGAUU	1269	UCAACAAACCCUUUGUCUU
812	AAGACAAAGGGUUUGUUGAAC	1269	UCAACAAACCCUUUGUCUU
812	AAGACAAAGGGUUUGUUGAAC	870	GUUCAACAAACCCUUUGUCUU
813	UAGACAAAGGGUUUGUUGAAC	871	GUUCAACAAACCCUUUGUCUA
814	AAGACAAAGGGUUUGUUGAACUU	870	GUUCAACAAACCCUUUGUCUU
815	UAGACAUGGGUAUGGCCUCUU	1270	GAGGCCAUACCCAUGUCUA
816	UAGACAUGGGUAUGGCCUCUA	1270	GAGGCCAUACCCAUGUCUA
816	UAGACAUGGGUAUGGCCUCUA	872	UAGAGGCCAUACCCAUGUCUA
817	UAGACAUGGGUAUGGCCUCUAAA	872	UAGAGGCCAUACCCAUGUCUA
818	UAGACAUGGGUAUGGCCUCUAUU	872	UAGAGGCCAUACCCAUGUCUA
819	UUUGAUCUGUUUCUUGGCCUU	1271	GGCCAAGAAACAGAUCAAA
820	UUUGAUCUGUUUCUUGGCCUC	1271	GGCCAAGAAACAGAUCAAA
820	UUUGAUCUGUUUCUUGGCCUC	873	GAGGCCAAGAAACAGAUCAAA

SEQ ID NO:	Antisense Base Sequence (5' → 3')	SEQ ID NO:	Sense Base Sequence (5' → 3')
821	UUUGAUCUGUUUCUUGGCCUCUU	873	GAGGCCAAGAAACAGAUCAAA
822	UGUUGGACUGGUGUGCCAGUU	1272	CUGGCACACCAGUCCAACA
823	UGUUGGACUGGUGUGCCAGCU	874	AGCUGGCACACCAGUCCAACA
824	UGUUGGACUGGUGUGCCAGCUGG	874	AGCUGGCACACCAGUCCAACA
825	UGUUGGACUGGUGUGCCAGCUG	875	GCUGGCACACCAGUCCAACA
826	AAAGGGUUUGUUGAACUUGUU	1273	CAAGUUCAACAAACCCUUU
827	AAAGGGUUUGUUGAACUUGAC	876	GUCAAGUUCAACAAACCCUUU
828	UAAGGGUUUGUUGAACUUGACCU	877	GUCAAGUUCAACAAACCCUUA
829	UAAGGGUUUGUUGAACUUGAC	877	GUCAAGUUCAACAAACCCUUA
830	UAUUGGUGCUGUUGGACUGUU	1274	CAGUCCAACAGCACCAAUA
831	UAUUGGUGCUGUUGGACUGGU	878	ACCAGUCCAACAGCACCAAUA
832	UAUUGGUGCUGUUGGACUGGUU	879	CCAGUCCAACAGCACCAAUA
833	UUGUUGGACUGGUGUGCCAG	880	CUGGCACACCAGUCCAACAA
834	UUGUUGGACUGGUGUGCCAGCU	880	CUGGCACACCAGUCCAACAA
835	UAUAGACAUGGGUAUGGCCUC	1275	GGCCAUACCCAUGUCUAUA
835	UAUAGACAUGGGUAUGGCCUC	881	GAGGCCAUACCCAUGUCUAUA
836	UCAAAGGGUUUGUUGAACUUGAC	882	GUCAAGUUCAACAAACCCUUUGA
836	UCAAAGGGUUUGUUGAACUUGAC	863	CAAGUUCAACAAACCCUUUGA
837	UUAUUGGUGCUGUUGGACUGG	883	CCAGUCCAACAGCACCAAUAA
838	UGUUAAACAUGCCUAAACGC	884	GCGUUUAGGCAUGUUUAACA
839	UGUUAAACAUGCCUAAACGCUU	884	GCGUUUAGGCAUGUUUAACA
839	UGUUAAACAUGCCUAAACGCUU	885	GCGUUUAGGCAUGUUUAACAUU
800	UGUUAAACAUGCCUAAACGCG	886	CGCGUUUAGGCAUGUUUAACAUU
801	UGUUAAACAUGCCUAAACGCU	887	AGCGUUUAGGCAUGUUUAACAUU
838	UGUUAAACAUGCCUAAACGC	885	GCGUUUAGGCAUGUUUAACAUU

The AAT RNAi agent sense strands and antisense strands that comprise or consist of the nucleotide sequences in Table 2 or Table 3 can be modified nucleotides or unmodified nucleotides. In some embodiments, the AAT RNAi agents having the sense and antisense strand sequences that comprise or consist of any of the nucleotide sequences in Table 2 or Table 3 are all or substantially all modified nucleotides.

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

As used herein, each N listed in a sequence disclosed in Table 2 may be independently selected. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is not complementary to the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is the same as the N nucleotide at the corresponding position on the other strand. In some embodiments, an N nucleotide listed in a sequence disclosed in Table 2 has a nucleobase that is different from the N nucleotide at the corresponding position on the other strand.

Certain modified AAT RNAi agent sense and antisense strands are provided in Table 4 and Table 5. Modified AAT RNAi agent antisense strands, as well as their underlying unmodified nucleobase sequences, are provided in Table 4. Modified AAT RNAi agent sense strands, as well as their underlying unmodified sequences, are provided in Table 5. In forming AAT RNAi agents, each of the nucleotides in each of the unmodified sequences listed in Tables 4 and 5, as well as in Table 2 and Table 3, above, can be a modified nucleotide.

The AAT RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2, Table 3, or Table 5, can be hybridized to any antisense strand containing a sequence listed in Table 2, Table 3, or Table 4, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

In some embodiments, an AAT RNAi agent antisense strand comprises a nucleotide sequence of any of the sequences in Table 2, Table 3, or Table 4.

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

Examples of antisense strands containing modified nucleotides are provided in Table 4. Examples of sense strands containing modified nucleotides are provided in Table 5.

As used in Tables 4 and 5, the following notations are used to indicate modified nucleotides, targeting groups, and linking groups. As the person of ordinary skill in the art would readily understand, unless otherwise indicated by the sequence, that when present in an oligonucleotide, the monomers are mutually linked by 5'-3'-phosphodiester bonds:

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

	Tf	=	2′-fluoro-5′-methyluridine-3′-phosphate
	Tfs	=	2′-fluoro-5′-methyluridine-3′-phosphorothioate
	Uf	=	2′-fluorouridine-3′-phosphate
	Ufs	=	2′-fluorouridine-3′-phosphorothioate
5	dN	=	any 2′-deoxyribonucleotide
	dT	=	2′-deoxythymidine-3′-phosphate
	N_{UNA}	=	2′,3′-seco nucleotide mimics (unlocked nucleobase analogs)-3′-Phosphate
	N_{UNAS}	=	2′,3′-seco nucleotide mimics (unlocked nucleobase analogs)-3′-
10			phosphorothioate
	U_{UNA}	=	2′,3′-seco-uridine-3′-phosphate
	U_{UNAS}	=	2′,3′-seco-uridine-3′-phosphorothioate
	a_2N	=	see Table 7
	a_2Ns	=	see Table 7
15	pu_2N	=	see Table 7
	pu_2Ns	=	see Table 7
	Npu	=	see Table 7
	Nus	=	see Table 7
	N_{LNA}	=	locked nucleotide
20	Nf_{ANA}	=	2'-F-Arabino nucleotide
	NM	=	2′-methoxyethyl nucleotide
	AM	=	2′-methoxyethyladenosine-3′-phosphate
	AMs	=	2′-methoxyethyladenosine-3′-phosphorothioate
	TM	=	2′-methoxyethylthymidine-3′-phosphate
25	TMs	=	2′-methoxyethylthymidine-3′-phosphorothioate
	R	=	ribitol
	(invdN)	=	any inverted deoxyribonucleotide (3′-3′ linked nucleotide)
	(invAb)	=	inverted (3′-3′ linked) abasic deoxyribonucleotide, see Table 7
	(invAb)s	=	inverted (3′-3′ linked) abasic deoxyribonucleotide-5′-
30			phosphorothioate, see Table 7
	(invn)	=	any inverted 2′-OMe nucleotide (3′-3′ linked nucleotide)
	s	=	phosphorothioate linkage
	vpdN	=	vinyl phosphonate deoxyribonucleotide
	(5Me-Nf)	=	5'-Me, 2'-fluoro nucleotide
35	cPrp	=	cyclopropyl phosphonate, see Table 7
	epTcPr	=	see Table 7
	epTM	=	see Table 7
	(Chol-TEG)	=	see Table 7
	(TEG-Biotin)	=	see Table 7
40	(PEG-C3-SS)	=	see Table 7
	(Alk-SS-C6)	=	see Table 7

(C6-SS-Alk) = see Table 7
(C6-SS-Alk-Me) = see Table 7

The person or 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*. Unless expressly indicated otherwise herein, such understandings of the person of ordinary skill in the art are used when describing the AAT RNAi agents and compositions of AAT RNAi agents disclosed herein.

Targeting groups and linking groups include the following, for which their chemical structures are provided below in Table 7: (PAZ), (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s. Each sense strand and/or antisense strand can have any targeting groups or linking groups listed above, as well as other targeting or linking groups, conjugated to the 5′ and/or 3′ end of the sequence.

Table 4. AAT RNAi Agent Antisense Strand Sequences

Antisense Strand ID:	SEQ ID NO.	Antisense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM00516-AS	888	dTGfgAfaCfUUNAUfgGfuGfaUfgAfuAfudTsdT	783	TGGAACUUGGUGAUGAUAUTT
AM02129-AS	889	dTsGfsgAfaCfUUNAUfgGfuGfaUfgAfuAfudTsdT	783	TGGAACUUGGUGAUGAUAUTT
AM02130-AS	890	dTsGfsgAfaCfUUNAUfgGfuGfaUfgAfuAfuCfgsusg	784	TGGAACUUGGUGAUGAUAUCGUG
AM04786-AS	891	aCfUUNAUfgGfuGfaUfgAfuAfudTsdT	785	ACUUGGUGAUGAUAUTT
AM05303-AS	892	dTdGdGdAdAdCdTdTdGdGdTdGdAdTdGdAdTdAdTdTsdT	786	TGGAACTTGGTGATGATATTT
AM05643-AS	893	usUfsusAfaAfcAfUfGfcCfuAfaAfcGfcusu	787	UUUAAACAUGCCUAAACGCUU
AM05645-AS	894	usGfscsAfuUfgCfCfCfaGfgUfaUfuUfcusu	788	UGCAUUGCCCAGGUAUUUCUU
AM05647-AS	895	usGfsgsAfaCfuUfGfGfuGfaUfgAfuAfuusu	789	UGGAACUUGGUGAUGAUAUUU
AM05649-AS	896	usGfsasUfcAfuAfGfGfuucCfaGfuAfausu	790	UGAUCAUAGGUUCCAGUAAUU
AM05651-AS	897	usAfscsAfgCfcUfUfAfuGfcAfcGfgCfcusu	791	UACAGCCUUAUGCACGGCCUU
AM05653-AS	898	usUfscsGfaUfgGfUfCfaGfcAfcAfgCfcusu	792	UUCGAUGGUCAGCACAGCCUU
AM05655-AS	899	usCfsasAfaGfgGfUfUfuGfuUfgAfaCfuusu	793	UCAAAGGGUUUGUUGAACUUU
AM05657-AS	900	usGfsusUfaAfaCfAfUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU
AM05659-AS	901	usUfuAfaAfcgugcCfuAfaAfcGfcsUfsg	795	UUUAAACGUGCCUAAACGCUG
AM05661-AS	902	usGfscAfuUfgcccaGfgUfaUfuUfcsAfsg	796	UGCAUUGCCCAGGUAUUUCAG
AM05663-AS	903	usGfsgAfaCfuugguGfaUfgAfuAfusCfsg	797	UGGAACUUGGUGAUGAUAUCG
AM05665-AS	904	usGfsaUfcAfuagguUfcCfaGfuAfasUfsg	798	UGAUCAUAGGUUCCAGUAAUG
AM05667-AS	905	usAfscAfgCfcuuauGfcAfcGfgCfcsUfsu	791	UACAGCCUUAUGCACGGCCUU
AM05669-AS	906	usUfscGfaUfggucaGfcAfcAfgCfcsUfsu	792	UUCGAUGGUCAGCACAGCCUU
AM05671-AS	907	usCfsaAfaGfgguuuGfuUfgAfaCfusUfsg	799	UCAAAGGGUUUGUUGAACUUG
AM05673-AS	908	usGfsuUfaAfacaugCfcUfaAfaCfgsCfsg	800	UGUUAAACAUGCCUAAACGCG
AM05677-AS	909	usUfsuAfaAfcgugcCfuAfaAfcGfcsUfsg	795	UUUAAACGUGCCUAAACGCUG
AM05884-AS	910	vpusGfsusUfaAfaCfAfUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU

Antisense Strand ID:	SEQ ID NO.	Antisense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM05885-AS	911	cPrpusGfsusUfaAfaCfAfUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU
AM05886-AS	912	usGfsusUfaAfaCfAfUfgCfcUfaAfaCfgcsu	801	UGUUAAACAUGCCUAAACGCU
AM05887-AS	913	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU
AM05888-AS	914	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcsu	801	UGUUAAACAUGCCUAAACGCU
AM05889-AS	915	usGfsusUfaAfaCfAfUfgCfcUfaAfaCfgCfsu	801	UGUUAAACAUGCCUAAACGCU
AM05890-AS	916	usGfsusUfaAfaCfAfUfgCfcUfaAfaCfgCfuusc	802	UGUUAAACAUGCCUAAACGCUUC
AM05891-AS	917	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgCfsu	801	UGUUAAACAUGCCUAAACGCU
AM05892-AS	918	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgCfuusc	802	UGUUAAACAUGCCUAAACGCUUC
AM05900-AS	919	cPrpusGfsuUfaAfacaugCfcUfaAfaCfgsCfsg	800	UGUUAAACAUGCCUAAACGCG
AM05901-AS	920	usGfsgsAfaCfUUNAUfGfGfuGfaUfgAfuAfuusu	789	UGGAACUUGGUGAUGAUAUUU
AM05954-AS	921	usGfscsUfgUfuggacUfgGfuGfuGfcusu	803	UGCUGUUGGACUGGUGUGCUU
AM05955-AS	922	usGfscsUfgUfuggacUfgGfuGfuGfccsa	804	UGCUGUUGGACUGGUGUGCCA
AM05956-AS	923	usGfscsUfgUfuggacUfgGfuGfuGfccausu	805	UGCUGUUGGACUGGUGUGCCAUU
AM05957-AS	924	usGfscsUfgUfuggacUfgGfuGfuGfccagsc	806	UGCUGUUGGACUGGUGUGCCAGC
AM05961-AS	925	usAfsasGfgCfuUfcUfgAfgUfgGfuAfcusu	807	UAAGGCUUCUGAGUGGUACUU
AM05962-AS	926	usAfsasGfgCfuUfcUfgAfgUfgGfuAfcasa	808	UAAGGCUUCUGAGUGGUACAA
AM05963-AS	927	usAfsasGfgCfuUfcUfgAfgUfgGfuAfcaacsu	809	UAAGGCUUCUGAGUGGUACAACU
AM05964-AS	928	gsAfsasGfgCfuUfcUfgAfgUfgGfuAfcusu	810	GAAGGCUUCUGAGUGGUACUU
AM05969-AS	929	asAfsgsAfcAfaAfgGfgUfuUfgUfuGfausu	811	AAGACAAAGGGUUUGUUGAUU
AM05970-AS	930	asAfsgsAfcAfaAfgGfgUfuUfgUfuGfaasc	812	AAGACAAAGGGUUUGUUGAAC
AM05973-AS	931	usAfsgsAfcAfaAfgGfgUfuUfgUfuGfaasc	813	UAGACAAAGGGUUUGUUGAAC
AM05974-AS	932	asAfsgsAfcAfaAfgGfgUfuUfgUfuGfaacusu	814	AAGACAAAGGGUUUGUUGAACUU
AM05976-AS	933	usAfsgsAfcAfuGfgGfuAfuGfgCfcUfcusu	815	UAGACAUGGGUAUGGCCUCUU
AM05977-AS	934	usAfsgsAfcAfuGfgGfuAfuGfgCfcUfcusa	816	UAGACAUGGGUAUGGCCUCUA
AM05979-AS	935	usAfsgsAfcAfuGfgGfuAfuGfgCfcUfcuaasa	817	UAGACAUGGGUAUGGCCUCUAAA
AM05980-AS	936	usAfsgsAfcAfuGfgGfuAfuGfgCfcUfcuausu	818	UAGACAUGGGUAUGGCCUCUAUU

Antisense Strand ID:	SEQ ID NO.	Antisense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM05982-AS	937	usUfsusGfaUfcUfgUfuUfcUfuGfgCfcusu	819	UUUGAUCUGUUUCUUGGCCUU
AM05983-AS	938	usUfsusGfaUfcUfgUfuUfcUfuGfgCfcusc	820	UUUGAUCUGUUUCUUGGCCUC
AM05985-AS	939	usUfsusGfaUfcUfgUfuUfcUfuGfgCfcucusu	821	UUUGAUCUGUUUCUUGGCCUCUU
AM05987-AS	940	usGfsusUfgGfacuggUfgUfgCfcAfgusu	822	UGUUGGACUGGUGUGCCAGUU
AM05989-AS	941	usGfsusUfgGfacuggUfgUfgCfcAfgcsu	823	UGUUGGACUGGUGUGCCAGCU
AM05990-AS	942	usGfsusUfgGfacuggUfgUfgCfcAfgcugsg	824	UGUUGGACUGGUGUGCCAGCUGG
AM05992-AS	943	usGfsusUfgGfacuggUfgUfgCfcAfgcusg	825	UGUUGGACUGGUGUGCCAGCUG
AM05994-AS	944	asAfsasGfgGfuUfuGfuUfgAfaCfuUfgusu	826	AAAGGGUUUGUUGAACUUGUU
AM05996-AS	945	asAfsasGfgGfuUfuGfuUfgAfaCfuUfgasc	827	AAAGGGUUUGUUGAACUUGAC
AM05998-AS	946	usAfsasGfgGfuUfuGfuUfgAfaCfuUfgaccsu	828	UAAGGGUUUGUUGAACUUGACCU
AM05999-AS	947	usAfsasGfgGfuUfuGfuUfgAfaCfuUfgasc	829	UAAGGGUUUGUUGAACUUGAC
AM06124-AS	948	usAfsusUfgGfuGfcUfgUfuGfgAfcUfgusu	830	UAUUGGUGCUGUUGGACUGUU
AM06125-AS	949	usAfsusUfgGfuGfcUfgUfuGfgAfcUfggsu	831	UAUUGGUGCUGUUGGACUGGU
AM06126-AS	950	usAfsusUfgGfuGfcUfgUfuGfgAfcUfggusu	832	UAUUGGUGCUGUUGGACUGGUU
AM06130-AS	951	usUfsgsUfuGfgacugGfuGfuGfcCfasg	833	UUGUUGGACUGGUGUGCCAG
AM06131-AS	952	usUfsgsUfuGfgacugGfuGfuGfcCfagcsu	834	UUGUUGGACUGGUGUGCCAGCU
AM06133-AS	953	usAfsusAfgAfcAfuGfgGfuAfuGfgCfcusc	835	UAUAGACAUGGGUAUGGCCUC
AM06134-AS	954	usAfsusAfgAfcauggGfuAfuGfgCfcusc	835	UAUAGACAUGGGUAUGGCCUC
AM06137-AS	955	usCfsasAfaGfgGfuUfuGfuUfgAfaCfuugasc	836	UCAAAGGGUUUGUUGAACUUGAC
AM06140-AS	956	usUfsasUfuGfgugcuGfuUfgGfaCfugsg	837	UUAUUGGUGCUGUUGGACUGG
AM06227-AS	957	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgsc	838	UGUUAAACAUGCCUAAACGC
AM06228-AS	958	usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcusu	839	UGUUAAACAUGCCUAAACGCUU
AM06234-AS	959	usGfsuUfaAfaCfaUfgCfcUfaAfaCfgsCfsg	800	UGUUAAACAUGCCUAAACGCG
AM06235-AS	960	usGfsuUfaAfacaugCfcUfaAfaCfgCfsu	801	UGUUAAACAUGCCUAAACGCU
AM06237-AS	961	usGfsuUfaAfaCfAfUfgCfcUfaAfaCfgsCfsg	800	UGUUAAACAUGCCUAAACGCG
AM06238-AS	962	NpusGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU

Antisense Strand ID:	SEQ ID NO.	Antisense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM06261-AS	963	NusGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu	794	UGUUAAACAUGCCUAAACGUU

Table 5. AAT RNAi Agent Sense Strand Sequences

Sense Strand ID:	SEQ ID NO.	Sense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM01887-SS	964	(Chol-TEG)uAuAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(invdT)(TEG-Biotin)	845	UAUAUAUCAUCACCAAGUUCCAT
AM01888-SS	965	(Chol-TEG)uAuAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(invdT)(PEG-C3-SS)	845	UAUAUAUCAUCACCAAGUUCCAT
AM01855-SS	966	(Alk-SS-C6)AfuAfuCfaUfcAfcCfaAfgUfuCfcAf(invdT)	846	AUAUCAUCACCAAGUUCCAT
AM02132-SS	967	CfsgsAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(C6-SS-Alk)	847	CGAUAUCAUCACCAAGUUCCA
AM02390-SS	968	CfsgsAfuAfuCfaUfcAfcCfaAfgUfuCfcAf(C6-SS-Alk-Me)	847	CGAUAUCAUCACCAAGUUCCA
AM04785-SS	969	uAfuCfaUfcAfcCfaAfgUfuCfcAf(invdT)	848	UAUCAUCACCAAGUUCCAT
AM05304-SS	970	(Chol-TEG)dTdAdTdAdTdAdTdCdAdTdCdAdCdCdAdAdGdTdTdCdCsdA(invdT)(TEG-Biotin)	849	TATATATCATCACCAAGTTCCAT
AM05599-SS	971	(Chol-TEG)dTdAdTdAdTdAdTdCdAdTdCdAdCdCdAdAdGdTdTdCdCsdA(invdT)	849	TATATATCATCACCAAGTTCCAT
AM05642-SS	972	(NAG25)(invAb)GfcGfuUfuAfGfGfcAfuGfuUfuAfaausu(invAb)	850	GCGUUUAGGCAUGUUUAAAUU
AM05644-SS	973	(NAG25)(invAb)GfaAfaUfaCfCfUfgGfgCfaAfuGfcausu(invAb)	851	GAAAUACCUGGGCAAUGCAUU
AM05646-SS	974	(NAG25)(invAb)AfuAfuCfaUfCfAfcCfaAfgUfuCfcausu(invAb)	852	AUAUCAUCACCAAGUUCCAUU
AM05648-SS	975	(NAG25)(invAb)UfuAfcUfgGfAfAfcCfuAfuGfaUfcausu(invAb)	853	UUACUGGAACCUAUGAUCAUU
AM05650-SS	976	(NAG25)(invAb)GfgCfcGfuGfCfAfuAfaGfgCfuGfuausu(invAb)	854	GGCCGUGCAUAAGGCUGUAUU
AM05652-SS	977	(NAG25)(invAb)GfgCfuGfuGfCfUfgAfcCfaUfcGfaausu(invAb)	855	GGCUGUGCUGACCAUCGAAUU

Sense Strand ID:	SEQ ID NO.	Sense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM05654-SS	978	(NAG25)(invAb)AfgUfuCfaAfCfAfaAfcCfcUfuUfgausu(invAb)	856	AGUUCAACAAACCCUUUGAUU
AM05656-SS	979	(NAG25)(invAb)CfgUfuUfaGfGfCfaUfgUfuUfaAfcausu(invAb)	857	CGUUUAGGCAUGUUUAACAUU
AM05658-SS	980	(NAG25)scsagcguuuAfGfGfcauguuuaasa(invAb)	858	CAGCGUUUAGGCAUGUUUAAA
AM05660-SS	981	(NAG25)scsugaaauaCfCfUfgggcaaugcsa(invAb)	859	CUGAAAUACCUGGGCAAUGCA
AM05662-SS	982	(NAG25)scsgauaucaUfCfAfccaaguuccsa(invAb)	847	CGAUAUCAUCACCAAGUUCCA
AM05664-SS	983	(NAG25)scsauuacugGfAfAfccuaugaucsa(invAb)	860	CAUUACUGGAACCUAUGAUCA
AM05666-SS	984	(NAG25)sasaggccguGfCfAfuaaggcugusa(invAb)	861	AAGGCCGUGCAUAAGGCUGUA
AM05668-SS	985	(NAG25)sasaggcuguGfCfUfgaccaucgasa(invAb)	862	AAGGCUGUGCUGACCAUCGAA
AM05670-SS	986	(NAG25)scsaaguucaAfCfAfaacccuuugsa(invAb)	863	CAAGUUCAACAAACCCUUUGA
AM05672-SS	987	(NAG25)scsgcguuuaGfGfCfauguuuaacsa(invAb)	864	CGCGUUUAGGCAUGUUUAACA
AM05658-SS	988	(NAG25)scsagcguuuAfGfGfcauguuuaasa(invAb)	858	CAGCGUUUAGGCAUGUUUAAA
AM05893-SS	989	(NAG25)s(invAb)scguuuaGfGfCfauguuuaacausu(invAb)	857	CGUUUAGGCAUGUUUAACAUU
AM05894-SS	990	(NAG25)s(invAb)sCfgUfuUfaGfGfCfaUfgUfuUfaAfcas(invAb)	429	CGUUUAGGCAUGUUUAACA
AM05895-SS	991	(NAG25)s(invAb)scguuuaGfGfCfauguuuaacas(invAb)	429	CGUUUAGGCAUGUUUAACA
AM05896-SS	992	(NAG25)s(invAb)saaCfgUfuUfaGfGfCfaUfgUfuUfaAfcas(invAb)	865	AACGUUUAGGCAUGUUUAACA
AM05897-SS	993	(NAG25)s(invAb)sagCfgUfuUfaGfGfCfaUfgUfuUfaAfcas(invAb)	866	AGCGUUUAGGCAUGUUUAACA
AM05898-SS	994	(NAG25)s(invAb)saacguuuaGfGfCfauguuuaacas(invAb)	865	AACGUUUAGGCAUGUUUAACA
AM05899-SS	995	(NAG25)s(invAb)sagcguuuaGfGfCfauguuuaacas(invAb)	866	AGCGUUUAGGCAUGUUUAACA
AM05958-SS	996	(NAG37)s(invAb)sgcacacCfAfGfuccaacagcas(invAb)	454	GCACACCAGUCCAACAGCA
AM05959-SS	997	(NAG37)s(invAb)suggcacacCfAfGfuccaacagcas(invAb)	867	UGGCACACCAGUCCAACAGCA
AM05960-SS	998	(NAG37)s(invAb)saagcacacCfAfGfuccaacagcas(invAb)	868	AAGCACACCAGUCCAACAGCA
AM05965-SS	999	(NAG37)s(invAb)sguaccaCfUfCfagaagccuuas(invAb)	519	GUACCACUCAGAAGCCUUA
AM05966-SS	1000	(NAG37)s(invAb)suuguaccaCfUfCfagaagccuuas(invAb)	869	UUGUACCACUCAGAAGCCUUA
AM05967-SS	1001	(NAG37)s(invAb)sguaccaCfUfCfagaagccuucs(invAb)	518	GUACCACUCAGAAGCCUUC
AM05968-SS	1002	(NAG37)s(invAb)sucaacaAfAfCfccuuugucuus(invAb)	738	UCAACAAACCCUUUGUCUU

Sense Strand ID:	SEQ ID NO.	Sense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM05971-SS	1003	(NAG37)s(invAb)sguucaacaAfAfCfccuuugucuus(invAb)	870	GUUCAACAAACCCUUUGUCUU
AM05972-SS	1004	(NAG37)s(invAb)sguucaacaAfAfCfccuuugucuas(invAb)	871	GUUCAACAAACCCUUUGUCUA
AM05975-SS	1005	(NAG37)s(invAb)sgaggccAfUfAfcccaugucuas(invAb)	707	GAGGCCAUACCCAUGUCUA
AM05978-SS	1006	(NAG37)s(invAb)suagaggccAfUfAfcccaugucuas(invAb)	872	UAGAGGCCAUACCCAUGUCUA
AM05981-SS	1007	(NAG37)s(invAb)sggccaaGfAfAfacagaucaaas(invAb)	537	GGCCAAGAAACAGAUCAAA
AM05984-SS	1008	(NAG37)s(invAb)sgaggccaaGfAfAfacagaucaaas(invAb)	873	GAGGCCAAGAAACAGAUCAAA
AM05986-SS	1009	(NAG37)s(invAb)scuggcaCfAfCfcaguccaacas(invAb)	445	CUGGCACACCAGUCCAACA
AM05988-SS	1010	(NAG37)s(invAb)sagcuggcaCfAfCfcaguccaacas(invAb)	874	AGCUGGCACACCAGUCCAACA
AM05991-SS	1011	(NAG37)s(invAb)sgcuggcaCfAfCfcaguccaacas(invAb)	875	GCUGGCACACCAGUCCAACA
AM05993-SS	1012	(NAG37)s(invAb)scaaguuCfAfAfcaaacccuuus(invAb)	725	CAAGUUCAACAAACCCUUU
AM05995-SS	1013	(NAG37)s(invAb)sgucaaguuCfAfAfcaaacccuuus(invAb)	876	GUCAAGUUCAACAAACCCUUU
AM05997-SS	1014	(NAG37)s(invAb)sgucaaguuCfAfAfcaaacccuuas(invAb)	877	GUCAAGUUCAACAAACCCUUA
AM06127-SS	1015	(NAG37)s(invAb)scaguccAfAfCfagcaccaauas(invAb)	458	CAGUCCAACAGCACCAAUA
AM06128-SS	1016	(NAG37)s(invAb)saccaguccAfAfCfagcaccaauas(invAb)	878	ACCAGUCCAACAGCACCAAUA
AM06129-SS	1017	(NAG37)s(invAb)sccaguccAfAfCfagcaccaauas(invAb)	879	CCAGUCCAACAGCACCAAUA
AM06132-SS	1018	(NAG37)s(invAb)scuggcacAfCfCfaguccaacaas(invAb)	880	CUGGCACACCAGUCCAACAA
AM06135-SS	1019	(NAG37)s(invAb)sggccauAfCfCfcaugucuauas(invAb)	712	GGCCAUACCCAUGUCUAUA
AM06136-SS	1020	(NAG37)s(invAb)sgaggccauAfCfCfcaugucuauas(invAb)	881	GAGGCCAUACCCAUGUCUAUA
AM06138-SS	1021	(NAG37)s(invAb)sgucaaguucaAfCfAfaacccuuugas(invAb)	882	GUCAAGUUCAACAAACCCUUUGA
AM06139-SS	1022	(NAG37)s(invAb)scaaguucaAfCfAfaacccuuugas(invAb)	863	CAAGUUCAACAAACCCUUUGA
AM06141-SS	1023	(NAG37)s(invAb)sccaguccaAfCfAfgcaccaauaas(invAb)	883	CCAGUCCAACAGCACCAAUAA
AM06195-SS	1024	(NAG37)s(invAb)scgcguuuaGfGfCfauguuuaacas(invAb)	864	CGCGUUUAGGCAUGUUUAACA
AM06223-SS	1025	(NAG37)s(invAb)scguuuaGfGfCfauguuuaacas(invAb)	429	CGUUUAGGCAUGUUUAACA
AM06224-SS	1026	(NAG37)s(invAb)scsgcguuuaGfGfCfauguuuaacsa(invAb)	864	CGCGUUUAGGCAUGUUUAACA
AM06225-SS	1027	(NAG37)s(invAb)sagCfgUfuUfaGfGfCfaUfgUfuUfaAfcas(invAb)	866	AGCGUUUAGGCAUGUUUAACA

Sense Strand ID:	SEQ ID NO.	Sense Sequence (Modified) (5' → 3')	SEQ ID NO.	Underlying Base Sequence (5' → 3')
AM06226-SS	1028	(NAG37)s(invAb)scguuuaGfGfCfauguuuaacausu(invAb)	857	CGUUUAGGCAUGUUUAACAUU
AM06229-SS	1029	(NAG37)s(invAb)sgcguuuaGfGfCfauguuuaacas(invAb)	884	GCGUUUAGGCAUGUUUAACA
AM06230-SS	1030	(NAG37)s(invAb)sgcguuuaGfGfCfauguuuaacausu(invAb)	885	GCGUUUAGGCAUGUUUAACAUU
AM06231-SS	1031	(NAG37)s(invAb)scgcguuuaGfGfCfauguuuaacsausu(invAb)	886	CGCGUUUAGGCAUGUUUAACAUU
AM06232-SS	1032	(NAG37)s(invAb)sagCfgUfuUfaGfGfCfaUfgUfuUfaAfcausu(invAb)	887	AGCGUUUAGGCAUGUUUAACAUU
AM06236-SS	1033	(NAG37)s(invAb)sagcguuuaGfGfCfauguuuaacas(invAb)	866	AGCGUUUAGGCAUGUUUAACA
AM06239-SS	1034	(NAG37)s(invAb)scgcguuuaGfGfCfauguuuaacsas(invAb)	864	CGCGUUUAGGCAUGUUUAACA

The AAT RNAi agents described herein are formed by annealing an antisense strand with a sense strand. A sense strand containing a sequence listed in Table 2, Table 3, or Table 5 can be hybridized to any antisense strand containing a sequence listed in Table 2, Table 3, or Table 4, provided the two sequences have a region of at least 85% complementarity over a contiguous 16, 17, 18, 19, 20, or 21 nucleotide sequence.

In some embodiments, the antisense strand of an AAT RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the antisense strand sequences in Table 4. In some embodiments, the sense strand of an AAT RNAi agent disclosed herein differs by 0, 1, 2, or 3 nucleotides from any of the sense strand sequences in Table 5.

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

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

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

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

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

A sense strand containing a sequence listed in Table 2, Table 3, or Table 5 can be hybridized to any antisense strand containing a sequence listed in Table 2, Table 3, or Table 5, 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 AAT RNAi agent has a sense strand consisting of the modified sequence of any of the modified sequences in Table 5, and an antisense strand consisting of the modified sequence of any of the modified sequences in Table 4. Representative sequence pairings are exemplified by the Duplex ID Nos. shown in Table 6.

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

of the Duplex ID Nos. presented herein and a targeting group and/or linking group, wherein the targeting group and/or linking group is covalently linked to the sense strand or the antisense strand.

In some embodiments, an AAT RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Table 2, Table 3, or Table 6, and comprises an asialoglycoprotein receptor ligand targeting group.

In some embodiments, an AAT RNAi agent comprises an antisense strand and a sense strand having the nucleotide sequences of any of the antisense strand/sense strand duplexes of Table 2 or Table 5, and further comprises a targeting group selected from the group consisting of (PAZ), (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s. In some embodiments, the targeting group is (NAG25) or (NAG25)s as defined in Table 7. In other embodiments, the targeting group is (NAG37) or (NAG37)s as defined in Table 7.

In some embodiments, an AAT 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 of any of the duplexes of Table 6.

In some embodiments, an AAT RNAi agent comprises an antisense strand and a sense strand having a modified nucleotide sequence of any of the antisense strand and/or sense strand nucleotide sequences of any of the duplexes of Table 6, and comprises an asialoglycoprotein receptor ligand targeting group.

In some embodiments, an AAT RNAi agent comprises the duplex structure of any of the duplexes in Table 6.

In some embodiments, an AAT RNAi agent consists of the duplex structure of any of the duplexes in Table 6.

Table 6. AAT RNAi Agents Identified by Duplex ID No. with Corresponding Sense and Antisense Strands

Duplex ID	Antisense Strand ID	Sense Strand ID
AD01131	AM00516-AS	AM01887-SS
AD01132	AM00516-AS	AM01888-SS
AD01174	AM00516-AS	AM01855-SS
AD01286	AM02129-AS	AM01855-SS
AD01287	AM02130-AS	AM02132-SS
AD01442	AM02130-AS	AM02390-SS
AD03752	AM04786-AS	AM04785-SS
AD04156	AM05303-AS	AM05304-SS
AD04406	AM05303-AS	AM05599-SS
AD04444	AM05643-AS	AM05642-SS
AD04445	AM05645-AS	AM05644-SS
AD04446	AM05647-AS	AM05646-SS
AD04447	AM05649-AS	AM05648-SS
AD04448	AM05651-AS	AM05650-SS
AD04449	AM05653-AS	AM05652-SS
AD04450	AM05655-AS	AM05654-SS
AD04451	AM05657-AS	AM05656-SS
AD04452	AM05659-AS	AM05658-SS
AD04453	AM05661-AS	AM05660-SS
AD04454	AM05663-AS	AM05662-SS
AD04455	AM05665-AS	AM05664-SS
AD04456	AM05667-AS	AM05666-SS
AD04457	AM05669-AS	AM05668-SS
AD04458	AM05671-AS	AM05670-SS
AD04459	AM05673-AS	AM05672-SS
AD04464	AM05677-AS	AM05658-SS
AD04601	AM05884-AS	AM05656-SS
AD04602	AM05885-AS	AM05656-SS
AD04603	AM05886-AS	AM05656-SS
AD04604	AM05887-AS	AM05893-SS
AD04605	AM05888-AS	AM05893-SS
AD04606	AM05657-AS	AM05894-SS
AD04607	AM05886-AS	AM05894-SS
AD04608	AM05887-AS	AM05895-SS
AD04609	AM05888-AS	AM05895-SS
AD04610	AM05657-AS	AM05896-SS
AD04611	AM05889-AS	AM05897-SS
AD04612	AM05890-AS	AM05897-SS
AD04613	AM05887-AS	AM05898-SS
AD04614	AM05891-AS	AM05899-SS
AD04615	AM05892-AS	AM05899-SS
AD04616	AM05900-AS	AM05672-SS
AD04617	AM05901-AS	AM05646-SS
AD04652	AM05954-AS	AM05958-SS
AD04653	AM05955-AS	AM05958-SS
AD04654	AM05955-AS	AM05959-SS
AD04655	AM05954-AS	AM05960-SS
AD04656	AM05956-AS	AM05959-SS
AD04657	AM05957-AS	AM05959-SS
AD04658	AM05961-AS	AM05965-SS
AD04659	AM05962-AS	AM05965-SS
AD04660	AM05962-AS	AM05966-SS
AD04661	AM05963-AS	AM05966-SS
AD04662	AM05964-AS	AM05967-SS
AD04663	AM05969-AS	AM05968-SS
AD04664	AM05970-AS	AM05968-SS

Duplex ID	Antisense Strand ID	Sense Strand ID
AD04665	AM05970-AS	AM05971-SS
AD04666	AM05973-AS	AM05972-SS
AD04667	AM05974-AS	AM05971-SS
AD04668	AM05976-AS	AM05975-SS
AD04669	AM05977-AS	AM05975-SS
AD04670	AM05977-AS	AM05978-SS
AD04671	AM05979-AS	AM05978-SS
AD04672	AM05980-AS	AM05978-SS
AD04673	AM05982-AS	AM05981-SS
AD04674	AM05983-AS	AM05981-SS
AD04675	AM05983-AS	AM05984-SS
AD04676	AM05985-AS	AM05984-SS
AD04677	AM05987-AS	AM05986-SS
AD04678	AM05989-AS	AM05988-SS
AD04679	AM05990-AS	AM05988-SS
AD04680	AM05992-AS	AM05991-SS
AD04681	AM05994-AS	AM05993-SS
AD04682	AM05996-AS	AM05995-SS
AD04683	AM05998-AS	AM05997-SS
AD04684	AM05999-AS	AM05997-SS
AD04761	AM06124-AS	AM06127-SS
AD04762	AM06125-AS	AM06128-SS
AD04763	AM06126-AS	AM06129-SS
AD04764	AM06130-AS	AM06132-SS
AD04765	AM06131-AS	AM06132-SS

Duplex ID	Antisense Strand ID	Sense Strand ID
AD04766	AM06133-AS	AM06135-SS
AD04767	AM06134-AS	AM06136-SS
AD04768	AM06137-AS	AM06138-SS
AD04769	AM06137-AS	AM06139-SS
AD04770	AM06140-AS	AM06141-SS
AD04805	AM05673-AS	AM06195-SS
AD04824	AM05887-AS	AM06223-SS
AD04825	AM05900-AS	AM06224-SS
AD04826	AM05889-AS	AM06225-SS
AD04827	AM05888-AS	AM06223-SS
AD04828	AM05887-AS	AM06226-SS
AD04829	AM06227-AS	AM06229-SS
AD04830	AM06228-AS	AM06229-SS
AD04831	AM06228-AS	AM06230-SS
AD04832	AM05673-AS	AM06231-SS
AD04833	AM05889-AS	AM06232-SS
AD04834	AM06227-AS	AM06230-SS
AD04836	AM06234-AS	AM06195-SS
AD04837	AM06235-AS	AM06236-SS
AD04838	AM06237-AS	AM06239-SS
AD04839	AM05673-AS	AM06239-SS
AD04840	AM06238-AS	AM06223-SS
AD04857	AM06261-AS	AM06223-SS

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

Targeting Groups, Linking Groups, and Delivery Vehicles

In some embodiments, an AAT RNAi agent is conjugated to one or more non-nucleotide groups including, but not limited to, a targeting group, linking group, delivery polymer, or a delivery vehicle. The non-nucleotide group can enhance targeting, delivery or attachment of the RNAi agent. Examples of targeting groups and linking groups are provided in Table 7. 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 AAT 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 AAT 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.

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

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

The AAT RNAi agents described herein can be synthesized having a reactive group, such as an amine group, at the 5′-terminus. The reactive group can be used to subsequently attach a targeting group using methods typical in the art.

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

Galactose derivatives have been used to target molecules to hepatocytes *in vivo* through their binding to the asialoglycoprotein receptor expressed on the surface of hepatocytes. Binding of asialoglycoprotein receptor ligands to the asialoglycoprotein receptor(s) facilitates cell-specific targeting to hepatocytes and endocytosis of the molecule into hepatocytes. Asialoglycoprotein receptor ligands can be monomeric (e.g., having a single galactose derivative) or multimeric (e.g., having multiple galactose derivatives). The galactose derivative or galactose derivative cluster can be attached to the 3′ or 5′ end of the sense or antisense strand of the RNAi agent using methods known in the art. The preparation of targeting groups, such as galactose derivative clusters, is described in, for example, U.S. Patent Application Serial No. 15/452,324 and U.S. Patent Publication No. US 2017/0253875, the contents of both of which are incorporated by reference herein in their entirety.

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

derivative tetramer (also referred to as tetra-antennary galactose derivative or tetra-valent galactose derivative). In some embodiments, the galactose derivative cluster comprises four N-acetyl-galactosamines.

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

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

Targeting groups include, but are not limited to, (PAZ), (NAG13), (NAG13)s, (NAG18), (NAG18)s, (NAG24), (NAG24)s, (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35),

(NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), and (NAG39)s as defined in Table 7. Other targeting groups, including galactose cluster targeting ligands, are known in the art.

In some embodiments, a linking group is conjugated to the RNAi agent. The linking group facilitates covalent linkage of the agent to a targeting group or delivery polymer or delivery vehicle. The linking group can be linked to the 3′ or the 5′ end of the RNAi agent sense strand or antisense strand. In some embodiments, the linking group is linked to the RNAi agent sense strand. In some embodiments, the linking group is conjugated to the 5′ or 3′ end of an RNAi agent sense strand. In some embodiments, a linking group is conjugated to the 5′ end of an RNAi agent sense strand. Examples of linking groups, can include, but are not limited to: reactive groups such a primary amines and alkynes, alkyl groups, abasic nucleotides, ribitol (abasic ribose), and/or PEG groups.

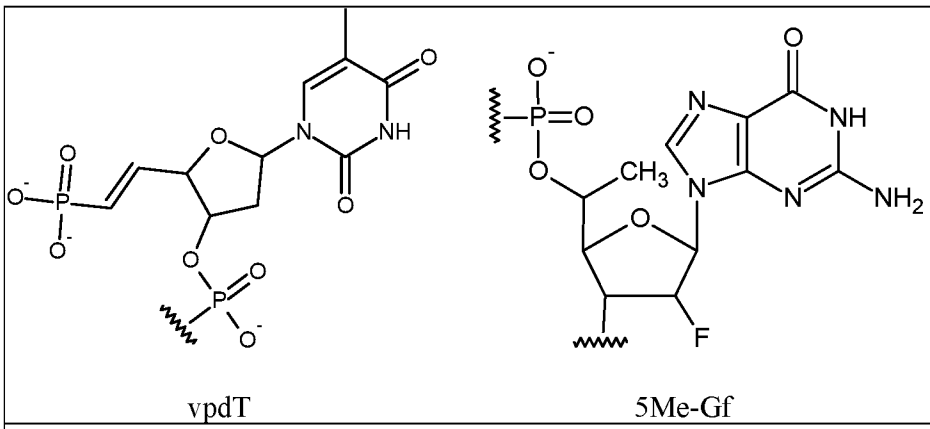
A linker or linking group is a connection between two atoms that links one chemical group (such as an RNAi agent) or segment of interest to another chemical group (such as a targeting group or delivery polymer) or segment of interest via one or more covalent bonds. A labile linkage contains a labile bond. A linkage may optionally include a spacer that increases the distance between the two joined atoms. A spacer can further add flexibility and/or length to the linkage. Spacers can include, but are not be limited to, alkyl groups, alkenyl groups, alkynyl groups, aryl groups, aralkyl groups, aralkenyl groups, and aralkynyl groups; each of which can contain one or more heteroatoms, heterocycles, amino acids, nucleotides, and saccharides. Spacer groups are well known in the art and the preceding list is not meant to limit the scope of the description.

Any of the AAT RNAi agent nucleotide sequences listed in Tables 2, 3, 4, or 5, whether modified or unmodified, may contain 3′ or 5′ targeting groups or linking groups. Any of the AAT RNAi agent sequences listed in Tables 4 or 5 which contain a 3′ or 5′ targeting group or linking group, may alternatively contain no 3′ or 5′ targeting group or linking group, or may contain a different 3′ or 5′ targeting group or linking group including, but not limited to, those depicted in Table 7. Any of the AAT RNAi agent duplexes listed in Table 2, Table 3, or Table 6, whether modified or unmodified, may further comprise a targeting group or linking group, including, but not limited to, those depicted in Table 7, and the targeting

group or linking group may be attached to the 3′ or 5′ terminus of either the sense strand or the antisense strand of the AAT RNAi agent duplex.

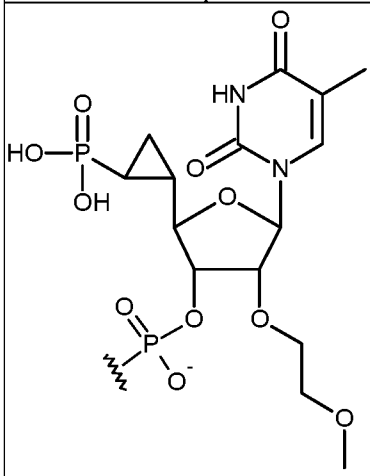
Examples of targeting groups and linking groups are provided in Table 7. Table 5 provides several embodiments of AAT RNAi agent sense strands having a targeting group or linking group linked to the 5′ or 3′ end.

Table 7. Structures Representing Various Modified Nucleotides, Targeting Groups, and Linking Groups

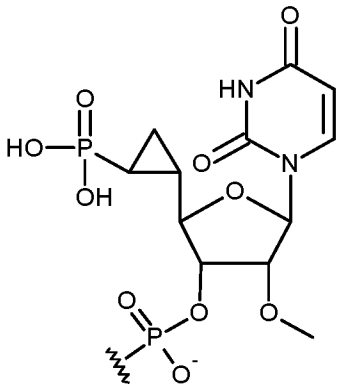


vpdT

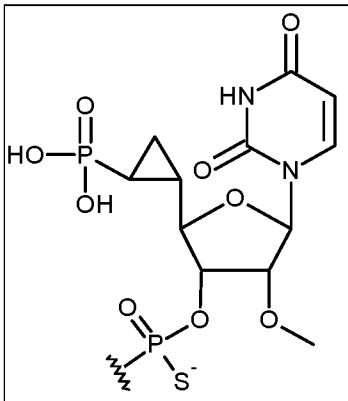
5Me-Gf



cPrpTM



(cPrp)u



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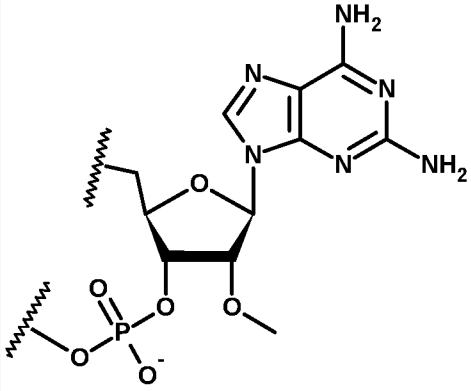

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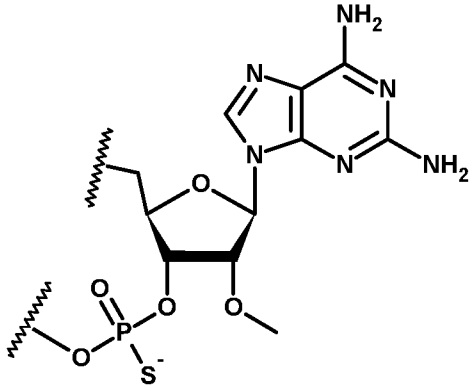
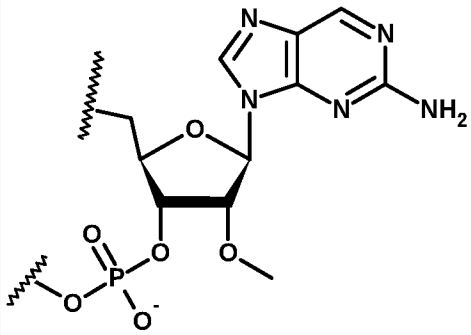
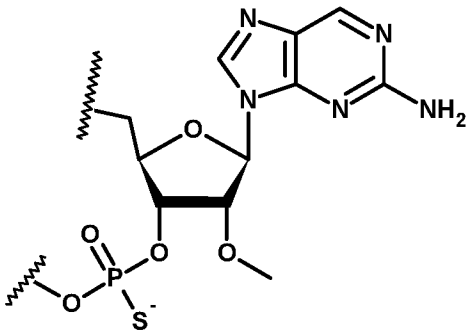


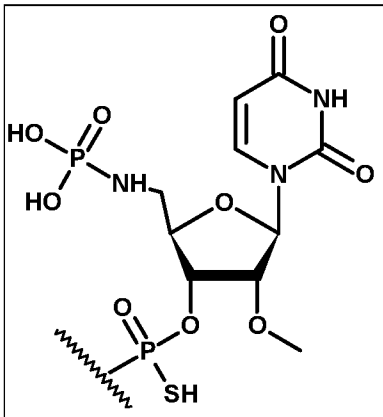
a_2Ns



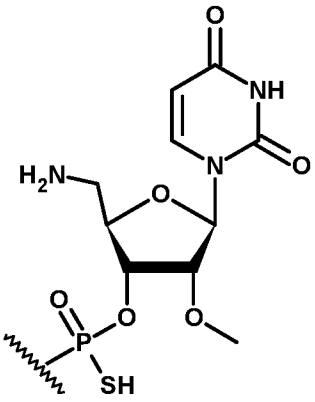
pu_2N



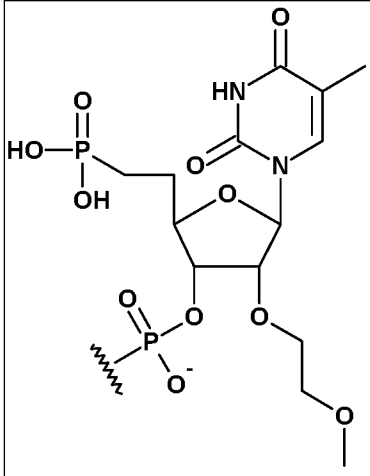
pu_2Ns



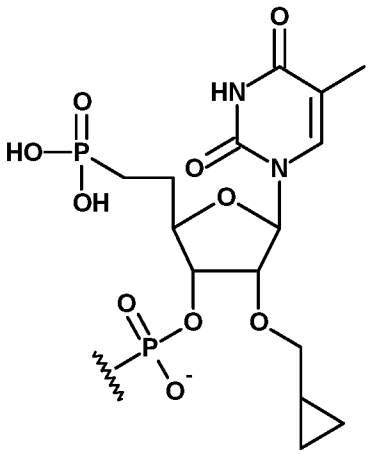
Npus



Nus

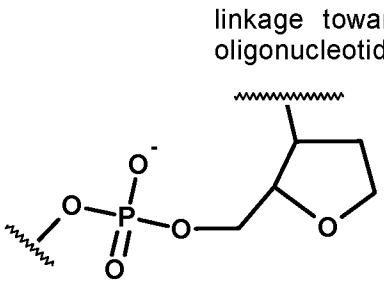


epTM



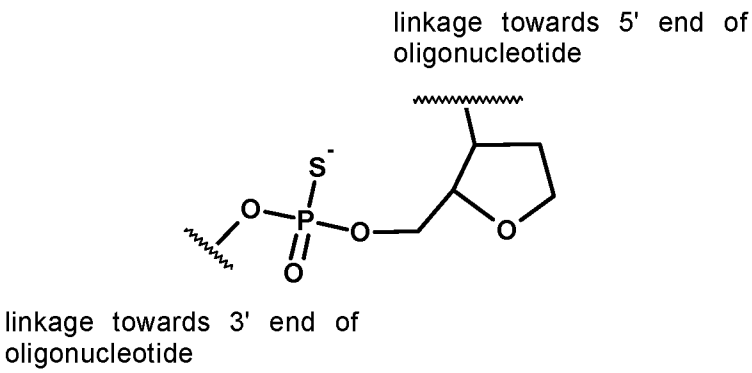
epTcPr

When positioned internally on oligonucleotide:



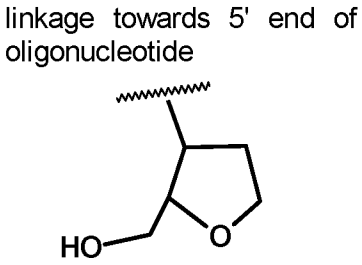
(invAb)

When positioned internally on oligonucleotide:

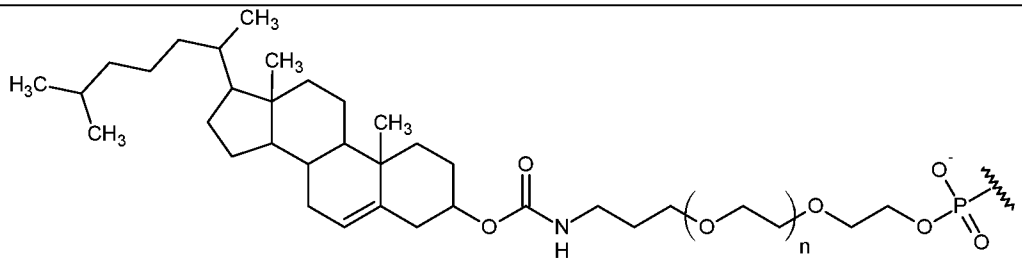


(invAb)s

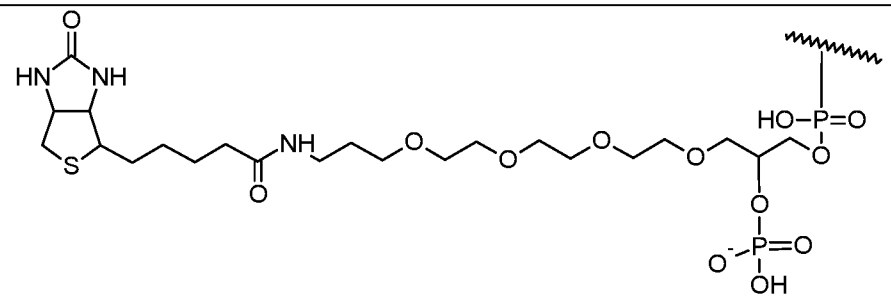
When positioned at the 3′ terminal end of oligonucleotide:



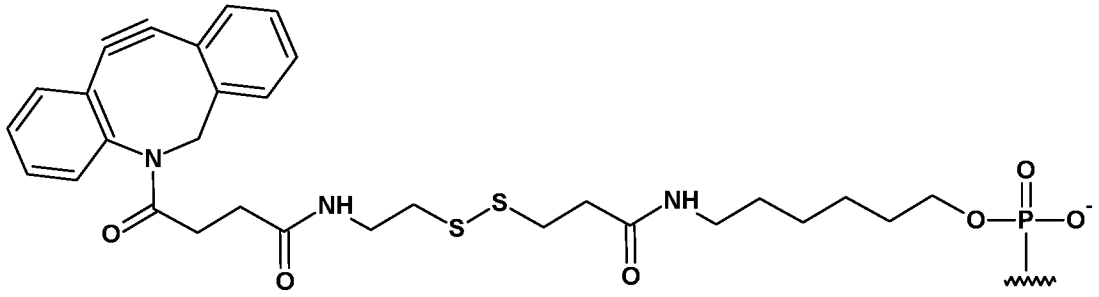
(invAb)



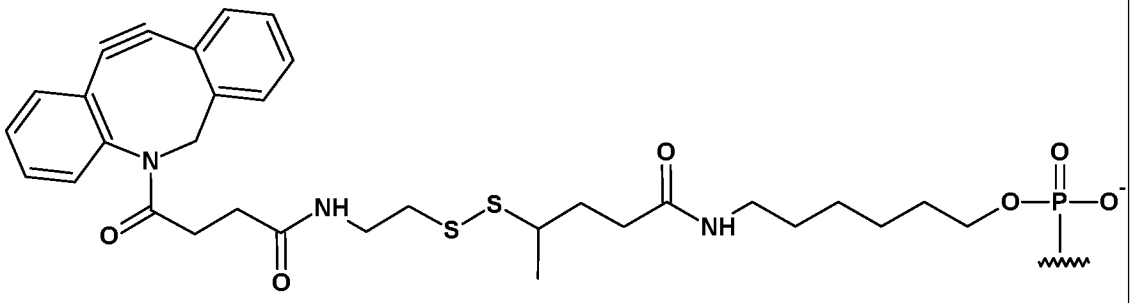
(Chol-TEG)



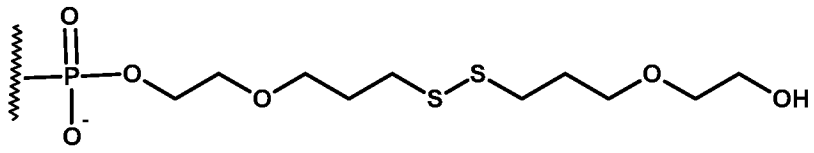
(TEG-Biotin)



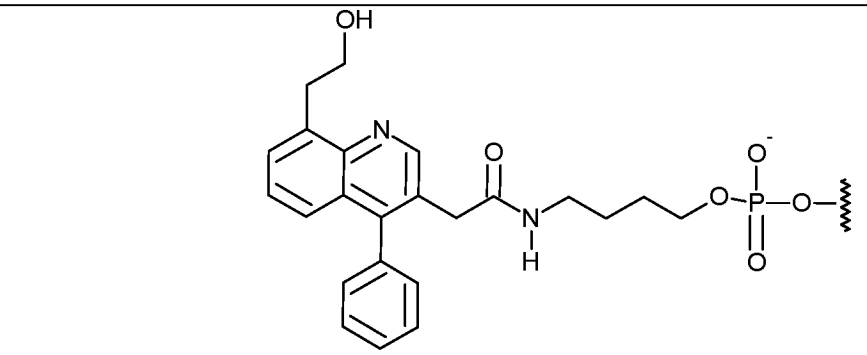
(C6-SS-Alk) or (Alk-SS-C6)



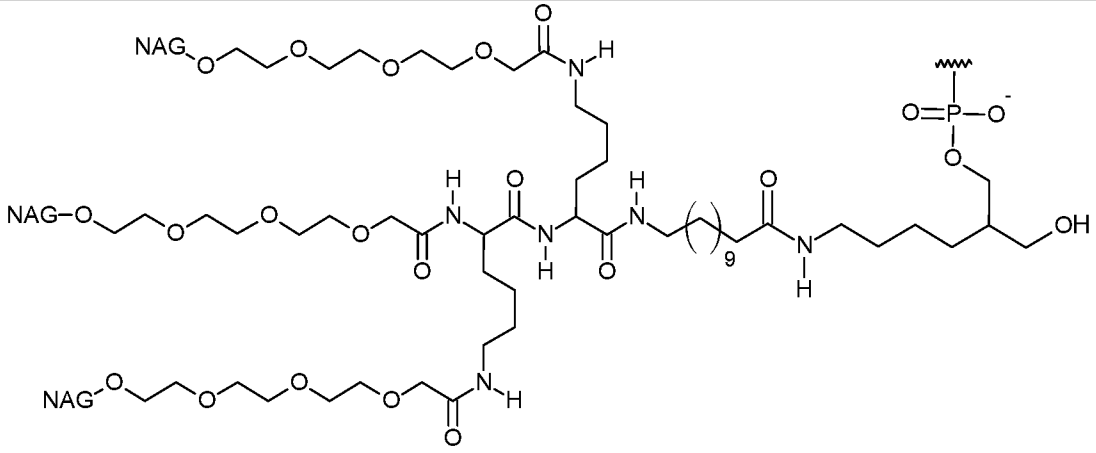
(C6-SS-Alk-Me)



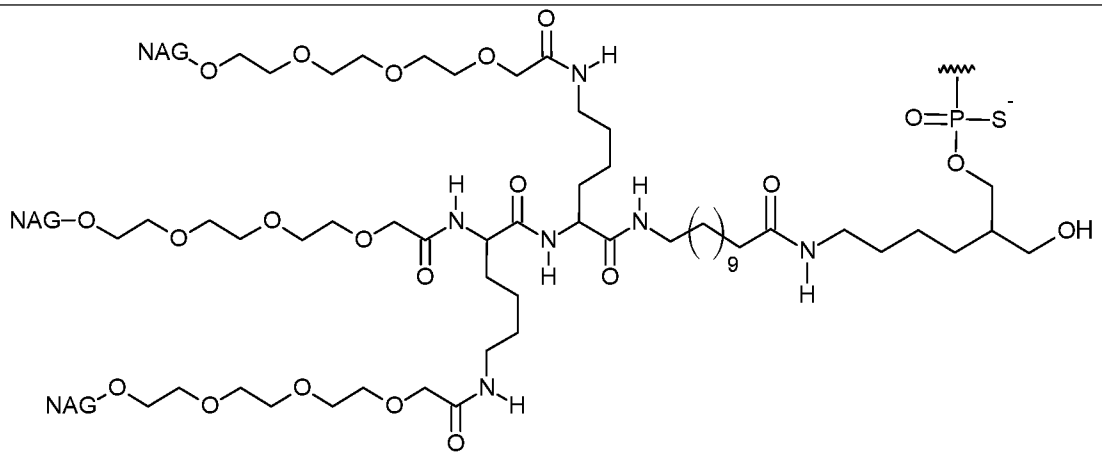
(PEG-C3-SS)



(PAZ)



(NAG13)



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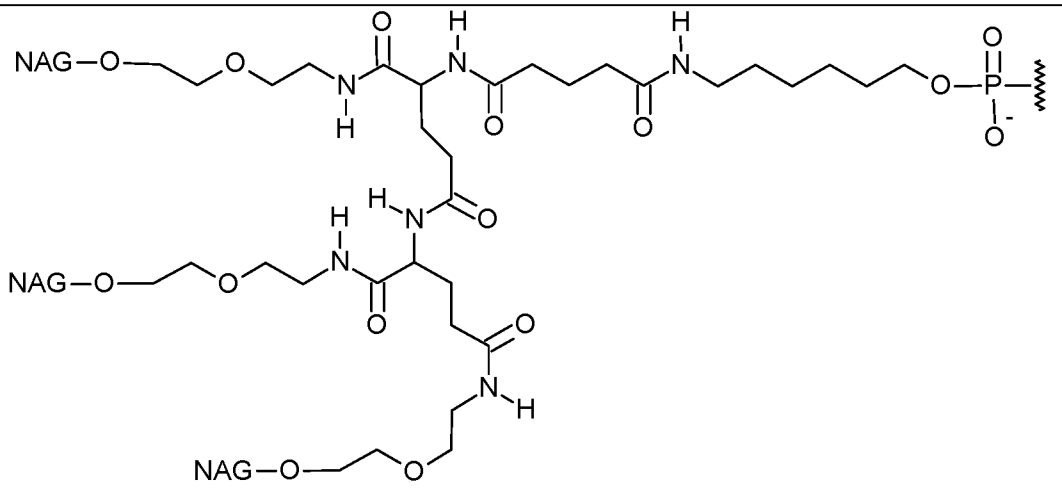

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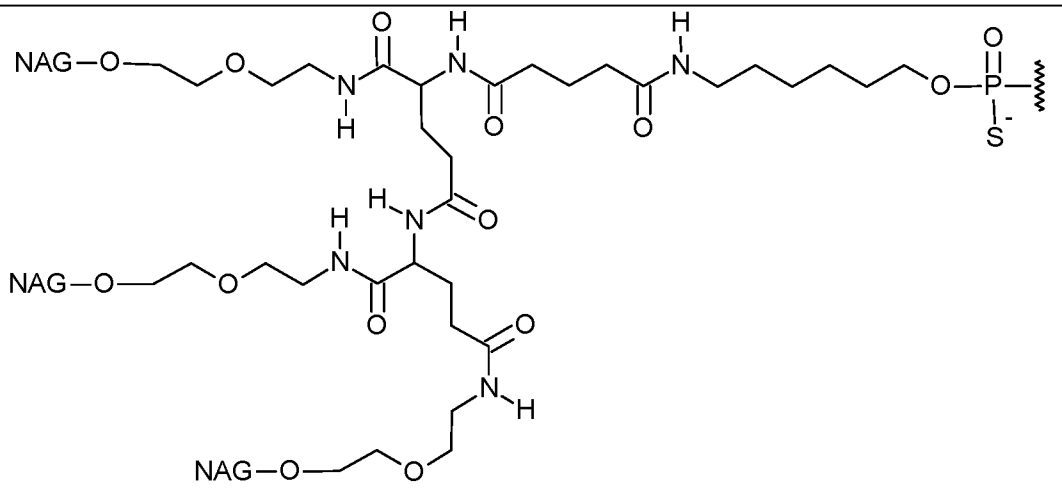

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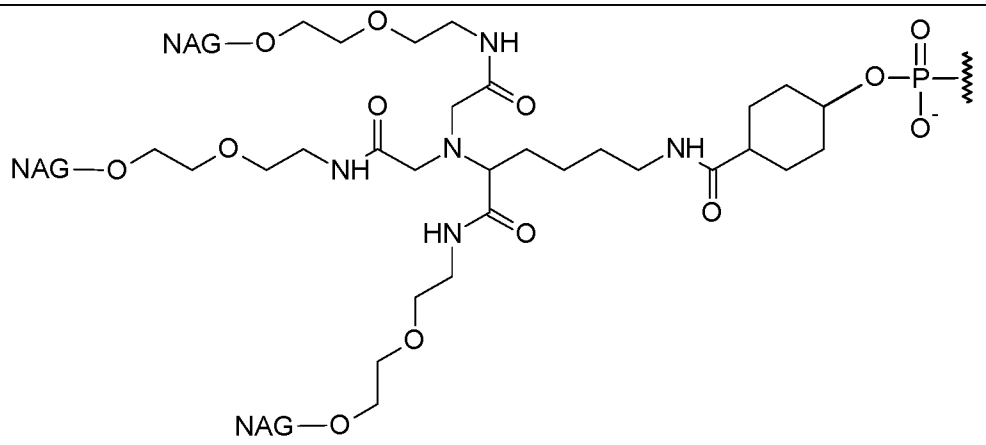

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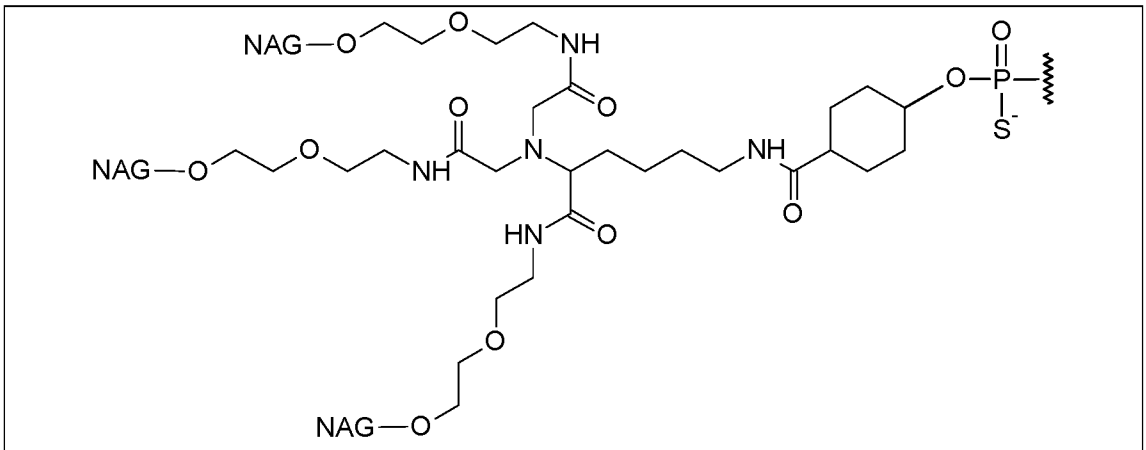

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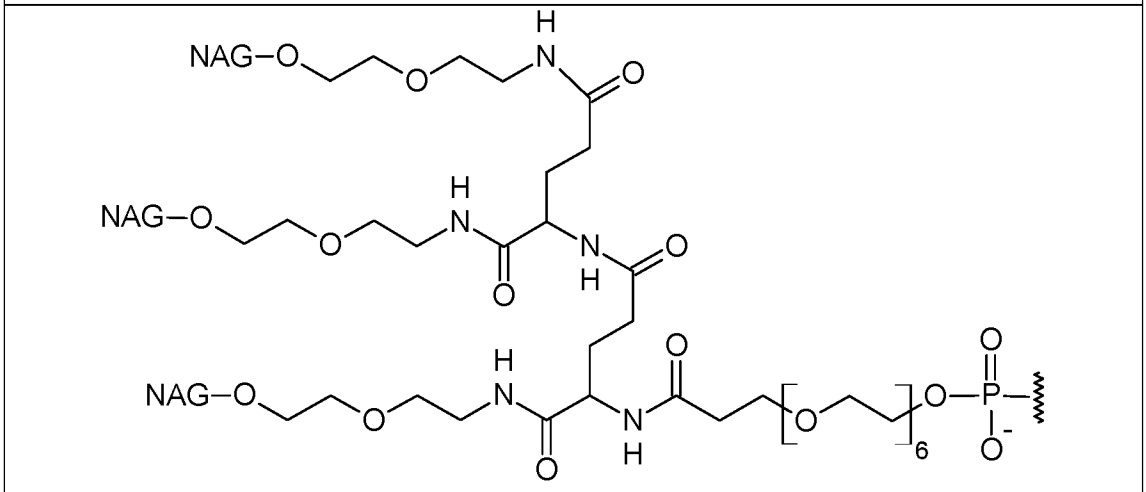

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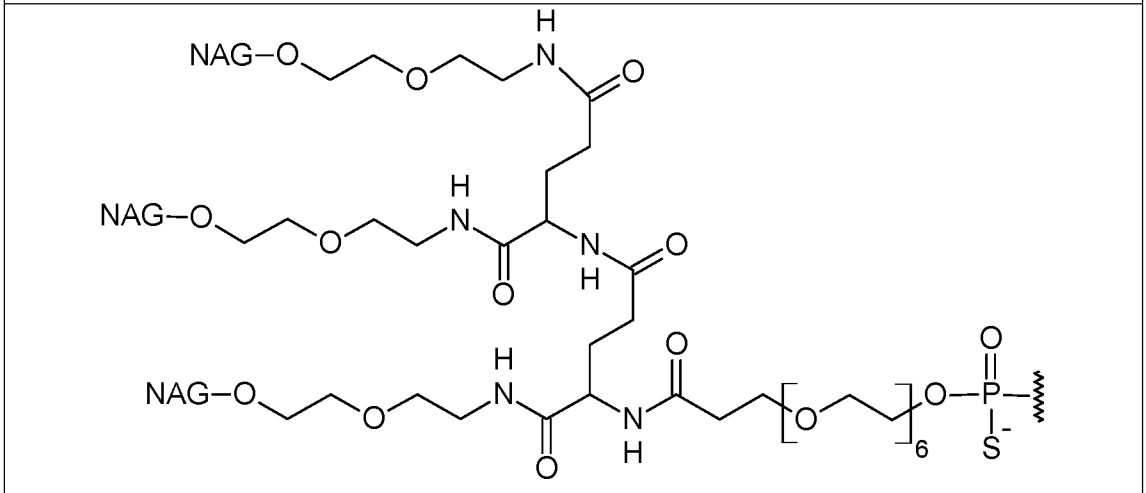

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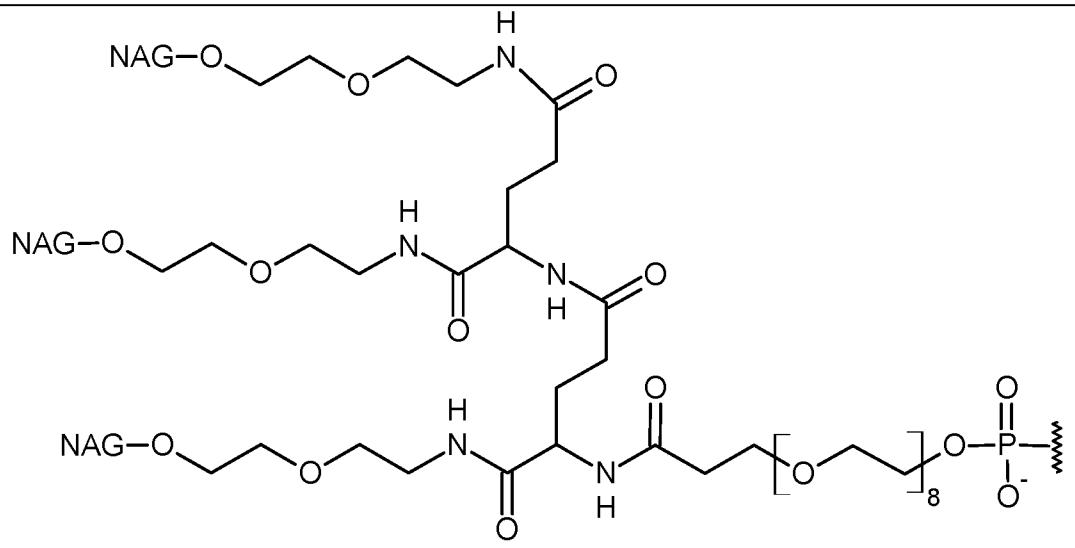

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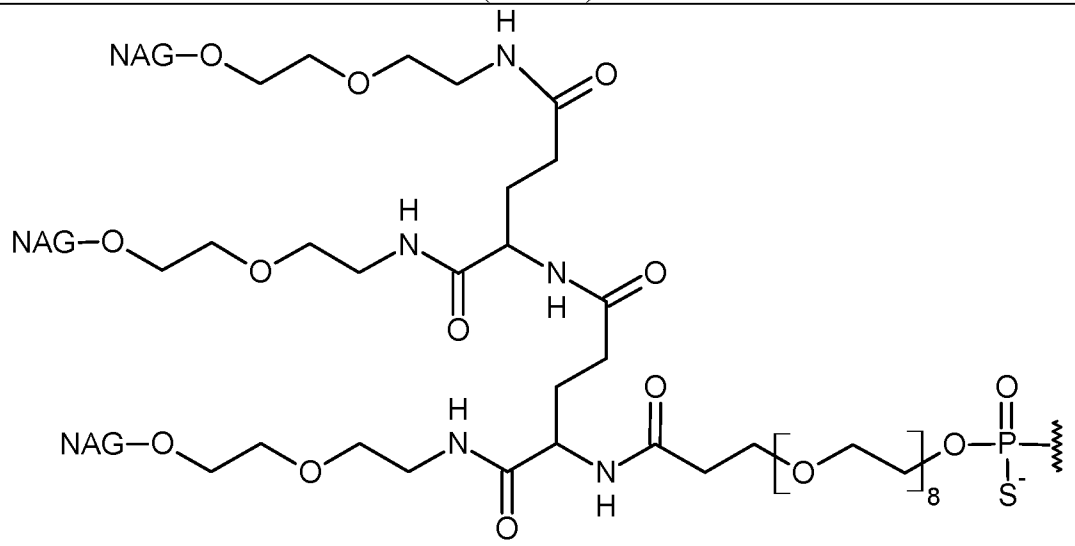

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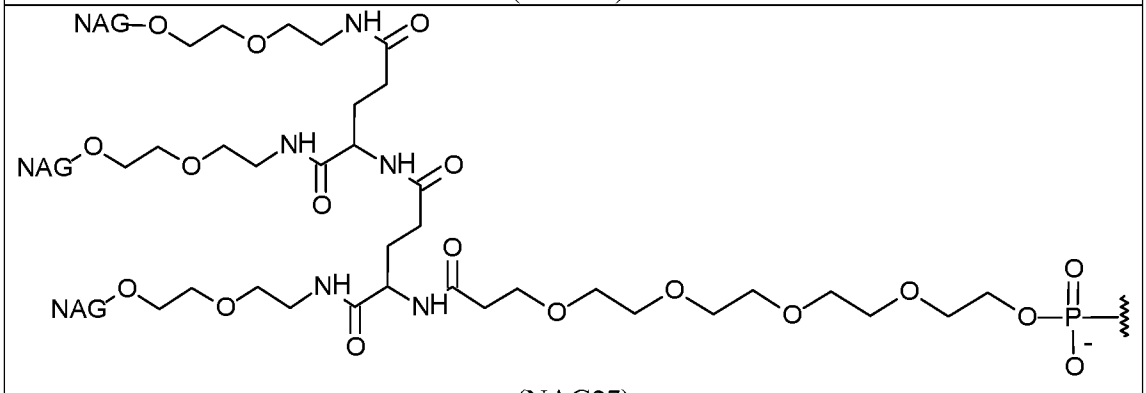

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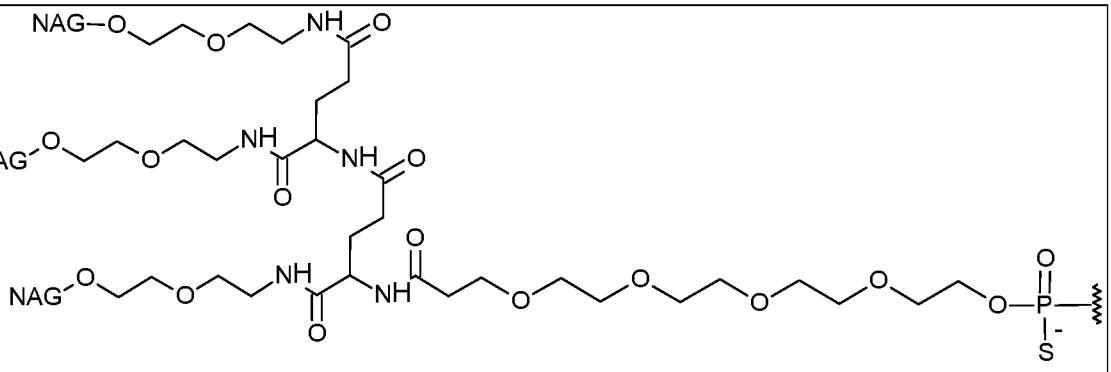

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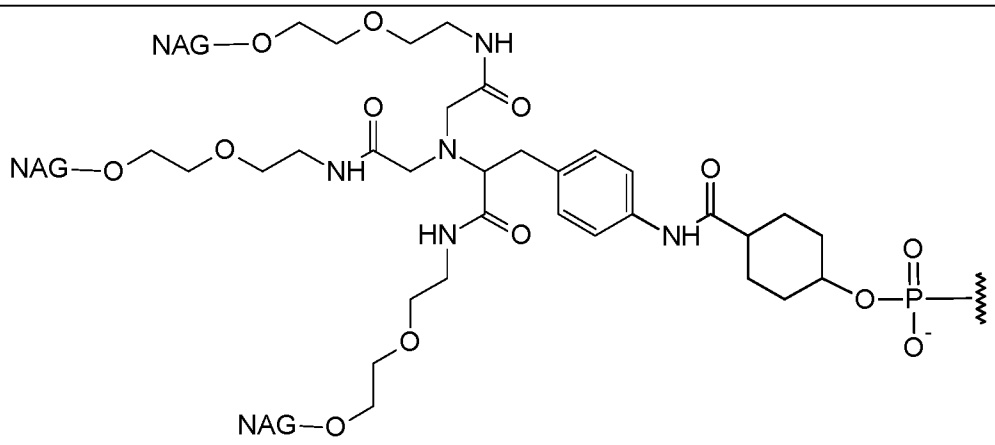

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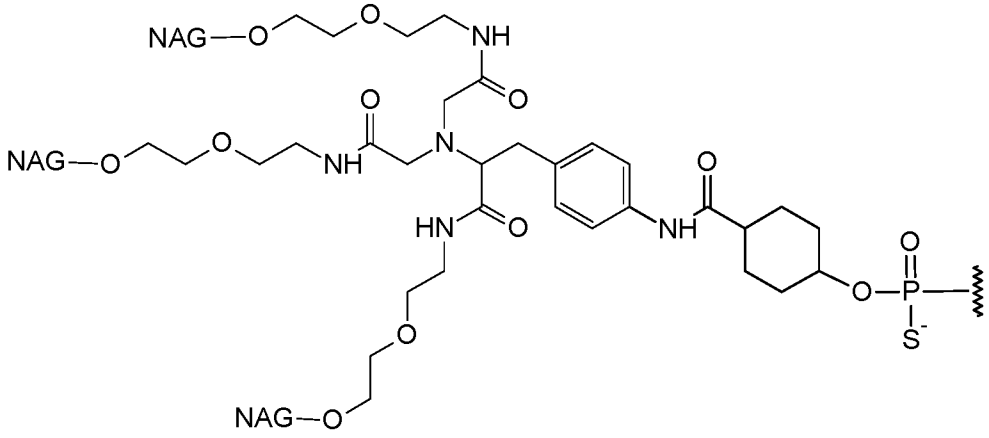

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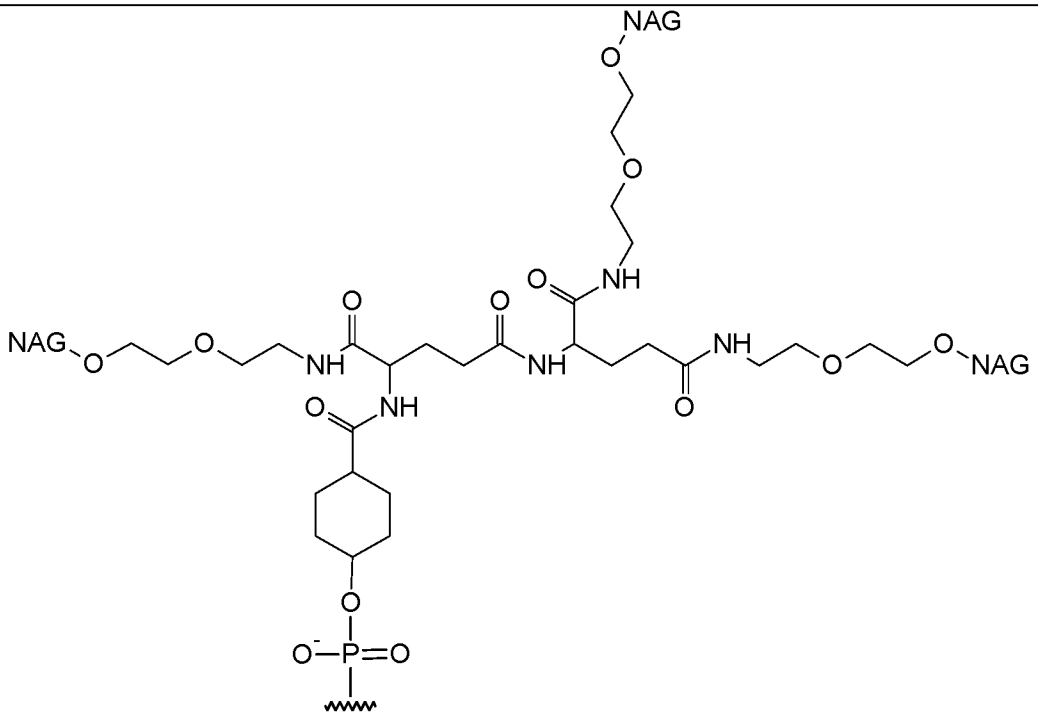

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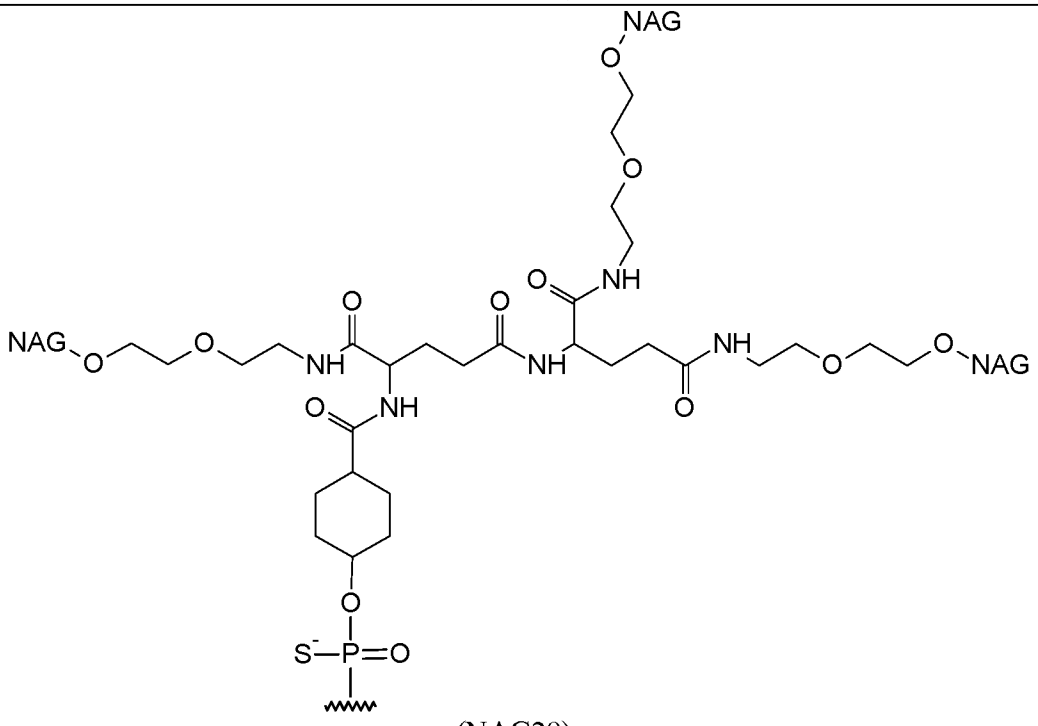

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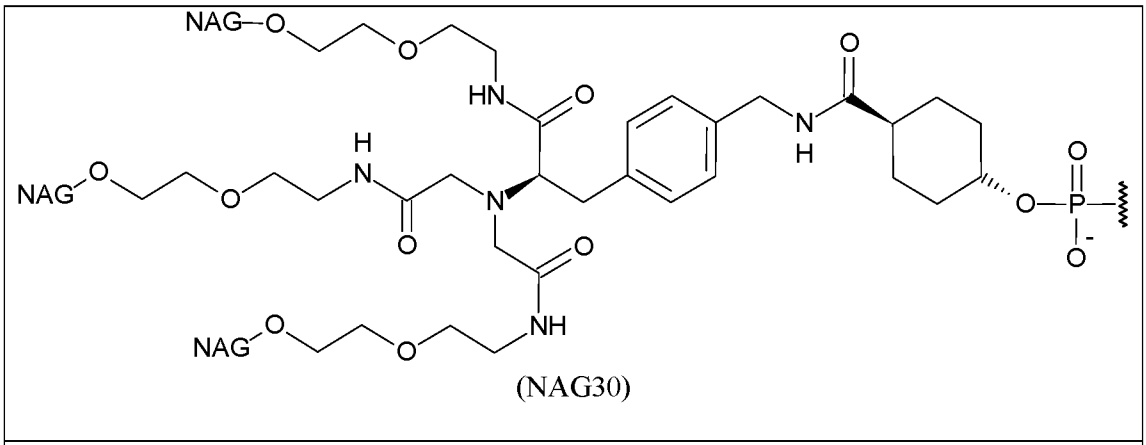

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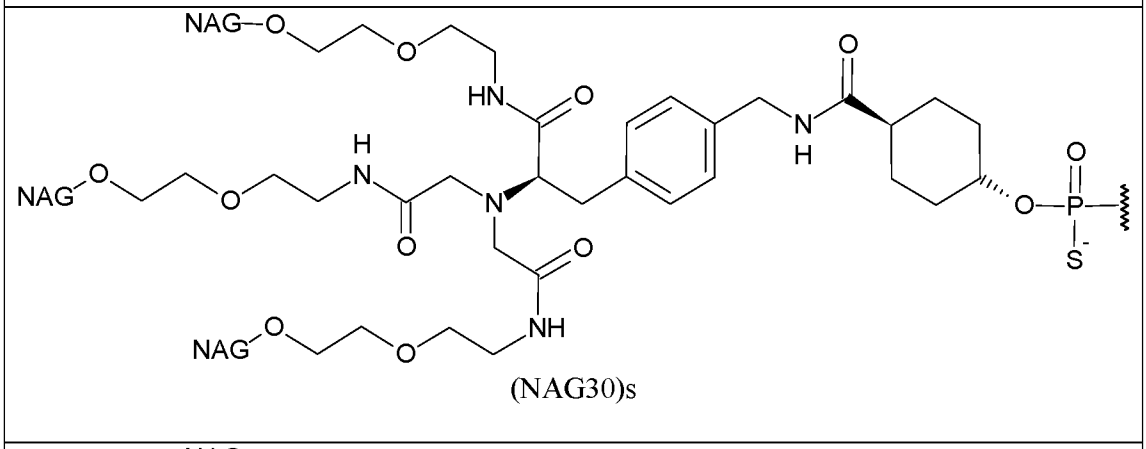

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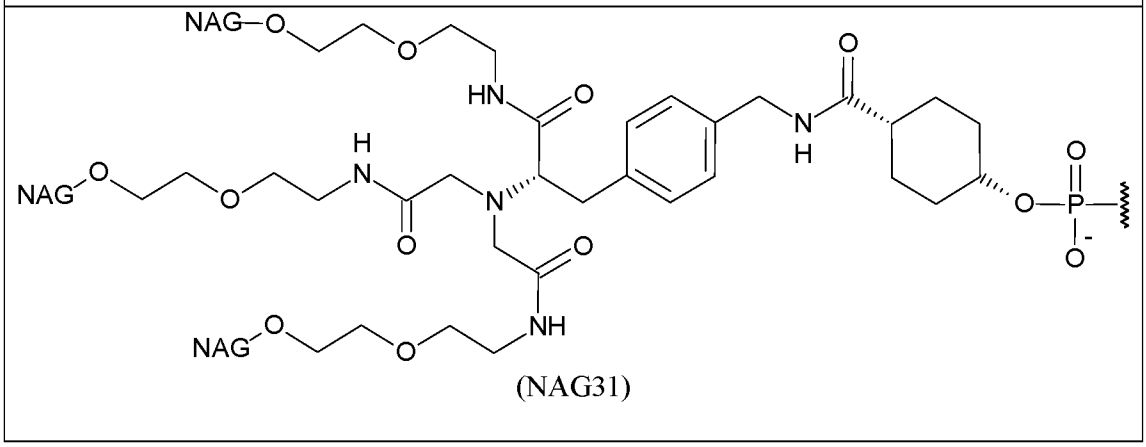

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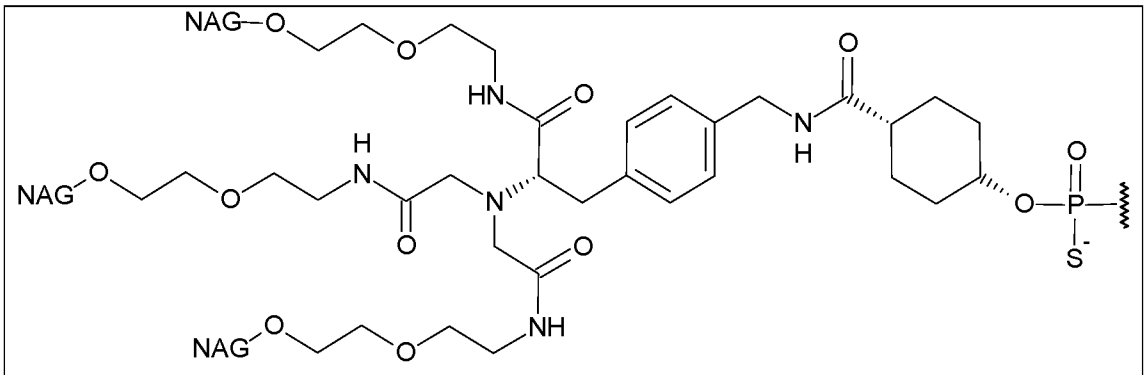

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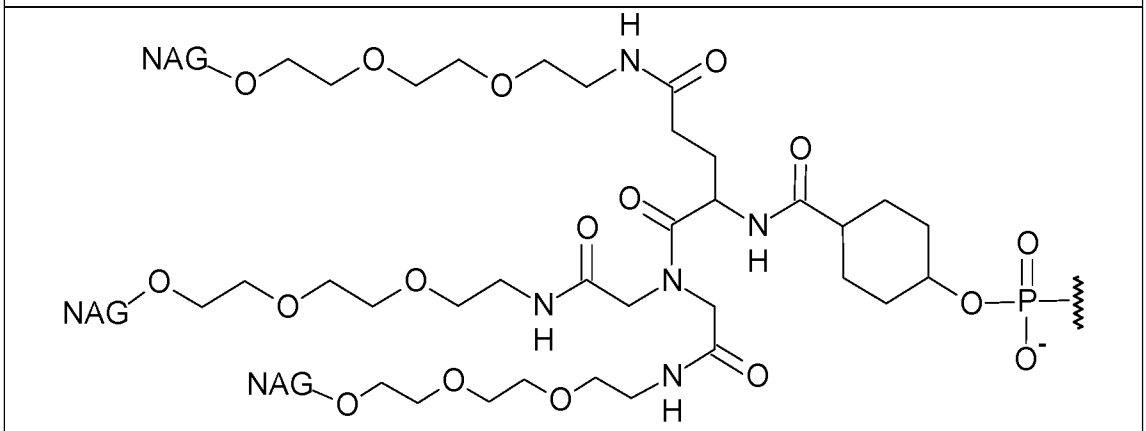

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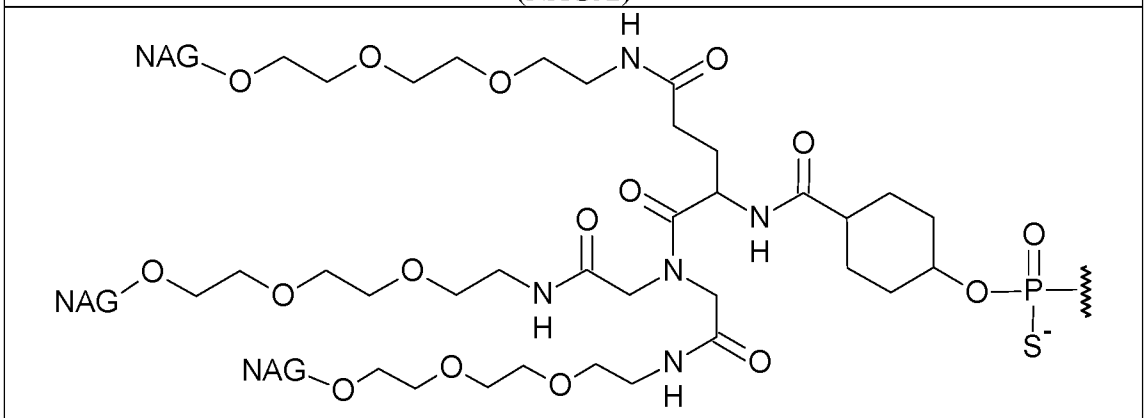

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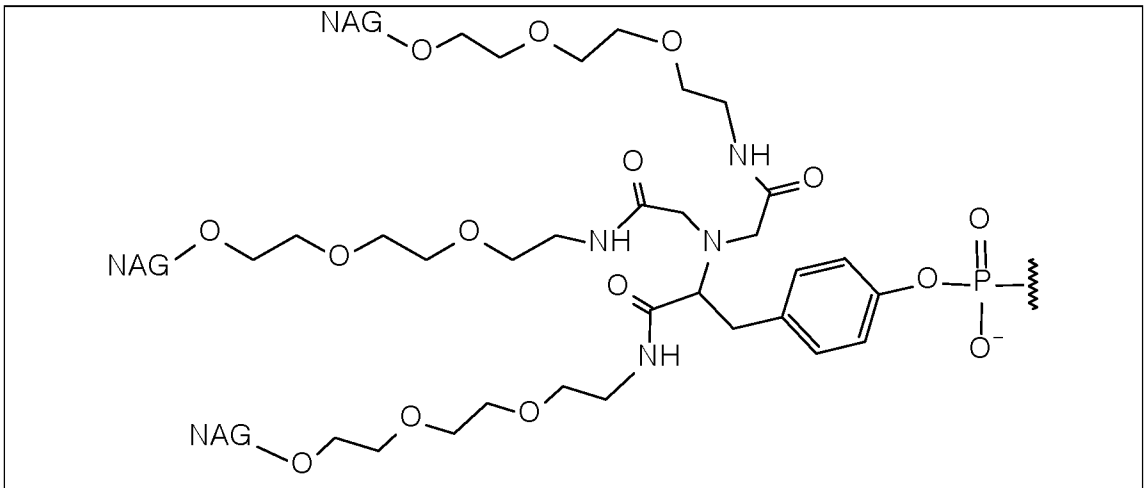

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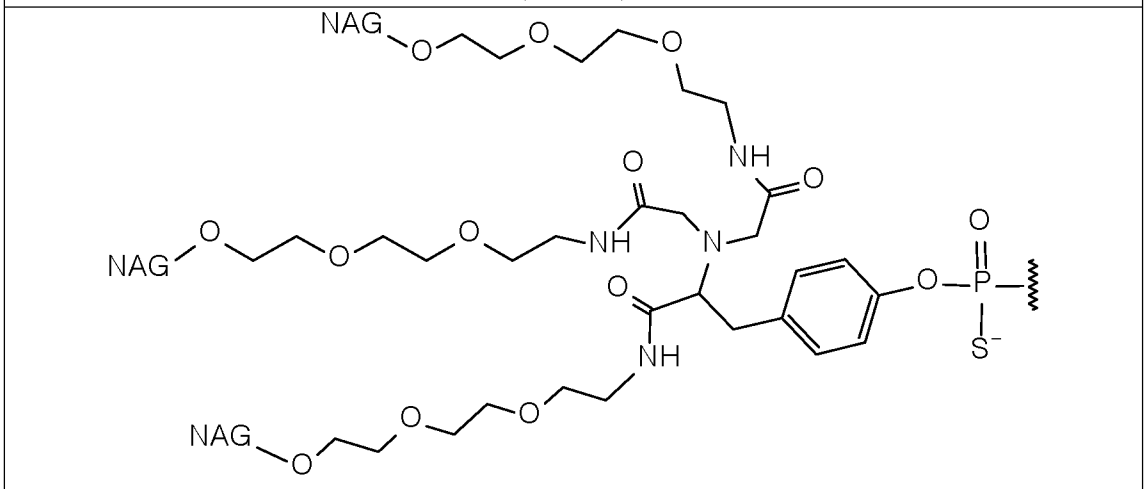

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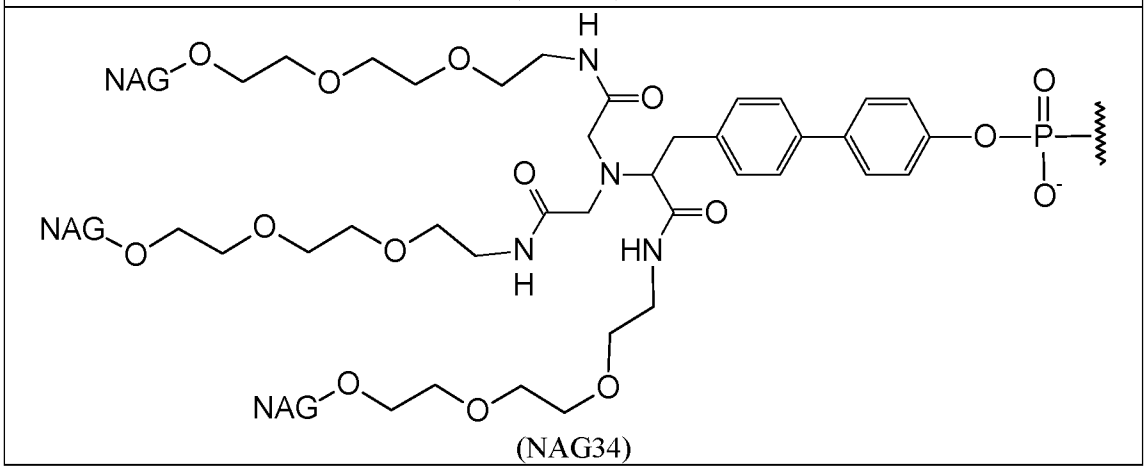

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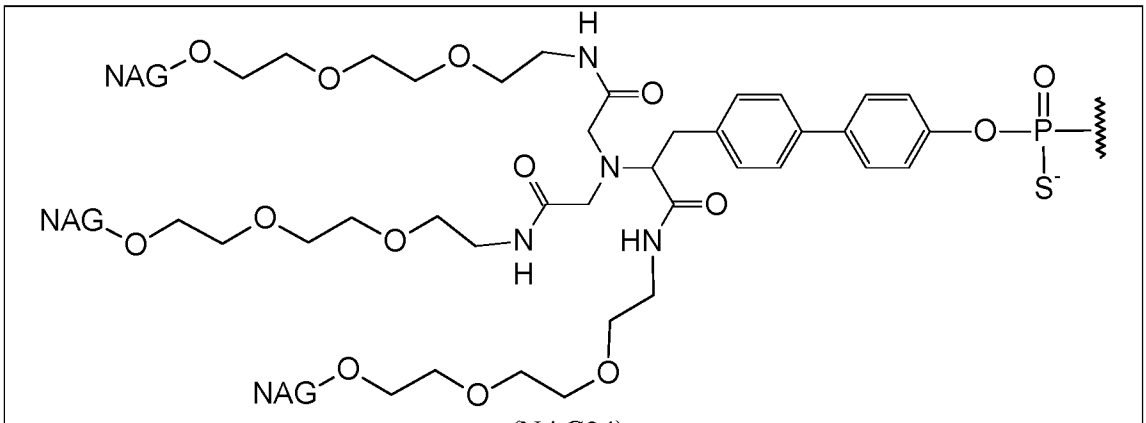

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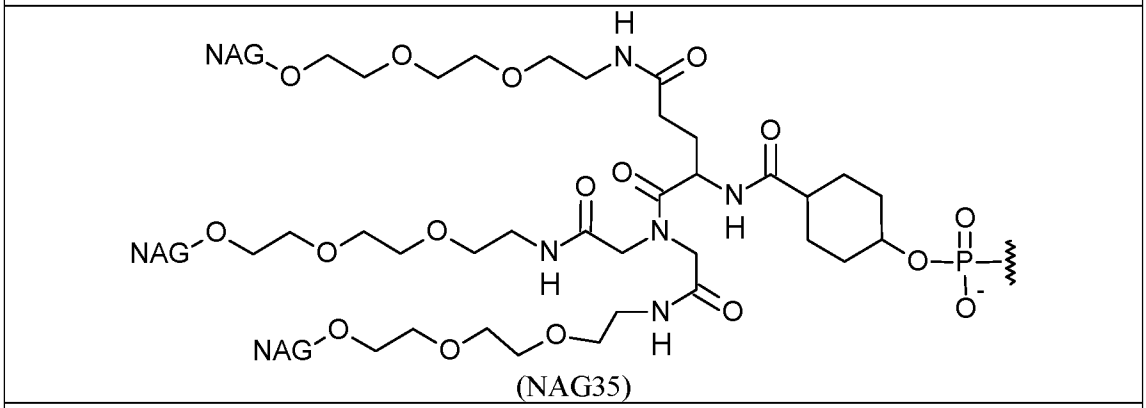

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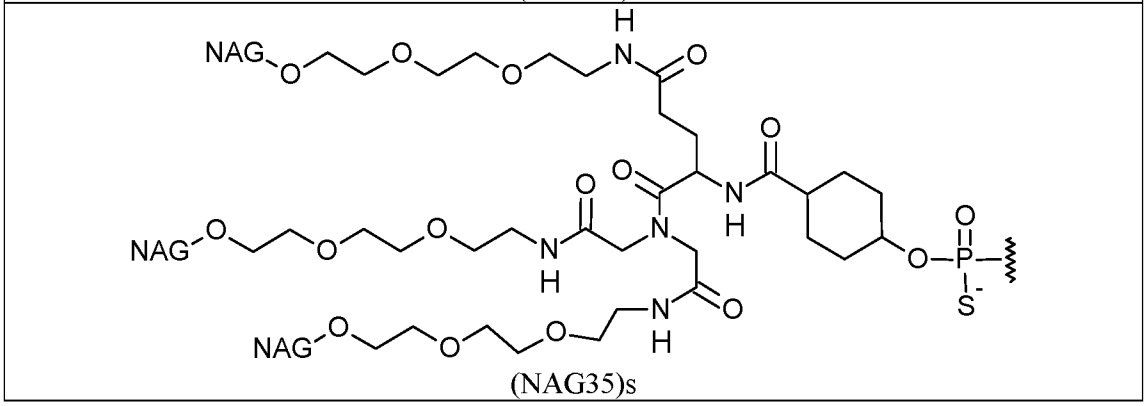

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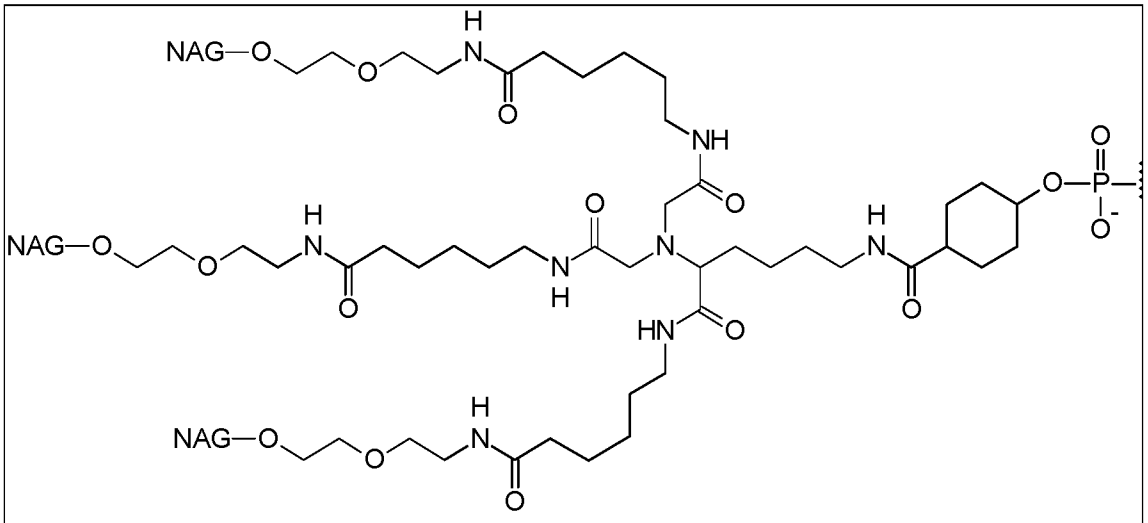
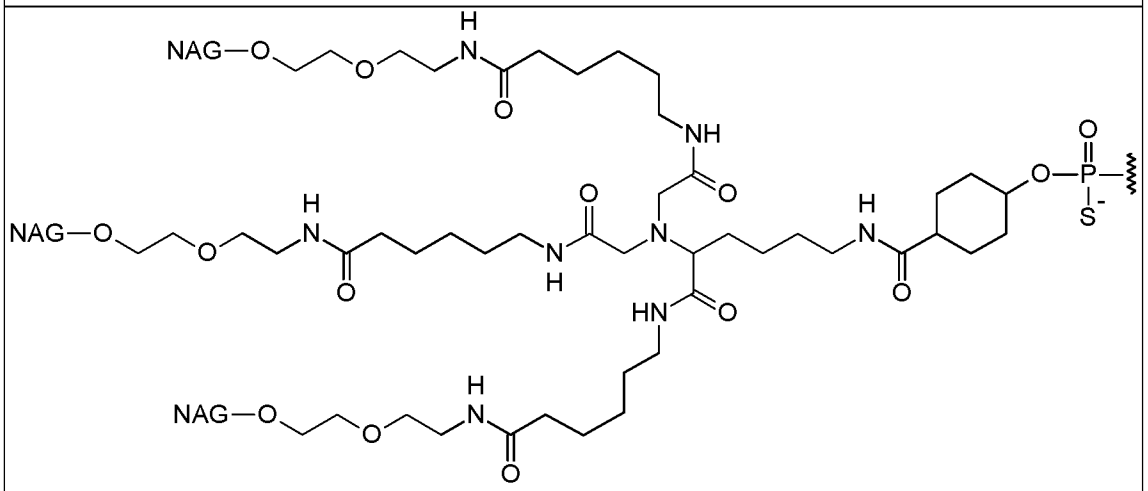


(NAG36)



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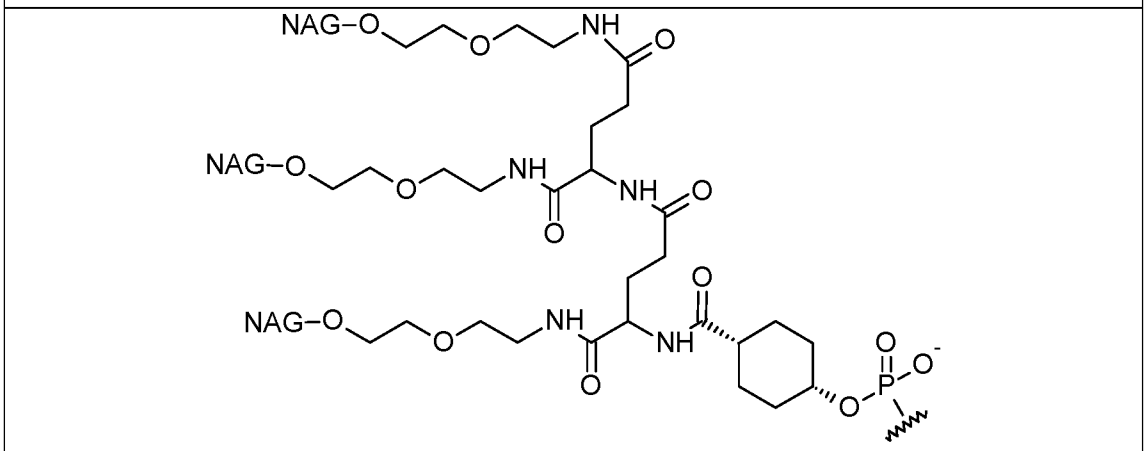

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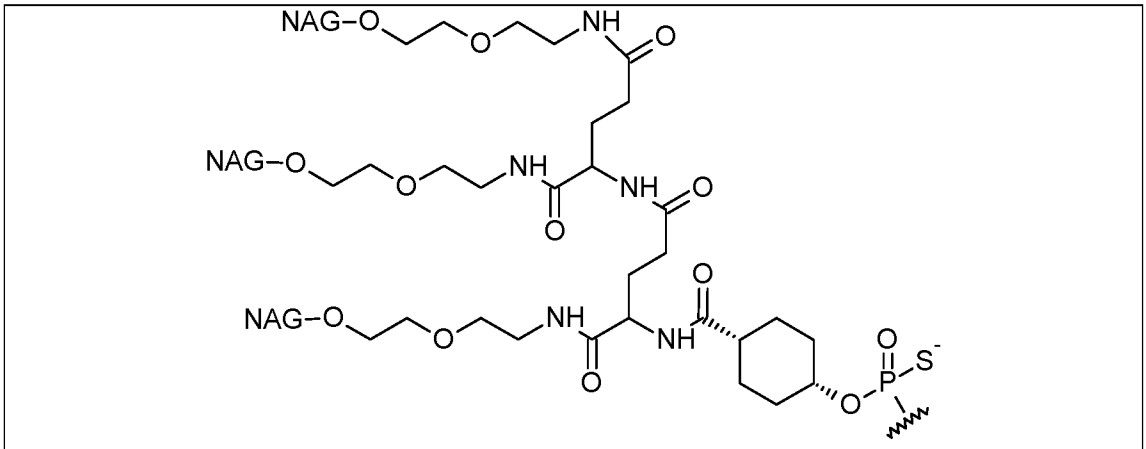

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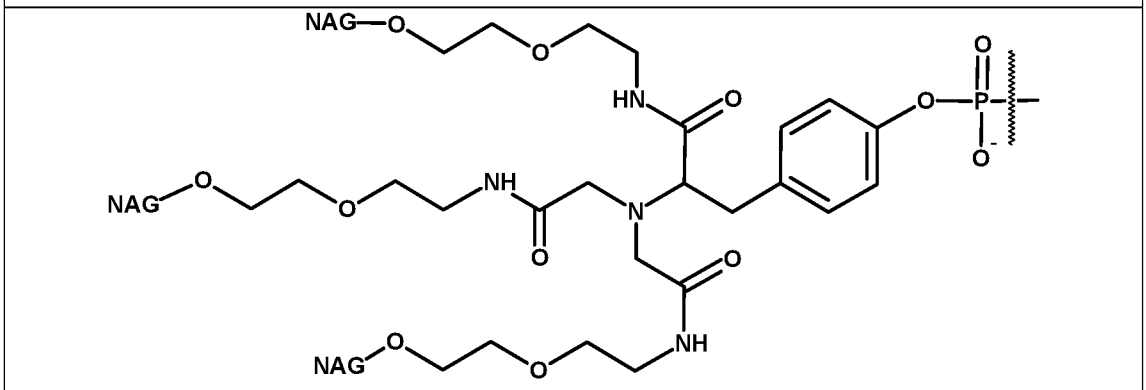
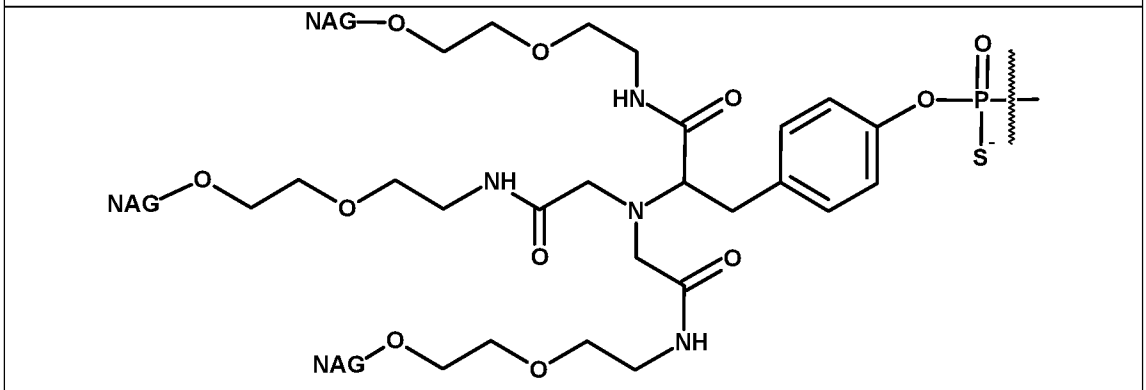


(NAG 38)



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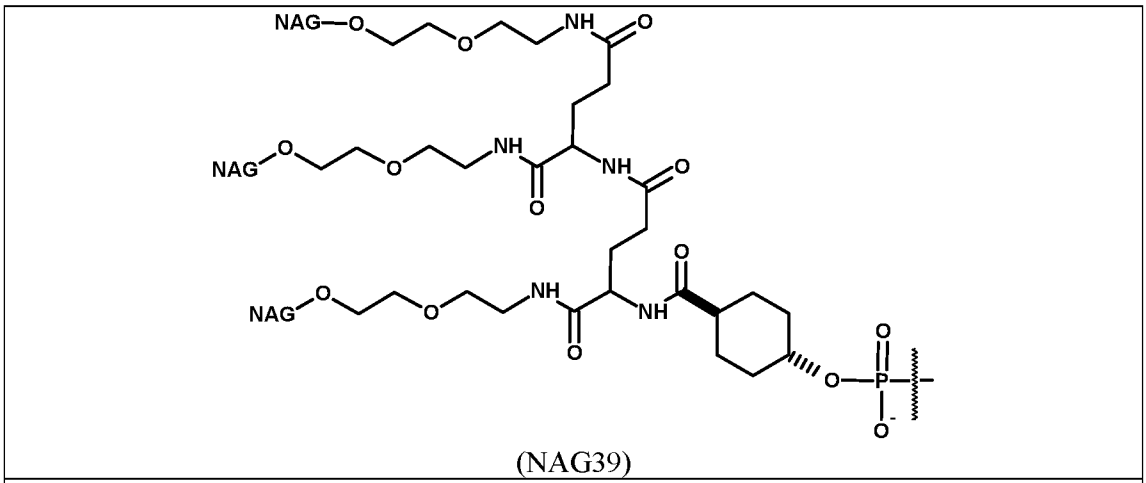
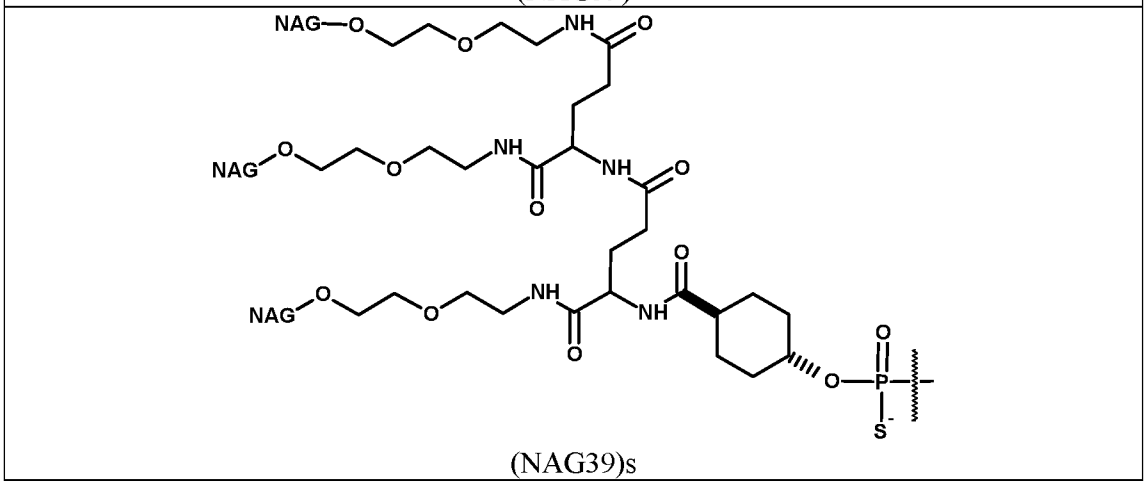
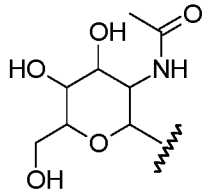


(NAG39)



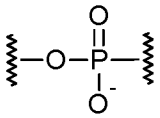
(NAG39)s

In each of the above structures in Table 7, NAG comprises an N-acetyl-galactosamine or another asialoglycoprotein receptor ligand, as would be understood by a person of ordinary skill in the art to be attached in view of the structures above and description provided herein. For example, in some embodiments, NAG in the structures provided in Table 7 is represented by the following structure:

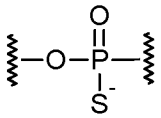


(N-acetyl-galactosamine)

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



Phosphate group



Phosphorothioate group

Other linking groups known in the art may be used.

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

Pharmaceutical Compositions and Formulations

The AAT RNAi agents disclosed herein can be prepared as pharmaceutical compositions or formulations. In some embodiments, pharmaceutical compositions include at least one AAT RNAi agent. These pharmaceutical compositions are particularly useful in the inhibition of the expression of the target mRNA in a target cell, a group of cells, a tissue, or an organism. The pharmaceutical compositions can be used to treat a subject having a disease or disorder that would benefit from reduction in the level of the target mRNA, or inhibition in expression of the target gene. The pharmaceutical compositions can be used to

treat a subject at risk of developing a disease or disorder 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 AAT 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 including an AAT RNAi agent, thereby forming a pharmaceutical formulation suitable for *in vivo* delivery to a subject, including a human.

The pharmaceutical compositions that include an AAT RNAi agent and methods disclosed herein decrease the level of the target mRNA in a cell, group of cells, group of cells, tissue, or subject, including: administering to the subject a therapeutically effective amount of a herein described AAT RNAi agent, thereby inhibiting the expression of AAT mRNA in the subject.

In some embodiments, the described pharmaceutical compositions including an AAT RNAi agent are used for treating or managing clinical presentations in a subject with AATD, such as chronic hepatitis, cirrhosis, hepatocellular carcinoma, transaminitis, cholestasis, fibrosis, and even fulminant hepatic failure. In some embodiments, a therapeutically or 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 AAT RNAi agents can be used to decrease the number, severity, and/or frequency of symptoms of a disease in a subject.

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

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

The pharmaceutical compositions including an AAT 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.

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

As used herein, a pharmaceutical composition or medicament includes a pharmacologically effective amount of at least one of the described AAT RNAi agents and one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients (excipients) are substances other than the Active Pharmaceutical Ingredient (API, therapeutic product, e.g., AAT RNAi agent) that are intentionally included in the drug delivery system. Excipients do not exert or are not intended to exert a therapeutic effect at the intended dosage. Excipients can act to a) aid in processing of the drug delivery system

during manufacture, b) protect, support, or enhance stability, bioavailability or patient acceptability of the API, c) assist in product identification, and/or d) enhance any other attribute of the overall safety, effectiveness, of delivery of the API during storage or use. A pharmaceutically acceptable excipient may or may not be an inert substance.

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

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor® ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). It should be stable under the conditions of manufacture and storage and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic

dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation include vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

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

The active compounds can be prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

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

A pharmaceutical composition can contain other additional components commonly found in pharmaceutical compositions. Such additional components include, but are not limited

to: anti-pruritics, astringents, local anesthetics, or anti-inflammatory agents (e.g., antihistamine, 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.

Generally, an effective amount of an active compound will be in the range of from about 0.1 to about 100 mg/kg of body weight/day, e.g., from about 1.0 to about 50 mg/kg of body weight/day. In some embodiments, an effective amount of an active compound will be in the range of from about 0.25 to about 5 mg/kg of body weight per dose. In some embodiments, an effective amount of an active ingredient will be in the range of from about 0.5 to about 4 mg/kg of body weight per dose. The amount administered will also likely depend on such variables as the overall health status of the patient, the relative biological efficacy of the compound delivered, the formulation of the drug, the presence and types of excipients in the formulation, and the route of administration. Also, it is to be understood that the initial dosage administered can, in some instances, be increased beyond the above upper level to rapidly achieve the desired blood-level or tissue level, or the initial dosage can, in some instances, be smaller than the optimum.

For treatment of disease or for formation of a medicament or composition for treatment of a disease, the pharmaceutical compositions described herein including an AAT 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 aptamer.

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

Methods of Treatment and Inhibition of Expression

The AAT 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

compound. In some embodiments, the RNAi agents disclosed herein can be used to treat a subject (e.g., a human) having AATD, or symptoms, diseases, or disorders that would benefit from reduction or inhibition in expression of AAT mRNA, such as AATD liver disease. The subject is administered a therapeutically effective amount of any one or more of the AAT RNAi agents described herein. The subject can be a human, patient, or human patient. The subject may be an adult, adolescent, child, or infant. The described pharmaceutical compositions including an AAT RNAi agent can be used to provide methods for the therapeutic treatment of diseases, such as AATD. Such methods include administration of a pharmaceutical composition described herein to a human being or animal.

In some embodiments, the AAT RNAi agents described herein are used to treat a subject with AATD, including symptoms, diseases or disorders related to AATD. AATD liver diseases or disorders include, but are not limited to, chronic hepatitis, cirrhosis, hepatocellular carcinoma, transaminitis, cholestasis, fibrosis, and fulminant hepatic failure. In some embodiments, the described AAT RNAi agents are used to treat at least one symptom in a subject having AATD. The subject is administered a therapeutically effective amount of any one or more of the described RNAi agents.

In certain embodiments, the present invention provides methods for treatment of AATD in a patient in need thereof, comprising administering to the patient any of the AAT RNAi agents described herein.

In some embodiments, the AAT RNAi agents are used to treat or manage a clinical presentation of a subject with an AATD liver disease or disorder. The subject is administered a therapeutically effective amount of one or more of the AAT RNAi agents or AAT RNAi agent-containing compositions described herein. In some embodiments, the method comprises administering a composition comprising an AAT RNAi agent described herein to a subject to be treated.

In some embodiments, the gene expression level and/or mRNA level of an AAT gene in a subject to whom a described AAT RNAi agent is administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 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 AAT RNAi agent or to a subject not receiving the AAT RNAi agent. The gene expression level and/or mRNA level in the subject is reduced in a cell, group of cells, and/or tissue of the subject.

In some embodiments, the protein level of AAT in a subject to whom a described AAT RNAi agent has been administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 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 AAT RNAi agent or to a subject not receiving the AAT RNAi agent. The protein level in the subject is reduced in a cell, group of cells, tissue, blood, and/or other fluid of the subject.

In some embodiments, the Z-AAT polymer protein level in a subject having AATD to whom a described AAT RNAi agent has been administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 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 AAT RNAi agent or to a subject not receiving the AAT RNAi agent. In some embodiments, the Z-AAT polymer protein level in a subject to whom a described AAT RNAi agent has been administered is reduced by at least about 5%, 10%, 15%, 20%, 25%, 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 AAT RNAi agent or to a subject not receiving the AAT RNAi agent.

A reduction in AAT gene expression, AAT mRNA, or AAT protein levels can be assessed and quantified by general methods known in the art. The Examples disclosed herein forth generally known methods for assessing inhibition of AAT gene expression and reduction in AAT protein levels. The reduction or decrease in AAT mRNA level and/or protein level (including Z-AAT polymer and/or monomer) are collectively referred to herein as a reduction or decrease in AAT or inhibiting or reducing the expression of AAT.

Cells and Tissues and non-Human organisms

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

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

EXAMPLES

Example 1. ***Identification of RNAi agent sequences and synthesis of RNAi agents***

A selection process for identifying lead sequences for inhibiting expression of the AAT gene began with *in silico* methods to identify conserved sequences across variants of an AAT gene (SEQ ID NO: 1). The AAT sequence was initially screened using bioinformatics for 19-nucleotide sequences having a complementary sequence in known variants of human AAT. Sequences known to have manufacturing challenges and those predicted to have poor RNAi activity based on known parameters were eliminated. Sequences were then subjected to cross-species reactivity analysis to select candidates that would cross-react with cynomolgus monkey AAT. The sequences were also evaluated for specificity to avoid off-target effects against the human and cynomolgus monkey genomes. One-hundred fifteen (115) sequence families of 19-mers were selected as candidates.

The duplexes in Table 6 herein were synthesized according to the following procedures:

Synthesis

The sense and antisense strands of the AAT RNAi agents were synthesized according to phosphoramidite technology on solid phase used in oligonucleotide synthesis. Depending on the scale, either a MerMade96E® (Bioautomation) or a MerMade12® (Bioautomation) was used. Syntheses were performed on a solid support made of controlled pore glass (CPG, 500 Å or 600Å, obtained from Prime Synthesis, Aston, PA, USA). All RNA and 2′-modified RNA phosphoramidites were purchased from Thermo Fisher Scientific (Milwaukee, WI, USA). Specifically, the following 2′-O-methyl phosphoramidites were used: (5′-O-dimethoxytrityl-N6-(benzoyl)-2′-O-methyl-adenosine-3′-O-(2-cyanoethyl-N,N-diisopropy-lamino) phosphoramidite, 5′-O-dimethoxy-trityl-N4-(acetyl)-2′-O-methyl-cytidine-3′-O-(2-cyanoethyl-N,N-diisopropyl-amino) phosphoramidite, (5′-O-

dimethoxytrityl-N2-(isobutyryl)-2′-O-methyl-guanosine-3′-O-(2-cyano-ethyl-N,N-diisopropylamino)phosphoramidite, and 5′-O-dimethoxy-trityl-2′-O-methyl-uridine-3′-O-(2-cyanoethyl-N,N-diisopropylamino)phosphoramidite. The 2′-deoxy-2′-fluoro-phosphoramidites carried the same protecting groups as the 2′-O-methyl RNA amidites. The following UNA phosphoramidites were used: 5′-(4,4'-Dimethoxytrityl)-N-benzoyl-2′,3′-seco-adenosine, 2′-benzoyl-3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphor-amidite, 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.

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) 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), 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.

Cleavage and Deprotection of Support Bound Oligomers.

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 two hours at 30°C. The solution was evaporated and the solid residue was reconstituted in water (see below).

Purification

Crude oligomers were purified by anionic exchange HPLC using a TKSgel SuperQ-5PW 13u 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 G-25 medium with a running buffer of 100mM ammonium bicarbonate, pH 6.7 and 20% Acetonitrile.

Annealing

Complementary strands were mixed by combining equimolar RNA solutions (sense and antisense) in 0.2× PBS (Phosphate-Buffered Saline, 1×, Corning, Cellgro) to form the RNAi agents. This solution was placed into a thermomixer at 70°C, heated to 95°C, held at 95°C for 5 min, and cooled to room temperature slowly. Some RNAi agents were lyophilized and stored at −15 to −25°C. Duplex concentration was determined by measuring the solution absorbance on a UV-Vis spectrometer in 0.2× PBS. The solution absorbance at 260 nm was then multiplied by a conversion factor and the dilution factor to determine the duplex concentration. Unless otherwise stated, all conversion factor was 0.037 mg/(mL·cm). For some experiments, a conversion factor was calculated from an experimentally determined extinction coefficient.

Example 2. ***In Vitro Testing of AAT RNAi Agents***

Candidate sequence duplexes were tested *in vitro*. The antisense strand sequences and sense strand sequences were annealed to form duplexes of 21-mer strands (having 19 base pairs and a di-nucleotide UU overhang on each 3' end) for *in vitro* testing, as shown in the following Table 8:

Table 8. Sequences of AAT RNAi Agents in Example 2

SEQ ID NO:	Antisense Sequence (5' → 3')	SEQ ID NO:	Sense Sequence (5' → 3')	Duplex ID No.
1035	AGAAGAUAUUGGUGCUGUUUU	1150	AACAGCACCAAUAUCUUCUUU	D1
1036	AGGAACUUGGUGAUGAUAUUU	1151	AUAUCAUCACCAAGUUCCUUU	D2
1037	UGUCUUCUGGGCAGCAUCUUU	1152	AGAUGCUGCCCAGAAGACAUU	D3
1038	UGUUGGACUGGUGUGCCAGUU	1153	CUGGCACACCAGUCCAACAUU	D4
1039	CUGUUGGACUGGUGUGCCAUU	1154	UGGCACACCAGUCCAACAGUU	D5
1040	UGCUGUUGGACUGGUGUGCUU	1155	GCACACCAGUCCAACAGCAUU	D6
1041	UAUUGGUGCUGUUGGACUGUU	1156	CAGUCCAACAGCACCAAUAUU	D7
1042	AUAUUGGUGCUGUUGGACUUU	1157	AGUCCAACAGCACCAAUAUUU	D8
1043	GAUAUUGGUGCUGUUGGACUU	1158	GUCCAACAGCACCAAUAUCUU	D9
1044	AAGAUAUUGGUGCUGUUGGUU	1159	CCAACAGCACCAAUAUCUUUU	D10
1045	GUAGCGAUGCUCACUGGGGUU	1160	CCCCAGUGAGCAUCGCUACUU	D11
1046	AAAGGCUGUAGCGAUGCUCUU	1161	GAGCAUCGCUACAGCCUUUUU	D12
1047	GCAAAGGCUGUAGCGAUGCUU	1162	GCAUCGCUACAGCCUUUGCUU	D13
1048	UGCAAAGGCUGUAGCGAUGUU	1163	CAUCGCUACAGCCUUUGCAUU	D14
1049	AUUGCAAAGGCUGUAGCGAUU	1164	UCGCUACAGCCUUUGCAAUUU	D15
1050	AGCAUUGCAAAGGCUGUAGUU	1165	CUACAGCCUUUGCAAUGCUUU	D16
1051	AGAGCAUUGCAAAGGCUGUUU	1166	ACAGCCUUUGCAAUGCUCUUU	D17
1052	GGAGUUCCUGGAAGCCUUCUU	1167	GAAGGCUUCCAGGAACUCCUU	D18
1053	UCCAAAAACUUAUCCACUAUU	1168	UAGUGGAUAAGUUUUUGGAUU	D19
1054	AAGGCUUCUGAGUGGUACAUU	1169	UGUACCACUCAGAAGCCUUUU	D20
1055	GAAGGCUUCUGAGUGGUACUU	1170	GUACCACUCAGAAGCCUUCUU	D21
1056	UUCUUGGCCUCUUCGGUGUUU	1171	ACACCGAAGAGGCCAAGAAUU	D22
1057	GUUUCUUGGCCUCUUCGGUUU	1172	ACCGAAGAGGCCAAGAAACUU	D23
1058	UUGAUCUGUUUCUUGGCCUUU	1173	AGGCCAAGAAACAGAUCAAUU	D24
1059	GUUGAUCUGUUUCUUGGCCUU	1174	GGCCAAGAAACAGAUCAACUU	D25
1060	CGUUGAUCUGUUUCUUGGCUU	1175	GCCAAGAAACAGAUCAACGUU	D26

SEQ ID NO:	Antisense Sequence (5' → 3')	SEQ ID NO:	Sense Sequence (5' → 3')	Duplex ID No.
1061	CACAAUUUUCCCUUGAGUAUU	1176	UACUCAAGGGAAAAUUGUGUU	D27
1062	UCCACAAUUUUCCCUUGAGUU	1177	CUCAAGGGAAAAUUGUGGAUU	D28
1063	AUCCACAAUUUUCCCUUGAUU	1178	UCAAGGGAAAAUUGUGGAUUU	D29
1064	UGUCAAGCUCCUUGACCAAUU	1179	UUGGUCAAGGAGCUUGACAUU	D30
1065	GUGUCUCUGUCAAGCUCCUUU	1180	AGGAGCUUGACAGAGACACUU	D31
1066	ACUGUGUCUCUGUCAAGCUUU	1181	AGCUUGACAGAGACACAGUUU	D32
1067	UGUAAUUCACCAGAGCAAAUU	1182	UUUGCUCUGGUGAAUUACAUU	D33
1068	UUAAACAUGCCUAAACGCUUU	1183	AGCGUUUAGGCAUGUUUAAUU	D34
1069	GUUAAACAUGCCUAAACGCUU	1184	GCGUUUAGGCAUGUUUAACUU	D35
1070	UGUUAAACAUGCCUAAACGUU	1185	CGUUUAGGCAUGUUUAACAUU	D36
1071	GGAUGUUAAACAUGCCUAAUU	1186	UUAGGCAUGUUUAACAUCCUU	D37
1072	AUUUCAUCAGCAGCACCCAUU	1187	UGGGUGCUGCUGAUGAAAUUU	D38
1073	GAAGAAGAUGGCGGUGGCAUU	1188	UGCCACCGCCAUCUUCUUCUU	D39
1074	GGUGAGUUCAUUUUCCAGGUU	1189	CCUGGAAAAUGAACUCACCUU	D40
1075	GAACUUGGUGAUGAUAUCGUU	1190	CGAUAUCAUCACCAAGUUCUU	D41
1076	CAUUUUCCAGGAACUUGGUUU	1191	ACCAAGUUCCUGGAAAAUGUU	D42
1077	CAUAGGUUCCAGUAAUGGAUU	1192	UCCAUUACUGGAACCUAUGUU	D43
1078	UCAUAGGUUCCAGUAAUGGUU	1193	CCAUUACUGGAACCUAUGAUU	D44
1079	UCAGAUCAUAGGUUCCAGUUU	1194	ACUGGAACCUAUGAUCUGAUU	D45
1080	UCUUCAGAUCAUAGGUUCCUU	1195	GGAACCUAUGAUCUGAAGAUU	D46
1081	CUCUUCAGAUCAUAGGUUCUU	1196	GAACCUAUGAUCUGAAGAGUU	D47
1082	GAGGUCAGCCCCAUUGCUGUU	1197	CAGCAAUGGGGCUGACCUCUU	D48
1083	GAGAGGUCAGCCCCAUUGCUU	1198	GCAAUGGGGCUGACCUCUCUU	D49
1084	CUUCAGGGGUGCCUCCUCUUU	1199	AGAGGAGGCACCCCUGAAGUU	D50
1085	GAGAGCUUCAGGGGUGCCUUU	1200	AGGCACCCCUGAAGCUCUCUU	D51
1086	UUAUGCACGGCCUUGGAGAUU	1201	UCUCCAAGGCCGUGCAUAAUU	D52
1087	CCUUAUGCACGGCCUUGGAUU	1202	UCCAAGGCCGUGCAUAAGGUU	D53
1088	GCCUUAUGCACGGCCUUGGUU	1203	CCAAGGCCGUGCAUAAGGCUU	D54

SEQ ID NO:	Antisense Sequence (5' → 3')	SEQ ID NO:	Sense Sequence (5' → 3')	Duplex ID No.
1089	AGCCUUAUGCACGGCCUUGUU	1204	CAAGGCCGUGCAUAAGGCUUU	D55
1090	CGAUGGUCAGCACAGCCUUUU	1205	AAGGCUGUGCUGACCAUCGUU	D56
1091	GUCGAUGGUCAGCACAGCCUU	1206	GGCUGUGCUGACCAUCGACUU	D57
1092	AAAAACAUGGCCCCAGCAGUU	1207	CUGCUGGGGCCAUGUUUUUUU	D58
1093	CUAAAAACAUGGCCCCAGCUU	1208	GCUGGGGCCAUGUUUUUAGUU	D59
1094	UCUAAAAACAUGGCCCCAGUU	1209	CUGGGGCCAUGUUUUUAGAUU	D60
1095	CCUCUAAAAACAUGGCCCCUU	1210	GGGGCCAUGUUUUUAGAGGUU	D61
1096	GCCUCUAAAAACAUGGCCCUU	1211	GGGCCAUGUUUUUAGAGGCUU	D62
1097	UAGACAUGGGUAUGGCCUCUU	1212	GAGGCCAUACCCAUGUCUAUU	D63
1098	GAUAGACAUGGGUAUGGCCUU	1213	GGCCAUACCCAUGUCUAUCUU	D64
1099	UGUUGAACUUGACCUCGGGUU	1214	CCCGAGGUCAAGUUCAACAUU	D65
1100	GGUUUGUUGAACUUGACCUUU	1215	AGGUCAAGUUCAACAAACCUU	D66
1101	AAAGGGUUUGUUGAACUUGUU	1216	CAAGUUCAACAAACCCUUUUU	D67
1102	ACAAAGGGUUUGUUGAACUUU	1217	AGUUCAACAAACCCUUUGUUU	D68
1103	GACAAAGGGUUUGUUGAACUU	1218	GUUCAACAAACCCUUUGUCUU	D69
1104	AAGACAAAGGGUUUGUUGAUU	1219	UCAACAAACCCUUUGUCUUUU	D70
1105	CAUUAAGAAGACAAAGGGUUU	1220	ACCCUUUGUCUUCUUAAUGUU	D71
1106	AUCAUUAAGAAGACAAAGGUU	1221	CCUUUGUCUUCUUAAUGAUUU	D72
1107	GAAGAGGGGAGACUUGGUAUU	1222	UACCAAGUCUCCCCUCUUCUU	D73
1108	CCAUGAAGAGGGGAGACUUUU	1223	AAGUCUCCCCUCUUCAUGGUU	D74
1109	CCCAUGAAGAGGGGAGACUUU	1224	AGUCUCCCCUCUUCAUGGGUU	D75
1110	UUCCCAUGAAGAGGGGAGAUU	1225	UCUCCCCUCUUCAUGGGAAUU	D76
1111	UUUCCCAUGAAGAGGGGAGUU	1226	CUCCCCUCUUCAUGGGAAAUU	D77
1112	AACCCUUCUUUAAUGUCAUUU	1227	AUGACAUUAAAGAAGGGUUUU	D78
1113	UUGUUGGACUGGUGUGCCAUU	1228	UGGCACACCAGUCCAACAAUU	D79
1114	UAUAUUGGUGCUGUUGGACUU	1229	GUCCAACAGCACCAAUAUAUU	D80
1115	UUAGCGAUGCUCACUGGGGUU	1230	CCCCAGUGAGCAUCGCUAAUU	D81
1116	UCAAAGGCUGUAGCGAUGCUU	1231	GCAUCGCUACAGCCUUUGAUU	D82

SEQ ID NO:	Antisense Sequence (5' → 3')	SEQ ID NO:	Sense Sequence (5' → 3')	Duplex ID No.
1117	UGAGUUCCUGGAAGCCUUCUU	1232	GAAGGCUUCCAGGAACUCAUU	D83
1118	UAAGGCUUCUGAGUGGUACUU	1233	GUACCACUCAGAAGCCUUAUU	D84
1119	UUUUCUUGGCCUCUUCGGUUU	1234	ACCGAAGAGGCCAAGAAAAUU	D85
1120	UUUGAUCUGUUUCUUGGCCUU	1235	GGCCAAGAAACAGAUCAAAUU	D86
1121	UGUUGAUCUGUUUCUUGGCUU	1236	GCCAAGAAACAGAUCAACAUU	D87
1122	UACAAUUUUCCCUUGAGUAUU	1237	UACUCAAGGGAAAAUUGUAUU	D88
1123	UUGUCUCUGUCAAGCUCCUUU	1238	AGGAGCUUGACAGAGACAAUU	D89
1124	UUUAAACAUGCCUAAACGCUU	1239	GCGUUUAGGCAUGUUUAAAUU	D90
1125	UGAUGUUAAACAUGCCUAAUU	1240	UUAGGCAUGUUUAACAUCAUU	D91
1126	UAAGAAGAUGGCGGUGGCAUU	1241	UGCCACCGCCAUCUUCUUAUU	D92
1127	UGUGAGUUCAUUUUCCAGGUU	1242	CCUGGAAAAUGAACUCACAUU	D93
1128	UAACUUGGUGAUGAUAUCGUU	1243	CGAUAUCAUCACCAAGUUAUU	D94
1129	UAUUUUCCAGGAACUUGGUUU	1244	ACCAAGUUCCUGGAAAAUAUU	D95
1130	UAUAGGUUCCAGUAAUGGAUU	1245	UCCAUUACUGGAACCUAUAUU	D96
1131	UUCUUCAGAUCAUAGGUUCUU	1246	GAACCUAUGAUCUGAAGAAUU	D97
1132	UAGGUCAGCCCCAUUGCUGUU	1247	CAGCAAUGGGGCUGACCUAUU	D98
1133	UAGAGGUCAGCCCCAUUGCUU	1248	GCAAUGGGGCUGACCUCUAUU	D99
1134	UUUCAGGGGUGCCUCCUCUUU	1249	AGAGGAGGCACCCCUGAAAUU	D100
1135	UAGAGCUUCAGGGGUGCCUUU	1250	AGGCACCCCUGAAGCUCUAUU	D101
1136	UCUUAUGCACGGCCUUGGAUU	1251	UCCAAGGCCGUGCAUAAGAUU	D102
1137	UCCUUAUGCACGGCCUUGGUU	1252	CCAAGGCCGUGCAUAAGGAUU	D103
1138	UGAUGGUCAGCACAGCCUUUU	1253	AAGGCUGUGCUGACCAUCAUU	D104
1139	UUCGAUGGUCAGCACAGCCUU	1254	GGCUGUGCUGACCAUCGAAUU	D105
1140	UUAAAAACAUGGCCCCAGCUU	1255	GCUGGGGCCAUGUUUUUAAUU	D106
1141	UCUCUAAAAACAUGGCCCCUU	1256	GGGGCCAUGUUUUUAGAGAUU	D107
1142	UCCUCUAAAAACAUGGCCCUU	1257	GGGCCAUGUUUUUAGAGGAUU	D108
1143	UAUAGACAUGGGUAUGGCCUU	1258	GGCCAUACCCAUGUCUAUAUU	D109
1144	UGUUUGUUGAACUUGACCUUU	1259	AGGUCAAGUUCAACAAACAUU	D110

SEQ ID NO:	Antisense Sequence (5' → 3')	SEQ ID NO:	Sense Sequence (5' → 3')	Duplex ID No.
1145	UACAAAGGGUUUGUUGAACUU	1260	GUUCAACAAACCCUUUGUAUU	D111
1146	UAUUAAGAAGACAAAGGGUUU	1261	ACCCUUUGUCUUCUUAAUAUU	D112
1147	UAAGAGGGGAGACUUGGUAUU	1262	UACCAAGUCUCCCCUCUUAUU	D113
1148	UCAUGAAGAGGGGAGACUUUU	1263	AAGUCUCCCCUCUUCAUGAUU	D114
1149	UCCAUGAAGAGGGGAGACUUU	1264	AGUCUCCCCUCUUCAUGGAUU	D115

AAT RNAi agents were evaluated by transfection of Hep3B cells, a human hepatocellular carcinoma line. Cells were plated at ~10,000 cells per well in 96-well format, and each of the 115 AAT RNAi agent duplexes was transfected at three concentrations (10 nM, 1 nM, and 0.1 nM), using LipoFectamine RNAiMax (Thermo Fisher) transfection reagent. Relative expression of each of the 115 AAT RNAi agents was determined by qRT-PCR by comparing the expression levels of AAT mRNA to an endogenous control, and normalized to untreated Hep3B cells ($\Delta\Delta C_T$ analysis), as shown in Table 9.

Table 9. *In Vitro* Data from Duplexes of Example 2

Duplex ID No. (From Table 8)	**Avg. Rel. Exp. 10 nM**	**Avg. Rel. Exp. 1 nM**	**Avg. Rel. Exp. 0.1 nM**
D1	1.037	0.896	0.709
D2	0.068	0.089	0.381
D3	0.046	0.064	0.403
D4	0.075	0.090	0.391
D5	0.408	0.424	0.743
D6	0.018	0.032	0.347
D7	0.069	0.125	0.666
D8	0.092	0.193	0.794
D9	0.206	0.228	0.839
D10	0.023	0.032	0.235
D11	0.309	0.522	0.894
D12	0.049	0.092	0.732
D13	0.549	0.665	0.955
D14	0.531	0.654	0.934
D15	0.108	0.197	0.820
D16	0.558	0.516	0.834
D17	0.626	0.606	0.841
D18	0.668	0.703	0.778
D19	0.624	0.803	0.785
D20	0.071	0.080	0.506
D21	0.022	0.037	0.345
D22	0.086	0.127	0.588

Duplex ID No. (From Table 8)	Avg. Rel. Exp. 10 nM	Avg. Rel. Exp. 1 nM	Avg. Rel. Exp. 0.1 nM
D23	0.175	0.238	0.893
D24	0.134	0.078	0.368
D25	0.056	0.075	0.687
D26	0.122	0.196	0.756
D27	0.517	0.560	0.846
D28	0.801	0.838	0.884
D29	0.820	0.870	0.903
D30	0.558	0.632	0.879
D31	1.112	1.110	0.922
D32	0.246	0.359	1.041
D33	0.107	0.355	0.967
D34	0.096	0.170	0.962
D35	0.317	0.552	0.949
D36	0.064	0.134	0.873
D37	0.463	1.005	1.006
D38	0.428	0.688	0.486
D39	0.730	0.918	1.258
D40	0.059	0.067	0.912
D41	0.093	0.095	0.952
D42	0.582	0.665	0.944
D43	0.196	0.283	1.004
D44	0.195	0.278	0.860
D45	0.053	0.103	0.817
D46	0.082	0.127	1.034
D47	0.089	0.156	0.821
D48	0.735	0.695	0.838
D49	0.604	0.610	0.838
D50	0.543	0.633	0.806
D51	0.114	0.144	0.775
D52	0.108	0.203	0.836

Duplex ID No. (From Table 8)	Avg. Rel. Exp. 10 nM	Avg. Rel. Exp. 1 nM	Avg. Rel. Exp. 0.1 nM
D53	1.062	0.836	0.931
D54	0.091	0.274	1.081
D55	0.526	0.623	0.914
D56	0.500	0.588	0.884
D57	0.049	0.126	0.797
D58	0.198	0.302	0.917
D59	0.732	0.745	0.953
D60	0.389	0.580	0.897
D61	0.585	0.624	1.802
D62	0.174	0.215	1.115
D63	0.093	0.074	0.917
D64	0.133	0.133	1.055
D65	0.395	0.362	0.986
D66	0.054	0.055	1.083
D67	0.105	0.118	1.018
D68	0.106	0.122	1.290
D69	0.201	0.194	1.062
D70	0.050	0.048	0.709
D71	0.231	0.216	0.767
D72	0.046	0.030	0.737
D73	0.521	0.423	0.782
D74	0.479	0.467	0.694
D75	0.531	0.583	0.794
D76	0.210	0.285	0.924
D77	0.152	0.181	0.803
D78	0.425	0.485	0.703
D79	0.120	0.127	0.711
D80	0.203	0.167	0.672
D81	0.477	0.402	0.611
D82	0.540	0.489	0.661

Duplex ID No. (From Table 8)	Avg. Rel. Exp. 10 nM	Avg. Rel. Exp. 1 nM	Avg. Rel. Exp. 0.1 nM
D83	0.315	0.316	0.838
D84	0.135	0.118	0.375
D85	0.209	0.270	1.050
D86	0.120	0.136	0.928
D87	0.172	0.207	1.056
D88	0.218	0.308	1.006
D89	0.605	0.643	0.925
D90	0.205	0.259	0.927
D91	0.594	1.097	1.052
D92	0.337	0.887	1.015
D93	0.068	0.503	0.864
D94	0.067	0.475	0.811
D95	0.186	0.770	0.931
D96	0.062	0.389	0.550
D97	0.066	0.470	0.896
D98	0.567	0.998	1.044
D99	0.451	1.092	1.359
D100	0.292	0.745	0.875
D101	0.049	0.320	0.659
D102	0.313	0.799	0.732
D103	0.068	0.541	0.630
D104	0.077	0.552	0.682
D105	0.071	0.355	0.459
D106	1.179	1.117	1.076
D107	0.328	0.597	0.876
D108	0.125	0.467	0.573
D109	0.141	0.545	0.753
D110	0.076	0.497	0.778
D111	0.132	0.511	0.634
D112	0.216	0.586	0.784

Duplex ID No. (From Table 8)	Avg. Rel. Exp. 10 nM	Avg. Rel. Exp. 1 nM	Avg. Rel. Exp. 0.1 nM
D113	0.462	0.687	1.021
D114	0.507	0.792	1.170
D115	0.259	0.797	1.027

Example 3. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in PiZ Mice.***

A transgenic PiZ mouse model (PiZ mice) was used to evaluate AAT RNAi agents *in vivo*. PiZ mice harbor the human PiZ AAT mutant allele and model human AATD (Carlson et al., Journal of Clinical Investigation 1989).

NAG-conjugated AAT RNAi agents were prepared in a pharmaceutically acceptable saline buffer and administered to PiZ mice to evaluate knockdown of AAT gene expression. On day 1, each mouse received a single subcutaneous (SQ) dose into the loose skin on the back between the shoulders of 5.0 mg/kg (mpk) of either AD04446, AD04447, AD04448, AD04449, AD04450, AD04451, AD04454, AD04455, AD04456, AD04457, AD04458, or AD04459. (*See* Tables 4-7 for the modified AAT RNAi agents and NAG ligand structures). AAT RNAi agents AD04451 and AD04459 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000; AAT RNAi agents AD04446 and AD04454 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1142; AAT RNAi agents AD04447 and AD04455 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1211; AAT RNAi agents AD04448 and AD04456 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1326; AAT RNAi agents AD04449 and AD04457 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1338; and AAT RNAi agents AD04450 and AD04458 included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1427. (*See also* Tables 1 and 2). Three mice were dosed with each AAT RNAi agent (n = 3).

Plasma samples were drawn and analyzed for AAT (Z-AAT) protein levels on day 1 (pre-dose), day 8, day 15, day 22, day 29, and day 36. AAT levels were normalized to day 1

(pre-dose) AAT plasma levels. Protein levels were measured by quantifying circulating human Z-AAT levels in plasma by a commercially available ELISA kit according to the manufacturer's recommendations. The average normalized AAT (Z-AAT) levels for each RNAi agent are reported in the following Table 10:

Table 10. Average Normalized AAT Protein (Normalized to Pre-Treatment) from Example 3

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 1 (5.0 mg/kg AD04447)	1.010	0.256	1.050	0.108	1.451	0.137	1.145	0.154	1.117	0.080
Group 2 (5.0 mg/kg AD04448)	0.884	0.262	0.866	0.276	1.306	0.112	1.147	0.119	1.076	0.172
Group 3 (5.0 mg/kg AD04449)	0.909	0.060	0.969	0.152	1.290	0.185	1.290	0.201	1.245	0.106
Group 4 (5.0 mg/kg AD04450)	0.595	0.083	0.799	0.131	1.099	0.256	1.090	0.346	1.229	0.444
Group 5 (5.0 mg/kg AD04451)	0.282	0.006	0.525	0.020	1.358	0.188	1.767	0.325	1.586	0.297
Group 6 (5.0 mg/kg AD04455)	0.656	0.126	0.639	0.039	0.741	0.089	0.738	0.235	0.819	0.156
Group 7 (5.0 mg/kg AD04456)	0.605	0.129	0.469	0.036	0.717	0.105	0.662	0.097	0.875	0.195
Group 8 (5.0 mg/kg AD04457)	0.501	0.108	0.663	0.091	1.031	0.324	1.176	0.368	1.603	0.597
Group 9 (5.0 mg/kg AD04458)	0.308	0.081	0.174	0.031	0.177	0.010	0.211	0.010	0.345	0.041
Group 10 (5.0 mg/kg AD04459)	0.256	0.021	0.134	0.045	0.174	0.084	0.234	0.174	0.315	0.336

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 11 (5.0 mg/kg AD04446)	0.686	0.178	0.739	0.130	0.973	0.263	0.955	0.107	0.885	0.119
Group 12 (5.0 mg/kg AD04454)	0.338	0.014	0.361	0.105	0.602	0.252	0.729	0.266	0.970	0.245

As shown from the data in Table 10, above, while AAT RNAi agent AD04447 showed essentially no reduction in AAT protein, AAT RNAi agents AD04458 (which included a modified nucleotide sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1427) and AD04459 (which included a modified nucleotide sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000) showed a substantial reduction in AAT protein across all timepoints. For example, AD04458 showed knockdown of approximately 69% at day 8 (0.308); approximately 83% at day 15 (0.174), and approximately 82% at day 22 (0.177). Additionally, for example, AD04459 showed a knockdown of approximately 74% at day 8 (0.256), approximately 87% at day 15 (0.134), and approximately 83% at day 22 (0.174).

Example 4. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in Cynomolgus Monkeys***

NAG-conjugated AAT RNAi agents were made and combined in a pharmaceutically acceptable saline buffer as known in the art for subcutaneous (SC) injection. On day 1, cynomolgus macaque (*Macaca fascicularis*) primates (referred to herein as "cynos" or "monkeys") were injected subcutaneously with 3 mg/kg of either AD04824, AD04825, AD04826, or AD04827 (*see* Tables 4-7 for the modified AAT RNAi agents and NAG ligand structures). Each of these AAT RNAi agents included a modified nucleotide sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000, and was cross-reactive with cynos. Three monkeys in each group were tested (n=3).

Serum samples from treated cynomolgus monkeys were taken on day -7 and day 1 (pre-dose), and on days 8, 15, 22, and 29 to monitor knockdown. Day 36 was also measured for cynos that were injected with AD04825 and AD04826. At the indicated time points, blood samples were drawn and analyzed for cynomolgus monkey AAT (cAAT). Blood was collected from the femoral vein. cAAT levels were determined on a Cobas Integra 400 Plus (Roche Diagnostics) according to the manufacturer's recommendations. AAT levels for each animal at a respective time point was divided by the pre-treatment level (average of day -7 and day 1 (pre-dose)) of expression in that animal to determine the ratio of expression "normalized to pre-dose."

Normalized cynomolgus monkey AAT (cAAT) protein levels after treatment with each

respective AAT RNAi agent are reported in the following Table 11:

Table 11. Normalized cAAT Protein (Normalized to Pre-Treatment) from Example 4 in Cynomolgus Monkeys.

	Day 8	**Day 15**	**Day 22**	**Day 29**	**Day 36**
Group ID	**cAAT**	**cAAT**	**cAAT**	**cAAT**	**cAAT**
Group 1, Cyno A (3.0 mg/kg AD04824)	0.62	0.52	0.45	0.52	
Group 1, Cyno B (3.0 mg/kg AD04824)	0.60	0.36	0.32	0.32	
Group 1, Cyno C (3.0 mg/kg AD04824)	0.62	0.44	0.41	0.41	
Group 2, Cyno A (3.0 mg/kg AD04825)	0.58	0.33	0.24	0.24	0.22
Group 2, Cyno B (3.0 mg/kg AD04825)	0.58	0.38	0.27	0.25	0.27
Group 2, Cyno C (3.0 mg/kg AD04825)	0.79	0.58	0.43	0.43	0.44
Group 3, Cyno A (3.0 mg/kg AD04826)	0.75	0.59	0.44	0.42	0.38
Group 3, Cyno B (3.0 mg/kg AD04826)	0.66	0.43	0.30	0.26	0.24
Group 3, Cyno C (3.0 mg/kg AD04826)	0.62	0.36	0.27	0.25	0.25
Group 4, Cyno A (3.0 mg/kg AD04827)	0.57	0.38	0.26	0.26	
Group 4, Cyno B (3.0 mg/kg AD04827)	0.61	0.37	0.34	0.34	
Group 4, Cyno C (3.0 mg/kg AD04827)	0.66	0.43	0.41	0.39	

Average normalized cAAT levels for each of the respective treatment groups is shown in the bar graph of Figure 9. As illustrated in Table 11, above, and in Figure 9, each of the AAT RNAi agents tested showed substantial knockdown of cAAT in cynomolgus monkeys across all time points measured.

Example 5. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in Cynomolgus Monkeys***

NAG-conjugated AAT RNAi agents were made and combined in a pharmaceutically acceptable saline buffer as known in the art for subcutaneous (SC) injection. On day 1, cynomolgus macaque (*Macaca fascicularis*) primates were injected subcutaneously with 3 mg/kg of either AD04828, AD04831, AD04836, or AD04837 (*see* Tables 4-7 for the modified AAT RNAi agents and NAG ligand structures). Each of these AAT RNAi agents included a modified nucleotide sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000, and was cross-reactive with cynos. Three monkeys in each group were tested (n=3) for AD04828 and AD04831, and two monkeys in each group were tested (n=2) for AD04836 and AD04837.

Serum samples from treated cynos were taken on day -35 and day 1 (pre-dose), and on days 8, 15, 21, and 29 to monitor knockdown. At the indicated time points, blood samples were drawn and analyzed for cAAT. Blood was collected from the femoral vein. cAAT levels were determined on a Cobas Integra 400 Plus (Roche Diagnostics) according to the manufacturer's recommendations. cAAT levels for each animal at a respective time point was divided by the pre-treatment level (average of day -35 and day 1 (pre-dose)) of expression in that animal to determine the ratio of expression "normalized to pre-treatment".

Normalized cynomolgus monkey AAT (cAAT) protein levels after treatment with each respective AAT RNAi agent are reported in the following Table 12:

Table 12. Normalized AAT Protein (Normalized to Pre-Treatment) from Example 5 in Cynomolgus Monkeys

	Day 8	**Day 15**	**Day 22**	**Day 29**
Group ID	**cAAT**	**cAAT**	**cAAT**	**cAAT**
Group 1, Cyno A (3.0 mg/kg AD04828)	0.60	0.32	0.25	0.23
Group 1, Cyno B (3.0 mg/kg AD04828)	0.67	0.59	0.61	0.76
Group 1, Cyno C (3.0 mg/kg AD04828)	0.51	0.35	0.29	0.29
Group 2, Cyno A (3.0 mg/kg AD04831)	0.68	0.43	0.32	0.28
Group 2, Cyno B (3.0 mg/kg AD04831)	0.71	0.49	0.47	0.44
Group 2, Cyno C (3.0 mg/kg AD04831)	0.61	0.43	0.34	0.30
Group 3, Cyno A (3.0 mg/kg AD04836)	0.61	0.37	0.27	0.23
Group 3, Cyno B (3.0 mg/kg AD04836)	0.67	0.43	0.32	0.27
Group 4, Cyno A (3.0 mg/kg AD04837)	0.65	0.40	0.28	0.24
Group 4, Cyno B (3.0 mg/kg AD04837)	0.55	0.29	0.20	0.17

Average normalized cAAT levels for each of the respective treatment groups is shown in the bar graph of Figure 10. As shown above in Table 12, as well as in the bar graph of Figure 10, each of the AAT RNAi agents tested showed substantial knockdown of cAAT in cynomolgus monkeys across all time points measured.

Example 6. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in PiZ Mice***

The transgenic PiZ mouse model (PiZ mice) as set forth in Example 3 was used to evaluate AAT RNAi agents *in vivo*. NAG-conjugated AAT RNAi agents were prepared in a pharmaceutically acceptable saline buffer and administered to PiZ mice to evaluate knockdown of AAT gene expression. On day 1, each mouse received a single subcutaneous (SQ) dose into the loose skin on the back between the shoulders of 2.0 mg/kg (mpk) of either AD04824, AD04828, AD04829, AD04830, AD04831, AD04832, AD04833, AD04834, AD04836, AD04837, AD04838, AD04839, or AD04857. (*See* Tables 4-7 for the modified AAT RNAi agents and NAG ligand structures). Each of the AAT RNAi agents in this study included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000. (*See also* Tables 1 and 2). Three mice were dosed with each AAT RNAi agent (n = 3).

Plasma samples were drawn and analyzed for AAT (Z-AAT) protein levels on days -2, day 1 (pre-dose), day 8, day 15, day 22, day 29, and day 36. AAT levels were normalized to day 1 (pre-dose) AAT plasma levels. Protein levels were measured by quantifying circulating human Z-AAT levels in plasma by a commercially available ELISA kit according to the manufacturer's recommendations. The average normalized AAT (Z-AAT) levels for each RNAi agent are reported in the following Table 13:

Table 13. Average Normalized AAT Protein (Normalized to Pre-Treatment) from Example 6

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 1 (2.0 mg/kg AD04824)	0.105	0.036	0.140	0.067	0.204	0.108	0.313	0.104	0.437	0.229
Group 2 (2.0 mg/kg AD04828)	0.141	0.055	0.236	0.111	0.304	0.138	0.624	0.289	0.814	0.139
Group 3 (2.0 mg/kg AD04829)	0.109	0.072	0.119	0.102	0.140	0.119	0.141	0.116	0.179	0.145
Group 4 (2.0 mg/kg AD04830)	0.147	0.095	0.190	0.148	0.307	0.192	0.521	0.424	0.547	0.202
Group 5 (2.0 mg/kg AD04831)	0.154	0.104	0.215	0.171	0.449	0.375	0.701	0.519	0.584	0.418
Group 6 (2.0 mg/kg AD04832)	0.088	0.032	0.089	0.048	0.090	0.046	0.117	0.071	0.193	0.131
Group 7 (2.0 mg/kg AD04833)	0.168	0.029	0.282	0.047	0.448	0.048	0.748	0.223	1.361	0.346
Group 8 (2.0 mg/kg AD04834)	0.159	0.037	0.255	0.159	0.470	0.315	0.662	0.346	0.728	0.141
Group 9 (2.0 mg/kg AD04836)	0.108	0.035	0.070	0.024	0.083	0.032	0.090	0.035	0.168	0.078
Group 10 (2.0 mg/kg AD04837)	0.157	0.071	0.209	0.104	0.242	0.097	0.417	0.198	0.550	0.193

	Day 8		Day 15		Day 22		Day 29		Day 36	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 11 (2.0 mg/kg AD04838)	0.106	0.017	0.099	0.022	0.108	0.039	0.158	0.072	0.188	0.050
Group 12 (2.0 mg/kg AD04839)	0.096	0.026	0.069	0.019	0.089	0.036	0.120	0.038	0.186	0.083
Group 12 (2.0 mg/kg AD04857)	0.272	0.130	0.302	0.145	0.478	0.187	0.815	0.436	1.772	1.412

As shown from the data in Table 13, above, each of the AAT RNAi agent showed a substantial reduction in AAT protein through at least day 29. For example, at day 15, each of the AAT RNAi agents tested achieved at least approximately 70% knockdown of protein compared to pre-treatment levels, with multiple groups achieving 90% or better knockdown.

Example 7. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in PiZ Mice***

The transgenic PiZ mouse model described in Example 3 was used. Each mouse was 5 weeks old at the beginning of the study. NAG-conjugated AAT RNAi agents were prepared in a pharmaceutically acceptable saline buffer and administered to PiZ mice to evaluate knockdown of AAT gene expression. Starting on day 1, each mouse received a subcutaneous (SQ) dose q2w (i.e., one injection every two weeks, for a total of 4 injections) into the loose skin on the back between the shoulders of 4.0 mg/kg (mpk) of either: (1) saline vehicle; (2) AD04837 (*see* Tables 4-7 for the modified AAT RNAi agent and NAG ligand structures), which as noted previously included a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000; or (3) a NAG-conjugated RNAi agent that included a nucleotide sequence targeting the HBV gene, to be used as a negative control. Single subcutaneous injections for the saline vehicle group, AAT RNAi agent group, and HBV RNAi agent group were performed on days 1, 15, 29, and 43. Seven (7) mice were dosed q2w with the saline vehicle (Group 1); nine (9) mice were dosed q2w with the AAT RNAi agent (Group 2); and six (6) mice were dosed with the HBV RNAi agent (Group 3). The mice in the three treatment groups were sacrificed on day 57 (13 weeks old). In addition to the treatment groups, seven (7) mice were sacrificed at week 1 of the study (i.e., 5-week old mice) to serve as a baseline control.

Plasma samples were drawn and analyzed for AAT (Z-AAT) protein levels on day 1 (pre-dose), day 8, day 15, day 22, day 29, and day 36 for all groups. Additional samples for the AAT RNAi agent group and the saline vehicle group were drawn on day 43, day 50, and day 57. AAT levels were normalized to day 1 (pre-dose) AAT plasma levels. Protein levels were measured by quantifying circulating human Z-AAT levels in plasma by a commercially available ELISA kit according to the manufacturer's recommendations. The average normalized AAT (Z-AAT) levels for the saline vehicle and each RNAi agent are reported in the following Table 14:

Table 14. Average Normalized AAT Protein (Normalized to Pre-Treatment) from Example 7

	Day 8		**Day 15**		**Day 22**		**Day 29**	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 1 (saline vehicle) (n=7)	0.876	0.172	1.264	0.386	1.234	0.457	1.319	0.453
Group 2 (AD04837) (n=9)	0.139	0.050	0.146	0.064	0.067	0.029	0.072	0.038
Group 3 (HBV RNAi agent – negative control) (n=6)	1.212	0.360	1.019	0.201	1.540	0.155	1.585	0.640
	Day 36		**Day 43**		**Day 50**		**Day 57**	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 1 (saline vehicle) (n=7)	1.267	0.491	1.441	0.416	1.172	0.340	1.058	0.299
Group 2 (AD04837) (n=9)	0.040	0.011	0.051	0.020	0.034	0.007	0.038	0.009
Group 3 (HBV RNAi agent – negative control) (n=6)	1.665	0.476	1.943	0.221	1.580	0.491	2.001	0.770

As shown from the data in Table 14, above, the HBV RNAi agent performed successfully as a negative control showing essentially no AAT inhibition. Further, the NAG-conjugated AAT RNAi agent (AD04837) achieved significant knockdown of expression compared to saline and the HBV RNAi agent negative control across all timepoints. In dosing q2w, the AAT RNAi agent in Example 7 showed a knockdown of approximately 96% of AAT protein at day 36 (0.040) and maintained a similar level of knockdown through day 57.

In addition to monitoring serum AAT levels, homogenized liver tissue from PiZ mice treated with NAG-conjugated AAT RNAi agent (AD04837) was further analyzed to determine if both soluble Z-AAT (which is expected to be predominantly monomeric protein), and insoluble polymers of Z-AAT (which is expected to be polymeric protein) were effectively reduced. A modified western blot protocol was used to separate the soluble and insoluble Z-AAT fractions under non-denaturing conditions as previously described and known in the art (*see, e.g.*, Mueller et al., Molecular Therapy, March 2012, 20(3): 590-600).

A western blot was prepared to examine certain livers of the sacrificed mice. Specifically, livers were examined of (i) 6 baseline mice; (ii) 5 AAT RNAi agent mice; and (iii) 4 saline mice. (The gels used for the western blot analysis included 15 wells). The samples for the animals used for this western blot were randomly selected from the various groups. Figures 11 and 12 show bar graphs reflecting the Z-AAT polymer and Z-AAT monomer levels quantified from the western blot analysis.

As seen from the bar graph in Figure 11, which reports the monomeric protein levels, when compared to baseline each of the mice dosed with AAT RNAi agent shown a significant reduction in AAT monomeric protein across all time points, indicating significant inhibition of the gene. Further, as shown in Figure 12, which reports the polymeric protein levels, the animals treated with the saline vehicle continued to have increased polymeric AAT burden after 8 weeks. Conversely, the animals treated with AAT RNAi agent showed a reduction in polymeric burden of approximately 50% over the course of 8 weeks as compared to the baseline (5-week-old) mice, indicating that the administration of NAG-conjugated AAT RNAi agent (AD04837) is capable of preventing and potentially reversing the production of polymeric AAT protein.

Example 8. ***In Vivo Testing of NAG-Conjugated AAT RNAi Agents in PiZ Mice***

The transgenic PiZ mouse model described in Example 3 was used to evaluate RNAi agents *in vivo*. Each mouse received a single subcutaneous (SQ) dose on day 1 into the loose skin on the back between the shoulders of either: (1) saline; (2) 1.0 mg/kg of the NAG-conjugated AAT RNAi agent of AD04837 (which includes a modified nucleotide antisense strand sequence designed to target an AAT gene (SEQ ID NO: 1) at position 1000); (3) 2.0 mg/kg of AD04837; (4) 4.0 mg/kg of AD04837; or (5) 8.0 mg/kg of AD04837. Four animals were dosed in group 1 (saline), and all four were sacrificed on day 43. Fifteen (15) animals were dosed in each of groups 2, 3, 4, and 5, and 3 animals from each group were sacrificed on day 8, day 15, day 22, day 29, and day 43, respectively.

Plasma samples were drawn and analyzed for AAT (Z-AAT) protein levels on day 1 (pre-dose), day 8, day 15, day 22, day 29, day 36, and day 43 for all groups. For the sacrificed mice, cardiac sticks were performed for serum isolation for Z-AAT protein level assessment (200 µl plasma). AAT levels were normalized to day 1 (pre-dose) AAT plasma levels. Protein levels were measured by quantifying circulating human Z-AAT levels in plasma by a commercially available ELISA kit according to the manufacturer's recommendations. The average normalized AAT (Z-AAT) levels for the saline vehicle and each RNAi agent dosing group are reported in the following Table 15:

Table 15. Average Normalized Plasma AAT Protein (Normalized to Pre-Treatment) from Example 8.

	Day 8		Day 15		Day 22		Day 29	
Group ID	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**	**Avg AAT**	**Std Dev (+/-)**
Group 1 (saline vehicle)	1.240	0.633	1.037	0.256	0.884	0.229	0.857	0.286
Group 2 (1.0 mg/kg AD04837)	0.266	0.100	0.250	0.107	0.259	0.060	0.412	0.191
Group 3 (2.0 mg/kg AD04837)	0.170	0.102	0.162	0.132	0.199	0.161	0.511	0.514
Group 4 (4.0 mg/kg AD04837)	0.051	0.015	0.038	0.010	0.051	0.021	0.110	0.045
Group 5 (8.0 mg/kg AD04837)	0.030	0.011	0.025	0.010	0.040	0.024	0.063	0.030

	Day 36		Day 43	
Group 1 (saline vehicle)	1.485	0.431	0.932	0.243
Group 2 (1.0 mg/kg AD04837)	0.791	0.207	0.560	0.111
Group 3 (2.0 mg/kg AD04837)	0.600	0.140	0.595	0.217
Group 4 (4.0 mg/kg AD04837)	0.156	0.008	0.148	0.022
Group 5 (8.0 mg/kg AD04837)	0.239	0.183	0.202	0.119

As shown from the data in Table 15, above, the NAG-conjugated AAT RNAi agent achieved significant knockdown of expression compared to saline across all timepoints measured at all dosing levels tested.

In addition, AAT mRNA levels were also assessed for the sacrificed mice at each respective timepoint. As described above, for Groups 2 through 5 (i.e., the RNAi agent groups), 3 mice were sacrificed on each of days 8, 15, 22, 29 and 43; and for Group 1, all 4 mice were sacrificed on day 43. Half of the left lateral liver lobe was collected and snap-frozen in liquid nitrogen for RNA isolation.

Table 16. Relative AAT mRNA Levels in PiZ Mice Following Administration of a Single SQ Injection of Saline or AAT RNAi Agent

Treatment Group	Day	Animals	Average Relative mRNA Expression	Low Variance	High Variance
Group 1 (saline vehicle)	43	n=4	1.000	0.071	0.076
Group 2 (1.0 mg/kg AD04837)	8	n=3	0.412	0.080	0.099
	15	n=3	0.419	0.037	0.040
	22	n=3	0.483	0.066	0.076
	29	n=3	0.696	0.069	0.076
	43	n=3	0.813	0.103	0.118
Group 3 (2.0 mg/kg AD04837)	8	n=3	0.272	0.101	0.160
	15	n=3	0.235	0.039	0.046
	22	n=3	0.327	0.099	0.141
	29	n=3	0.587	0.155	0.210
	43	n=3	0.845	0.123	0.145
Group 4 (4.0 mg/kg AD04837)	8	n=3	0.129	0.025	0.031
	15	n=3	0.161	0.017	0.020
	22	n=3	0.222	0.048	0.061
	29	n=3	0.247	0.067	0.093
	43	n=3	0.454	0.051	0.057
Group 5 (8.0 mg/kg AD04837)	8	n=3	0.078	0.013	0.015
	15	n=3	0.055	0.014	0.019
	22	n=3	0.077	0.009	0.010
	29	n=3	0.116	0.038	0.056

Treatment Group	Day	Animals	Average Relative mRNA Expression	Low Variance	High Variance
	43	n=3	0.332	0.122	0.193

As shown in Table 16, above, relative AAT mRNA expression levels were significantly reduced across all timepoints measured compared to saline vehicle. For example, on day 15, Group 2 (1.0 mg/kg AAT RNAi agent) showed approximately 58% reduction of AAT mRNA levels (0.419); Group 3 (2.0 mg/kg AAT RNAi agent) showed approximately 67% reduction of AAT mRNA levels (0.327); Group 4 (4.0 mg/kg AAT RNAi agent) showed approximately 84% reduction of AAT mRNA levels (0.161); and Group 5 (8.0 mg/kg AAT RNAi agent) showed approximately 94% reduction of Z-AAT mRNA levels (0.055) upon a single SQ dose at day 1.

OTHER EMBODIMENTS

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

CLAIMS

1. An RNAi agent for inhibiting the expression of an alpha-1 antitrypsin (AAT) gene, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the antisense strand comprises nucleotides 2-18 of any of the antisense strand sequences in Table 2, Table 3, or Table 4, and wherein the sense strand is at least substantially complementary to the antisense strand.

2. An RNAi agent for inhibiting the expression of an alpha-1 antitrypsin (AAT) gene, wherein the RNAi agent comprises a sense strand and an antisense strand, wherein the sense strand comprises nucleotides 2-18 of any of the sense strand sequences in Table 2, Table 3, or Table 5, and wherein the antisense strand is at least substantially complementary to the sense strand.

3. The RNAi agent of claim 1, wherein antisense strand comprises the nucleotide sequence of any of the antisense strand sequences in Table 2, Table 3, or Table 4.

4. The RNAi agent of claim 2, wherein sense strand comprises the nucleotide sequence of any of the antisense strand sequences in Table 2, Table 3, or Table 5.

5. The RNAi agent of claim 1, wherein the sense strand comprises the nucleotide sequence of any of the sense strand sequences in Table 4, and the antisense strand comprises the nucleotide sequence of any of the antisense strand sequences in Table 5.

6. The RNAi agent of any of claims 1-5, wherein the RNAi agent comprises at least one modified nucleotide.

7. The RNAi agent of any of claim 6, wherein the at least one 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 abasic nucleotide, inverted 2′-OMe nucleotide, inverted 2′-deoxy nucleotide, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholino nucleotide, vinyl phosphonate deoxyribonucleotide, and 3′-OMe nucleotide.

8. The RNAi agent of any of claims 1-5, wherein the RNAi agent comprises at least one phosphorothioate internucleoside linkage.

9. The RNAi agent of any of claims 1-5, wherein the sense strand comprises at least one phosphorothioate internucleoside linkage.

10. The RNAi agent of any of claims 1-5, wherein the antisense strand contains one, two, three, or four phosphorothioate internucleoside linkages.

11. The RNAi agent of any of claims 1-5, wherein all or substantially all of the nucleotides of the sense strand and the antisense strand are modified nucleotides.

12. The RNAi agent of any of claims 1-11, wherein the sense strand is no more than 30 nucleotides in length, and the antisense strand is no more than 30 nucleotides in length.

13. The RNAi agent of any of claims 1-12, wherein the sense strand is no more than 24 nucleotides in length, and the antisense strand is no more than 24 nucleotides in length.

14. The RNAi agent of any of claims 1-13, wherein the sense strand and the antisense strand are each between 18 and 24 nucleotides in length.

15. The RNAi agent of any of claims 1-14, wherein the sense strand and the antisense strand are each 21 nucleotides in length.

16. The RNAi agent of any of claims 1-15, wherein the RNAi agent has two blunt ends.

17. The RNAi agent of any of claims 1-16, wherein the RNAi agent comprises at least one modified nucleotide and further comprises one or more targeting groups or linking groups.

18. The RNAi agent of any of claims 1-17, wherein the RNAi agent comprises at least one modified nucleotide and further comprises one or more targeting groups or linking groups, and wherein the targeting groups or linking groups have the structure of any of the structures represented in Table 7.

19. The RNAi agent of claim 18, wherein the one or more targeting groups or linking groups are conjugated to the sense strand.

20. The RNAi agent of any of claims 1-19, wherein the RNAi agent comprises a targeting group that includes an asialoglycoprotein receptor ligand.

21. The RNAi agent of claim 20, wherein the asialoglycoprotein receptor ligand comprises N-acetyl-galactosamine.

22. The RNAi agent of claim 21, wherein the asialoglycoprotein receptor ligand comprises an N-acetyl-galactosamine trimer.

23. The RNAi agent of any of claims 1-22, wherein the RNAi agent comprises at least one modified nucleotide and further comprises one or more targeting groups, wherein the targeting group has a structure selected from the group consisting of: (NAG25), (NAG25)s, (NAG26), (NAG26)s, (NAG27), (NAG27)s, (NAG28), (NAG28)s, (NAG29), (NAG29)s, (NAG30), (NAG30)s, (NAG31), (NAG31)s, (NAG32), (NAG32)s, (NAG33), (NAG33)s, (NAG34), (NAG34)s, (NAG35), (NAG35)s, (NAG36), (NAG36)s, (NAG37), (NAG37)s, (NAG38), (NAG38)s, (NAG39), (NAG39)s.

24. The RNAi agent of any of claims 17-23, wherein the RNAi agent comprises a targeting group that is conjugated to the 5' terminal end of the sense strand.

25. The RNAi agent of claim 1 or claim 2, wherein the RNAi agent is comprised of a sense strand and antisense strand forming a duplex having the structure of any of the duplexes in Table 6.

26. The RNAi agent of claim 1 or claim 2, wherein the RNAi agent has the duplex structure selected from the group consisting of: AD04824, AD04825, AD04826, AD04827, AD04828, AD04829, AD04830, AD04831, AD04832, AD04833, AD04834, AD04835, AD04836, and AD04837.

27. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein all or substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand comprises the sequence (5′ → 3′) AGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 866), wherein all or substantially all of the nucleotides of the sense strand are modified nucleotides.

28. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein all or substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand comprises the sequence (5′ → 3′) CGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 857), wherein all or substantially all of the nucleotides of the sense strand are modified nucleotides.

29. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein all or substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand comprises the sequence (5′ → 3′) GCGUUUAGGCAUGUUUAACAUU (SEQ ID NO: 885), wherein all or substantially all of the nucleotides of the sense strand are modified nucleotides.

30. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein all or substantially all of the nucleotides of the antisense strand are modified nucleotides, and wherein the sense strand comprises the sequence (5′ → 3′) CGCGUUUAGGCAUGUUUAACA (SEQ ID NO: 864), wherein all or substantially all of the nucleotides of the sense strand are modified nucleotides.

31. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCU (SEQ ID NO: 801), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 801 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

32. The RNAi agent of claim 31, wherein the antisense strand comprises the nucleotide sequence (5′ → 3′) usGfsuUfaAfacaugCfcUfaAfaCfgCfsu (SEQ ID NO: 960), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage.

33. The RNAi agent of claim 32, wherein the sense strand comprises the sequence (5′ → 3′) agcguuuaGfGfCfauguuuaaca (SEQ ID NO: 1279), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one or two inverted abasic deoxyribose residues (invAb) and/or one, two, three, or four phosphorothioate internucleoside linkages; and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

34. The RNAi agent of claim 33, wherein the RNAi agent has the duplex structure of AD04837 (SEQ ID PAIR NOs: 960/1033).

35. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGUU (SEQ ID NO: 794), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 794 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

36. The RNAi agent of claim 35, wherein the antisense strand of the RNAi agent comprises the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgusu (SEQ ID NO: 913), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage.

37. The RNAi agent of claim 36, wherein the sense strand comprises the sequence (5′ → 3′) cguuuaGfGfCfauguuuaacausu (SEQ ID NO: 1276), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one or two inverted abasic deoxyribose residues (invAb) and/or one, two, three, or four phosphorothioate internucleoside linkages; and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

38. The RNAi agent of claim 37, wherein the RNAi agent has the duplex structure of AD04828 (SEQ ID PAIR NOs: 913/1028).

39. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCUU (SEQ ID NO: 839), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 839 is located at positions 1 to 22 (5′ → 3′) of the antisense strand.

40. The RNAi agent of claim 39, wherein the antisense strand of the RNAi agent comprises the sequence (5′ → 3′) usGfsusUfaAfaCfaUfgCfcUfaAfaCfgcusu (SEQ ID NO: 958), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage.

41. The RNAi agent of claim 40, wherein the sense strand comprises the sequence (5′ → 3′) gcguuuaGfGfCfauguuuaacausu (SEQ ID NO: 1277), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one or two inverted abasic deoxyribose residues (invAb) and/or one, two, three, or four phosphorothioate internucleoside linkages; and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

42. The RNAi agent of claim 41, wherein the RNAi agent has the duplex structure of AD04831 (SEQ ID PAIR NOs: 958/1030).

43. The RNAi agent of claim 1, wherein the antisense strand comprises the sequence (5′ → 3′) UGUUAAACAUGCCUAAACGCG (SEQ ID NO: 800), wherein all or substantially all of the nucleotides are modified nucleotides, and wherein SEQ ID NO: 800 is located at positions 1 to 21 (5′ → 3′) of the antisense strand.

44. The RNAi agent of claim 43, wherein the antisense strand of the RNAi agent comprises the sequence (5′ → 3′) usGfsuUfaAfaCfaUfgCfcUfaAfaCfgsCfsg (SEQ ID NO: 959), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; and s is a phosphorothioate linkage.

45. The RNAi agent of claim 44, wherein the sense strand comprises the sequence (5′ → 3′) cgcguuuaGfGfCfauguuuaaca (SEQ ID NO: 1278), wherein a, c, g, and u are 2′-O-methyl adenosine, cytidine, guanosine, or uridine, respectively; Af, Cf, Gf, and Uf are 2′-fluoro adenosine, cytidine, guanosine, or uridine, respectively; s is a phosphorothioate linkage; wherein optionally present on the sense strand is one or two inverted abasic deoxyribose residues (invAb) and/or one, two, three, or four phosphorothioate internucleoside linkages; and wherein optionally linked to the 5′ terminal end of the sense strand is a targeting ligand that includes N-acetyl-galactosamine.

46. The RNAi agent of claim 45, wherein the RNAi agent has the duplex structure of AD04836 (SEQ ID PAIR NOs: 959/1024).

47. A composition comprising the RNAi agent of any of claims 1-46, and at least one pharmaceutically acceptable excipient.

48. The composition of claim 47, further comprising one or more additional therapeutics or treatments.

49. The composition of any of claims 47-48, wherein the composition is packaged in a kit, container, pack, dispenser, pre-filled syringes, or vials.

50. The composition of any of claims 47-49, wherein the composition is formulated for administration by subcutaneous injection.

51. The composition of claim 47, wherein the composition includes one or more duplexes having the structure selected from the group consisting of: AD04824, AD04825, AD04826, AD04827, AD04828, AD04829, AD04830, AD04831, AD04832, AD04833, AD04834, AD04835, AD04836, and AD04837.

52. A method for inhibiting the expression of an AAT gene in a cell, the method comprising administering an effective amount of an RNAi agent of any of claims 1-46.

53. A method for inhibiting the expression of an AAT gene in a subject, the method comprising administering to the subject an effective amount of an RNAi agent of any of claims 1-46 or a composition of any of claims 47-51.

54. A method for the treatment of alpha-1 antitrypsin deficiency (AATD), the method comprising administering to a subject in need thereof a therapeutically effective amount of an RNAi agent of any of claims 1-46 or a composition of claims 47-51.

55. A method for the treatment of a condition or disease caused by alpha-1 antitrypsin deficiency (AATD), the method comprising administering to a subject in need thereof a therapeutically effective amount of an RNAi agent of any of claims 1-46 or a composition of claims 47-51.

56. The method of claim 55, wherein the condition or disease caused by AAT is a liver disease.

57. The method of claim 56, wherein the liver disease is chronic hepatitis, cirrhosis, hepatocellular carcinoma, transaminitis, cholestasis, fibrosis, or fulminant hepatic failure.

58. A method of decreasing or reducing the level of insoluble AAT protein in a subject compared to baseline pre-treatment levels, the method comprising administering to the subject an effective amount an RNAi agent of any of claims 1-46 or a composition of claims 47-51.

59. The method of any of claims 52-58, wherein the RNAi agent is administered to the subject subcutaneously.

60. The method of any of claims 52-59, further comprising measuring serum AAT protein levels (soluble and/or insoluble) or AAT mRNA levels in the subject.

61. The method of any of claims 52-60, wherein the serum AAT protein levels (soluble and/or insoluble) and/or the AAT mRNA levels in the subject are decreased.

62. The RNAi agent of any of claims 1-46 for use in a method of treating a subject.

63. The RNAi agent of any of claims 1-46 for use in a method of treating a subject having AATD.

64. The composition of any of claims 47-51 for use in a method of treating a subject.

65. The composition of any of claims 47-51 for use in a subject having AATD.

66. The RNAi agent of any of claims 1-46 for use in use in the inhibition of expression of AAT mRNA in a cell.