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(54) Title: RNAI AGENTS, COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING TRANSTHYRETIN (TTR) ASSOCIATED DISEASES

(57) Abstract: The present invention provides RNAi agents, e.g., double stranded RNAi agents, that target the transthyretin (TTR) gene and methods of using such RNAi agents for treating or preventing TTR- associated diseases.

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RNAi AGENTS, COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING TRANSTHYRETIN (TTR) ASSOCIATED DISEASES

Related Applications

This application claims priority to U.S. Provisional Application No. 61/561,710, filed on November 18, 2011, U.S. Provisional Application No. 61/615,618, filed on March 26, 2012, and U.S. Provisional Application No. 61/680,098, filed on August 6, 2012, the entire contents of each of which are hereby incorporated herein by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 13, 2012, is named 121301WO.txt and is 541,508 bytes in size.

Background of the Invention

Transthyretin (TTR) (also known as prealbumin) is found in serum and cerebrospinal fluid (CSF). TTR transports retinol-binding protein (RBP) and thyroxine (T4) and also acts as a carrier of retinol (vitamin A) through its association with RBP in the blood and the CSF. Transthyretin is named for its **trans**port of **thy**roxine and **retin**ol. TTR also functions as a protease and can cleave proteins including apoA-I (the major HDL apolipoprotein), amyloid β-peptide, and neuropeptide Y. See Liz, M.A. *et al.* (2010) *IUBMB Life*, 62(6):429-435.

TTR is a tetramer of four identical 127-amino acid subunits (monomers) that are rich in beta sheet structure. Each monomer has two 4-stranded beta sheets and the shape of a prolate ellipsoid. Antiparallel beta-sheet interactions link monomers into dimers. A short loop from each monomer forms the main dimer-dimer interaction. These two pairs of loops separate the opposed, convex beta-sheets of the dimers to form an internal channel.

The liver is the major site of TTR expression. Other significant sites of expression include the choroid plexus, retina (particularly the retinal pigment epithelium) and pancreas.

Transthyretin is one of at least 27 distinct types of proteins that is a precursor protein in the formation of amyloid fibrils. See Guan, J. *et al.* (Nov. 4, 2011) Current perspectives on cardiac amyloidosis, *Am J Physiol Heart Circ Physiol*, doi:10.1152/ajpheart.00815.2011. Extracellular deposition of amyloid fibrils in organs and tissues is the hallmark of amyloidosis. Amyloid fibrils are composed of misfolded protein aggregates, which may result from either excess production of or specific mutations in precursor proteins. The amyloidogenic potential of TTR may be related to its extensive beta sheet structure; X-ray crystallographic studies indicate that certain amyloidogenic mutations destabilize the tetrameric structure of the protein. See, *e.g.*, Saraiva M.J.M. (2002) *Expert Reviews in Molecular Medicine*, 4(12):1-11.

Amyloidosis is a general term for the group of amyloid diseases that are characterized by amyloid deposits. Amyloid diseases are classified based on their precursor protein; for example, the name starts with "A" for amyloid and is followed by an abbreviation of the precursor protein, *e.g.*, ATTR for **a**mloidogenic **t**rans**thyr**etin. *Ibid.*

There are numerous TTR-associated diseases, most of which are amyloid diseases. Normal-sequence TTR is associated with cardiac amyloidosis in people who are elderly and is termed senile systemic amyloidosis (SSA) (also called senile cardiac amyloidosis (SCA) or cardiac amyloidosis). SSA often is accompanied by microscopic deposits in many other organs. TTR amyloidosis manifests in various forms. When the peripheral nervous system is affected more prominently, the disease is termed familial amyloidotic polyneuropathy (FAP). When the heart is primarily involved but the nervous system is not, the disease is called familial amyloidotic cardiomyopathy (FAC). A third major type of TTR amyloidosis is leptomeningeal amyloidosis, also known as leptomeningeal or meningocerebrovascular amyloidosis, central nervous system (CNS) amyloidosis, or amyloidosis VII form. Mutations in TTR may also cause amyloidotic vitreous opacities, carpal tunnel syndrome, and euthyroid hyperthyroxinemia, which is a non-amyloidotic disease thought to be secondary to an increased association of

thyroxine with TTR due to a mutant TTR molecule with increased affinity for thyroxine. See, *e.g.*, Moses *et al.* (1982) *J. Clin. Invest.*, 86, 2025-2033.

Abnormal amyloidogenic proteins may be either inherited or acquired through somatic mutations. Guan, J. *et al.* (Nov. 4, 2011) Current perspectives on cardiac amyloidosis, *Am J Physiol Heart Circ Physiol*, doi:10.1152/ajpheart.00815.2011. Transthyretin associated ATTR is the most frequent form of hereditary systemic amyloidosis. Lobato, L. (2003) *J. Nephrol.*, 16:438-442. TTR mutations accelerate the process of TTR amyloid formation and are the most important risk factor for the development of ATTR. More than 85 amyloidogenic TTR variants are known to cause systemic familial amyloidosis. TTR mutations usually give rise to systemic amyloid deposition, with particular involvement of the peripheral nervous system, although some mutations are associated with cardiomyopathy or vitreous opacities. *Ibid.*

The V30M mutation is the most prevalent TTR mutation. See, *e.g.*, Lobato, L. (2003) *J Nephrol*, 16:438-442. The V122I mutation is carried by 3.9% of the African American population and is the most common cause of FAC. Jacobson, D.R. et al. (1997) *N. Engl. J. Med.* 336 (7): 466–73. It is estimated that SSA affects more than 25% of the population over age 80. Westermark, P. et al. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87** (7): 2843–5.

Accordingly, there is a need in the art for effective treatments for TTR-associated diseases.

Summary of the Invention

The present invention provides RNAi agents, e.g., double stranded RNAi agents, targeting the Transthyretin (TTR) gene. The present invention also provides methods of inhibiting expression of TTR and methods of treating or preventing a TTR-associated disease in a subject using the RNAi agents, e.g. double stranded RNAi agents, of the invention. The present invention is based, at least in part, on the discovery that RNAi agents that comprise particular chemical modifications show a superior ability to inhibit expression of TTR. Agents including a certain pattern of chemical modifications (*e.g.*, an alternating pattern) and a ligand are shown herein to be effective in silencing the activity of the TTR gene. Furthermore, agents including one or more motifs of three

identical modifications on three consecutive nucleotides, including one such motif at or near the cleavage site of the agents, show surprisingly enhanced TTR gene silencing activity. When a single such chemical motif is present in the agent, it is preferred to be at or near the cleavage region for enhancing of the gene silencing activity. Cleavage region is the region surrounding the cleavage site, *i.e.*, the site on the target mRNA at which cleavage occurs.

Accordingly, in one aspect, the present invention features RNAi agents, e.g., double stranded RNAi agents, for inhibiting expression of a transthyretin (TTR). The double stranded RNAi agent includes a sense strand complementary to an antisense strand. The antisense strand includes a region complementary to a part of an mRNA encoding transthyretin. Each strand has 14 to 30 nucleotides, and the double stranded RNAi agent is represented by formula (III):

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

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

(III).

In Formula III, i, j, k, and l are each independently 0 or 1; p, p', q, and q' are each independently 0-6; each N_a and N_a' independently represents an oligonucleotide sequence including 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence including at least two differently modified nucleotides; each N_b and N_b' independently represents an oligonucleotide sequence including 0-10 nucleotides which are either modified or unmodified or combinations thereof; each n_p, n_p', n_q, and n_q' independently represents an overhang nucleotide; XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represents one motif of three identical modifications on three consecutive nucleotides; modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'. In some embodiments, the sense strand is conjugated to at least one ligand, *e.g.*, at least one ligand, *e.g.*, at least one ligand attached to the 3' end of the sense strand. In other embodiments, the ligand may be conjugated to the antisense strand.

In some embodiments, i is 1; j is 1; or both i and j are 1.

In some embodiments, k is 1; l is 1; or both k and l are 1.

In some embodiments, i is 0; j is 1.

In some embodiments, i is 1, j is 0.

In some embodiments, k is 0; l is 1.

In some embodiments, k is 1; l is 0.

In some embodiments, XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand.

In some embodiments, the Y′Y′Y′ motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

In some embodiments, the Y′ is 2′-O-methyl.

In some embodiments, the Y' is 2'-fluoro.

In some embodiments, formula (III) is represented as formula (IIIa):

sense: 5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

antisense: 3' n_p′-N_a′-Y′Y′Y′-N_b′-Z′Z′Z′-N_a′n_q′ 5'

(IIIa).

In formula IIIa, each N_b and N_b′ independently represents an oligonucleotide sequence including 1-5 modified nucleotides.

In some embodiments, formula (III) is represented as formula (IIIb):

sense: 5' n_p-N_a-X X X -N_b-Y Y Y -N_a-n_q 3'

antisense: 3' n_p′-N_a′-X′X′X′-N_b′-Y′Y′Y′-N_a′-n_q′ 5'

(IIIb).

In formula IIIb each N_b and N_b′ independently represents an oligonucleotide sequence including 1-5 modified nucleotides.

In some embodiments, formula (III) is represented as formula (IIIc):

sense: 5' n_p-N_a-X X X -N_b-Y Y Y -N_b-Z Z Z -N_a-n_q 3'

antisense: 3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_b'-Z′Z′Z′-N_a'-n_q' 5'

(IIIc).

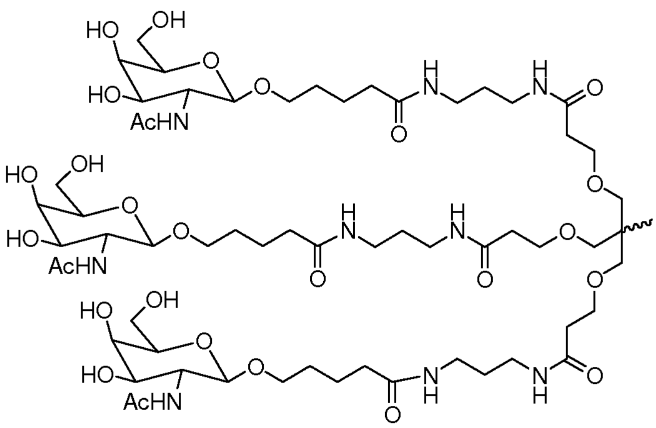
In formula IIIc, each N_b and -N_b' independently represents an oligonucleotide sequence including 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence including 2-10 modified nucleotides.

In many embodiments, the duplex region is 15-30 nucleotide pairs in length. In some embodiments, the duplex region is 17-23 nucleotide pairs in length, 17-25 nucleotide pairs in length, 23-27 nucleotide pairs in length, 19-21 nucleotide pairs in length, or 21-23 nucleotide pairs in length.

In certain embodiments, each strand has 15-30 nucleotides.

In some embodiments, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C-allyl, 2′-fluoro, 2′-deoxy, 2’-hydroxyl, and combinations thereof. In some preferred embodiments, the modifications on the nucleotides are 2′-O-methyl or 2′-fluoro.

In some embodiments, the ligand is one or more N-acetylgalactosamine (GalNAc) derivatives attached through a bivalent or trivalent branched linker. In particular embodiments, the ligand is



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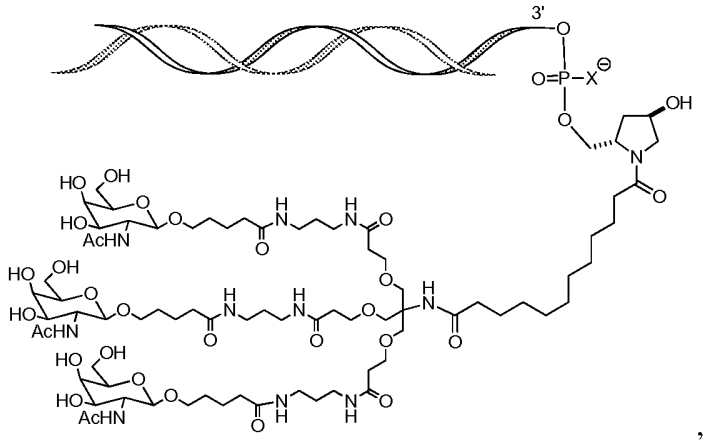
In some embodiments, the ligand is attached to the 3′ end of the sense strand.

In some embodiments, the RNAi agent is conjugated to the ligand as shown in the following schematic



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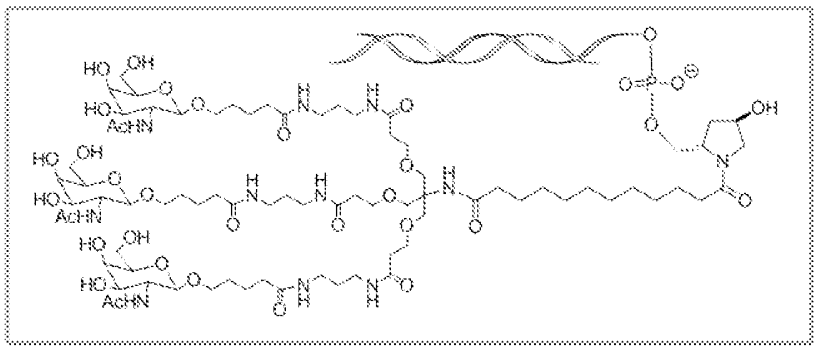
wherein X is O or S.

In some embodiments, the RNAi agent is conjugated to the ligand as shown in the following schematic



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In some embodiments, the RNAi agent further includes at least one phosphorothioate or methylphosphonate internucleotide linkage. In some embodiments, the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminal of one strand. In some embodiments, the strand is the antisense strand. In other embodiments, the strand is the sense strand.

In certain embodiments, the base pair at the 1 position of the 5′-end of the duplex is an AU base pair.

In some embodiments, the Y nucleotides contain a 2′-fluoro modification.

In some embodiments, the Y′ nucleotides contain a 2′-O-methyl modification.

In some embodiments, p′>0. In some such embodiments, each n is complementary to the target mRNA. In other such embodiments, each n is non-complementary to the target mRNA. In some embodiments, p, p', q and q' are 1-6. In some preferred embodiments, p' = 1 or 2. In some preferred embodiments, p′=2. In some such embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA. In other such embodiments, q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

In some embodiments, the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

In certain embodiments, linkages between n_p' include phosphorothioate linkages. In some such embodiments, the linkages between n_p' are phosphorothioate linkages.

In some embodiments, the RNAi agent is selected from the group of agents listed in Table 1.

In preferred embodiments, the RNAi agent is selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547.

In an even more preferred embodiment, the RNAi agent is AD-51547 having the following structure:

sense: 5'- UfgGfgAfuUfuCfAfUfgUfaacCfaAfgAfL96-3' (SEQ ID NO:2)

antisense: 5'- uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc-3' (SEQ ID NO:3)

wherein lowercase nucleotides (a, u, g, c) indicate 2'-O-methyl nucleotides; Nf (e.g., Af) indicates a 2'-fluoro nucleotide; s indicates a phosphothiorate linkage; L96 indicates a $GalNAc_3$ ligand.

In another aspect, the present invention features a cell containing the RNAi agent for inhibiting expression of TTR.

In a further aspect, the present invention features a pharmaceutical composition comprising an RNAi agent for inhibiting expression of TTR. In some embodiments, the pharmaceutical composition is a solution comprising the RNAi agent. In some embodiments, the solution comprising the RNAi agent is an unbuffered solution, e.g., saline solution or water. In other embodiments, the solution is a buffered solution, e.g., a solution of phosphate buffered saline (PBS). In other embodiments, the pharmaceutical composition is a liposome or a lipid formulation. In some embodiments, the lipid formulation comprises a XTC or MC3.

In yet another aspect, the present invention features methods of inhibiting expression of transthyretin (TTR) in a cell. The methods include contacting a cell with an RNAi agent, e.g., a double stranded RNAi agent, in an amount effective to inhibit expression of TTR in the cell, thereby inhibiting expression of TTR in the cell.

In some embodiments, the expression of TTR is inhibited by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

In other embodiments, the cell is contacted *in vitro* with the RNAi agent. In other embodiments, the cell is present within a subject. In preferred embodiments, the subject is a human.

In further embodiments, the subject is a subject suffering from a TTR-associated disease and the effective amount is a therapeutically effective amount. In other embodiments, the subject is a subject at risk for developing a TTR-associated disease and the effective amount is a prophylactically effective amount. In some embodiments,

a subject at risk for develping a TTR-associated disease is a subject who carries a TTR gene mutation that is associated with the development of a TTR-associated disease.

In certain embodiments, the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

In some embodiments, the subject has a TTR-associated amyloidosis and the method reduces an amyloid TTR deposit in the subject.

In other embodiments, the RNAi agent is administered to the subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In certain embodiments, the RNAi agent is administered to the subject via subcutaneous or intravenous administration. In preferred embodiments, the RNAi agent is administered to the subject via subcutaneous administration. In some such embodiments, the subcutaneous administration includes administration via a subcutaneous pump or subcutaneous depot.

In certain embodiments, the RNAi agent is administered to the subject such that the RNAi agent is delivered to a specific site within the subject. In some embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. In preferred embodiments, the site is the liver. In some embodiments, the delivery of the RNAi agent is mediated by asialoglycoprotein receptor (ASGP-R) present in hepatocytes.

In some embodiments, the RNAi agent is administered at a dose of between about 0.25 mg/kg to about 50 mg/kg, *e.g.*, between about 0.25 mg/kg to about 0.5 mg/kg, between about 0.25 mg/kg to about 1 mg/kg, between about 0.25 mg/kg to about 5 mg/kg, between about 0.25 mg/kg to about 10 mg/kg, between about 1 mg/kg to about 10 mg/kg, between about 5 mg/kg to about 15 mg/kg, between about 10 mg/kg to about 20 mg/kg, between about 15 mg/kg to about 25 mg/kg, between about 20 mg/kg to about

30 mg/kg, between about 25 mg/kg to about 35 mg/kg, or between about 40 mg/kg to about 50 mg/kg.

In some embodiments, the RNAi agent is administered at a dose of about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 26 mg/kg, about 27 mg/kg, about 28 mg/kg, about 29 mg/kg, 30 mg/kg, about 31 mg/kg, about 32 mg/kg, about 33 mg/kg, about 34 mg/kg, about 35 mg/kg, about 36 mg/kg, about 37 mg/kg, about 38 mg/kg, about 39 mg/kg, about 40 mg/kg, about 41 mg/kg, about 42 mg/kg, about 43 mg/kg, about 44 mg/kg, about 45 mg/kg, about 46 mg/kg, about 47 mg/kg, about 48 mg/kg, about 49 mg/kg or about 50 mg/kg.

In some embodiments, the RNAi agent is administered in two or more doses. In particular embodiments, the RNAi agent is administered at intervals selected from the group consisting of once every about 2 hours, once every about 3 hours, once every about 4 hours, once every about 6 hours, once every about 8 hours, once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, once every about 96 hours, once every about 120 hours, once every about 144 hours, once every about 168 hours, once every about 240 hours, once every about 336 hours, once every about 504 hours, once every about 672 hours and once every about 720 hours.

In other embodiments, the method further includes assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

In preferred embodiments, administering the RNAi agent does not result in an inflammatory response in the subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from the subject.

In some embodiments, the RNAi agent is administered using a pharmaceutical composition

In preferred embodiments, the RNAi agent is administered in a solution. In some such embodiments, the siRNA is administered in an unbuffered solution. In one embodiment, the siRNA is administered in water. In other embodiments, the siRNA is administered with a buffer solution, such as an acetate buffer, a citrate buffer, a prolamine buffer, a carbonate buffer, or a phosphate buffer or any combination thereof. In some embodiments, the buffer solution is phosphate buffered saline (PBS).

In another embodiment, the pharmaceutical composition is a liposome or a lipid formulation comprising SNALP or XTC. In one embodiment, the lipid formulation comprises an MC3.

In another aspect, the invention provides methods of treating or preventing a TTR-associated disease in a subject. The methods include administering to the subject a therapeutically effective amount or prophylactically effective amount of an RNAi agent, e.g., a double stranded RNAi agent, thereby treating or preventing the TTR-associated disease in the subject.

In some embodiments, TTR expression in a sample derived from the subject is inhibited by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% or at least about 70% at least about 80%, or at least about 90%.

In some embodiments, the subject is a human.

In some embodiments, the subject is a subject suffering from a TTR-associated disease. In other embodiments, the subject is a subject at risk for developing a TTR-associated disease.

In some embodiments, the subject is a subject who carries s a TTR gene mutation that is associated with the development of a TTR-associated disease.

In certain embodiments, the TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

In some embodiments, the subject has a TTR-associated amyloidosis and the method reduces an amyloid TTR deposit in the subject.

In some embodiments, the RNAi agent is administered to the subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In certain embodiments, the RNAi agent is administered to the subject via subcutaneous or intravenous administration. In preferred embodiments, the RNAi agent is administered to the subject via subcutaneous administration. In some such embodiments, the subcutaneous administration includes administration via a subcutaneous pump or subcutaneous depot.

In certain embodiments, the RNAi agent is administered to the subject such that the RNAi agent is delivered to a specific site within the subject. In some such embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. In preferred embodiments, the site is the liver. In some embodiments, the delivery of the RNAi agent is mediated by asialoglycoprotein receptor (ASGP-R) present in hepatocytes.

In some embodiments, the RNAi agent is administered at a dose of between about 0.25 mg/kg to about 50 mg/kg, *e.g.*, between about 0.25 mg/kg to about 0.5 mg/kg, between about 0.25 mg/kg to about 1 mg/kg, between about 0.25 mg/kg to about 5 mg/kg, between about 0.25 mg/kg to about 10 mg/kg, between about 1 mg/kg to about 10 mg/kg, between about 5 mg/kg to about 15 mg/kg, between about 10 mg/kg to about 20 mg/kg, between about 15 mg/kg to about 25 mg/kg, between about 20 mg/kg to about 30 mg/kg, between about 25 mg/kg to about 35 mg/kg, or between about 40 mg/kg to about 50 mg/kg.

In some embodiments, the RNAi agent is administered at a dose of about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 26 mg/kg, about 27 mg/kg, about 28 mg/kg, about 29 mg/kg, 30 mg/kg, about 31 mg/kg, about 32 mg/kg, about 33 mg/kg, about 34 mg/kg, about 35 mg/kg, about 36 mg/kg, about 37 mg/kg, about 38 mg/kg, about 39 mg/kg, about 40 mg/kg, about 41 mg/kg, about 42 mg/kg, about 43 mg/kg, about 44 mg/kg, about 45 mg/kg, about 46 mg/kg, about 47 mg/kg, about 48 mg/kg, about 49 mg/kg or about 50 mg/kg.

In some embodiments, the RNAi agent is administered in two or more doses. In particular embodiments, the RNAi agent is administered at intervals selected from the group consisting of once every about 2 hours, once every about 3 hours, once every about 4 hours, once every about 6 hours, once every about 8 hours, once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, once every about 96 hours, once every about 120 hours, once every about 144 hours, once every about 168 hours, once every about 240 hours, once every about 336 hours, once every about 504 hours, once every about 672 hours and once every about 720 hours.

In other embodiments, the method further includes assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

In preferred embodiments, administering the RNAi agent does not result in an inflammatory response in the subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from the subject.

In some embodiments, the RNAi agent is administered using a pharmaceutical composition, *e.g.*, a liposome.

In some embodiments, the RNAi agent is administered in a solution. In some such embodiments, the siRNA is administered in an unbuffered solution. In one embodiment, the siRNA is administered in saline or water. In other embodiments, the siRNA is administred with a buffer solution, such as an acetate buffer, a citrate buffer, a prolamine buffer, a carbonate buffer, or a phosphate buffer or any combination thereof. In some embodiments, the buffer solution is phosphate buffered saline (PBS).

In another aspect, the present invention provides a method of inhibiting expression of transthyretin (TTR) in a cell, including contacting a cell with an RNAi agent, e.g., a double stranded RNAi agent, in an amount effective to inhibit expression of TTR in the cell. In one aspect, the double stranded RNAi agent is selected from the group of agents listed in Table 1, thereby inhibiting expression of transthyretin (TTR) in the cell.

In another aspect, the present invention provides a method of inhibiting expression of transthyretin (TTR) in a cell, including contacting a cell with an RNAi agent, e.g., a double stranded RNAi agent, in an amount effective to inhibit expression of TTR in the cell. In one aspect, the double stranded RNAi agent is selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547, thereby inhibiting expression of transthyretin (TTR) in the cell.

In a further aspect, the present invention provides a method of treating or preventing a TTR-associated disease in a subject, including administering to the subject a therapeutically effective amount or a prophylactically effective amount of an RNAi agent, e.g., a double stranded RNAi agent. In one aspect, the double stranded RNAi agent is selected from the group of agents listed in Table 1, thereby treating or preventing a TTR-associated disease in the subject.

In yet another aspect, the present invention provides a method of treating or preventing a TTR-associated disease in a subject, including administering to the subject a therapeutically effective amount or a prophylactically effective amount of an RNAi agent, e.g., a double stranded RNAi agent. In one aspect, the double stranded RNAi

agent is selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547, thereby treating or preventing a TTR-associated disease in the subject.

In further aspects, the invention provides kits for performing the methods of the invention. In one aspect, the invention provides a kit for performing a method of inhibiting expression of transthyretin (TTR) in a cell comprising contacting a cell with an RNAi agent, e.g., a double stranded RNAi agent, in an amount effective to inhibit expression of said TTR in said cell, thereby inhibiting the expression of TTR in the cell. The kit comprises an RNAi agent and instructions for use and, optionally, means for administering the RNAi agent to the subject.

The present invention is further illustrated by the following detailed description and drawins.

Brief Description of the Drawings

Figure 1 is a graph depicting that administering to mice a single subcutaneous dose of a GalNAc-conjugated RNAi agent targeting TTR resulted in dose-dependent suppression of TTR mRNA.

Figure 2 is a graph depicting that administering to mice a single subcutaneous dose of 7.5 mg/kg or 30 mg/kg of a GalNAc conjugated RNAi agent targeting TTR resulted in long lasting suppression of TTR mRNA.

Figure 3 depicts the human TTR mRNA sequence.

Figure 4 is a graph depicting improved silencing activity of RNAi agents modified relative to the parent AD-45163.

Figure 5 is a graph depicting improved silencing activity of RNAi agents modified relative to the parent AD-45165.

Figure 6 is a graph depicting improved free uptake silencing following 4 hour incubation with RNAi agents modified relative to the parent AD-45163.

Figure 7 is a graph depicting improved free uptake silencing following 24 hour incubation with RNAi agents modified relative to the parent AD-45163.

Figure 8 is a graph depicting improved free uptake silencing following 4 hour incubation with RNAi agents modified relative to the parent AD-45165.

Figure 9 is a graph depicting improved free uptake silencing following 24 hour incubation with RNAi agents modified relative to the parent AD-45165.

Figure 10 is a graph depicting silencing of TTR mRNA in transgenic mice that express hTTR V30M following administration of a single subcutaneous dose of RNAi agents AD-51544, AD-51545, AD-45163, AD-51546, AD-51547, or AD-45165.

Figure 11 is a graph depicting TTR protein suppression in transgenic mice that express hTTR V30M following administration of a single subcutaneous dose of 5 mg/kg or 1mg/kg of RNAi agents AD-51544, AD-51545, or AD-45163.

Figure 12 is a graph depicting TTR protein suppression in transgenic mice that express hTTR V30M following administration of a single subcutaneous dose of 5 mg/kg or 1mg/kg of RNAi agents AD-51546, AD-51547, or AD-45165.

Figure 13 depicts the protocol for post-dose blood draws in monkeys that received 5x5mg/kg RNAi agent (top line) or 1x25mg/kg RNAi agent (bottom line).

Figure 14 is a graph depicting suppression of TTR protein in non-human primates following subcutaneous administration of five 5 mg/kg doses (top panel) or a single 25mg/kg dose (bottom panel) of AD-45163, AD-51544, AD-51545, AD-51546, or AD-51547.

Figure 15 is a graph depicting suppression of TTR protein in non-human primates following subcutaneous administration of AD-51547 at 2.5 mg/kg (white squares), 5 mg/kg (black squares) or 10 mg/kg (patterned squares) per dose, or administration of PBS as a negative control (gray squares).

Detailed Description of the Invention

The present invention provides RNAi agents, e.g., double stranded RNAi agents, and compositions targeting the Transthyretin (TTR) gene. The present invention also provides methods of inhibiting expression of TTR and methods of treating or preventing a TTR-associated disease in a subject using the RNAi agents, e.g., double stranded RNAi agents, of the invention. The present invention is based, at least in part, on the discovery that RNAi agents that comprise particular chemical modifications show a superior ability to inhibit expression of TTR. Agents including a certain pattern of chemical modifications (*e.g.*, an alternating pattern) and a ligand are shown herein to be effective in silencing the activity of the TTR gene. Furthermore, agents including one or more motifs of three identical modifications on three consecutive nucleotides, including one such motif at or near the cleavage site of the agents, show surprisingly enhanced TTR gene silencing activity. When a single such chemical motif is present in the agent, it is preferred to be at or near the cleavage region for enhancing of the gene silencing activity. Cleavage region is the region surrounding the cleavage site, *i.e.*, the site on the target mRNA at which cleavage occurs.

I. Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

As used herein, a “transthyretin” (“TTR”) refers to the well known gene and protein. TTR is also known as prealbumin, HsT2651, PALB, and TBPA. TTR functions as a transporter of retinol-binding protein (RBP), thyroxine (T4) and retinol, and it also acts as a protease. The liver secretes TTR into the blood, and the choroid plexus secretes TTR into the cerebrospinal fluid. TTR is also expressed in the pancreas and the retinal pigment epithelium. The greatest clinical relevance of TTR is that both

normal and mutant TTR protein can form amyloid fibrils that aggregate into extracellular deposits, causing amyloidosis. See, *e.g.*, Saraiva M.J.M. (2002) *Expert Reviews in Molecular Medicine*, 4(12):1-11 for a review. The molecular cloning and nucleotide sequence of rat transthyretin, as well as the distribution of mRNA expression, was described by Dickson, P.W. et al. (1985) *J. Biol. Chem.* 260(13)8214-8219. The X-ray crystal structure of human TTR was described in Blake, C.C. et al. (1974) *J Mol Biol 88*, 1-12. The sequence of a human TTR mRNA transcript can be found at National Center for Biotechnology Information (NCBI) RefSeq accession number NM_000371. The sequence of mouse TTR mRNA can be found at RefSeq accession number NM_013697.2, and the sequence of rat TTR mRNA can be found at RefSeq accession number NM_012681.1

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a TTR gene, including mRNA that is a product of RNA processing of a primary transcription product.

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

"G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. "T" and "dT" are used interchangeably herein and refer to a deoxyribonucleotide wherein the nucleobase is thymine, *e.g.*, deoxyribothymine, 2'-deoxythymidine or thymidine. However, it will be understood that the term "ribonucleotide" or "nucleotide" or "deoxyribonucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

A "double stranded RNAi agent," double-stranded RNA (dsRNA) molecule, also referred to as "dsRNA agent," "dsRNA", "siRNA", "iRNA agent," as used interchangeably herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined below, nucleic acid strands. In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. In addition, as used in this specification, an "RNAi agent" may include ribonucleotides with chemical modifications; an RNAi agent may include substantial modifications at multiple nucleotides. Such modifications may include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "RNAi agent" for the purposes of this specification and claims.

In another embodiment, the RNAi agent may be a single-stranded siRNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides and are chemically modified. The design and testing of single-stranded siRNAs are described in U.S. Patent No. 8,101,348 and in Lima *et al.*, (2012) *Cell* 150: 883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima *et al.*, (2012) *Cell* 150;:883-894.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of

the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi agent may comprise one or more nucleotide overhangs. The term "siRNA" is also used herein to refer to an RNAi agent as described above.

In another aspect, the agent is a single-stranded antisense RNA molecule. An antisense RNA molecule is complementary to a sequence within the target mRNA. Antisense RNA can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. *et al.*, (2002) *Mol Cancer Ther* 1:347-355. The antisense RNA molecule may have about 15-30 nucleotides that are complementary to the target mRNA. For example, the antisense RNA molecule may have a sequence of at least 15, 16, 17, 18, 19, 20 or more contiguous nucleotides from one of the antisense sequences of Table 1.

As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of an RNAi agent when a 3'-end of one strand of the RNAi agent extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNAi agent, *i.e.*, no nucleotide overhang. A "blunt ended" RNAi agent is a dsRNA that is double-stranded over its entire length, *i.e.*, no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with nucleotide overhangs at one end (*i.e.*, agents with one overhang and one blunt end) or with nucleotide overhangs at both ends.

The term "antisense strand" refers to the strand of a double stranded RNAi agent which includes a region that is substantially complementary to a target sequence (*e.g.*, a human TTR mRNA). As used herein, the term "region complementary to part of an mRNA encoding transthyretin" refers to a region on the antisense strand that is substantially complementary to part of a TTR mRNA sequence. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are

most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, *e.g.,* within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Sequences can be "fully complementary" with respect to each when there is base-pairing of the nucleotides of the first nucleotide sequence with the nucleotides of the second nucleotide sequence over the entire length of the first and second nucleotide sequences. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize

under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes described herein.

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

The terms "complementary," "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (*e.g.*, an mRNA encoding TTR) including a 5' UTR, an open reading frame (ORF), or a 3' UTR. For example, a polynucleotide is complementary to at least a part of a TTR mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding TTR.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating," "suppressing" and other similar terms, and includes any level of inhibition.

The phrase "inhibiting expression of a TTR," as used herein, includes inhibition of expression of any TTR gene (such as, *e.g.*, a mouse TTR gene, a rat TTR gene, a monkey TTR gene, or a human TTR gene) as well as variants or mutants of a TTR gene. Thus, the TTR gene may be a wild-type TTR gene, a mutant TTR gene (such as a

mutant TTR gene giving rise to systemic amyloid deposition), or a transgenic TTR gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of a TTR gene" includes any level of inhibition of a TTR gene, *e.g.*, at least partial suppression of the expression of a TTR gene, such as an inhibition of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

The expression of a TTR gene may be assessed based on the level of any variable associated with TTR gene expression, *e.g.*, TTR mRNA level, TTR protein level, retinol binding protein level, vitamin A level, or the number or extent of amyloid deposits. Inhibition may be assessed by a decrease in an absolute or relative level of one or more of these variables compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

The phrase "contacting a cell with an RNAi agent," as used herein, includes contacting a cell by any possible means. Contacting a cell with an RNAi agent, e.g., a double stranded RNAi agent, includes contacting a cell *in vitro* with the RNAi agent or contacting a cell *in vivo* with the RNAi agent. The contacting may be done directly or indirectly. Thus, for example, the RNAi agent may be put into physical contact with the cell by the individual performing the method, or alternatively, the RNAi agent may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell *in vitro* may be done, for example, by incubating the cell with the RNAi agent. Contacting a cell *in vivo* may be done, for example, by injecting the RNAi agent into or near the tissue where the cell is located, or by injecting the RNAi agent into another area, *e.g.*, the bloodstream or the subcutaneous space, such that the

agent will subsequently reach the tissue where the cell to be contacted is located. For example, the RNAi agent may contain and/or be coupled to a ligand, *e.g.*, a $GalNAc_3$ ligand, that directs the RNAi agent to a site of interest, *e.g.*, the liver. Combinations of *in vitro* and *in vivo* methods of contacting are also possible. In connection with the methods of the invention, a cell might also be contacted *in vitro* with an RNAi agent and subsequently transplanted into a subject.

A "patient" or "subject," as used herein, is intended to include either a human or non-human animal, preferably a mammal, *e.g.*, a monkey. Most preferably, the subject or patient is a human.

A "TTR-associated disease," as used herein, is intended to include any disease associated with the TTR gene or protein. Such a disease may be caused, for example, by excess production of the TTR protein, by TTR gene mutations, by abnormal cleavage of the TTR protein, by abnormal interactions between TTR and other proteins or other endogenous or exogenous substances. A "TTR-associated disease" includes any type of TTR amyloidosis (ATTR) wherein TTR plays a role in the formation of abnormal extracellular aggregates or amyloid deposits. TTR-associated diseases include senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, amyloidotic vitreous opacities, carpal tunnel syndrome, and hyperthyroxinemia. Symptoms of TTR amyloidosis include sensory neuropathy (*e.g.*, paresthesia, hypesthesia in distal limbs), autonomic neuropathy (*e.g.*, gastrointestinal dysfunction, such as gastric ulcer, or orthostatic hypotension), motor neuropathy, seizures, dementia, myelopathy, polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, vitreous opacities, renal insufficiency, nephropathy, substantially reduced mBMI (modified Body Mass Index), cranial nerve dysfunction, and corneal lattice dystrophy.

"Therapeutically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a patient for treating a TTR associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease). The "therapeutically effective amount" may vary depending on the RNAi agent, how the

agent is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by TTR expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject who does not yet experience or display symptoms of a TTR-associated disease, but who may be predisposed to the disease, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Symptoms that may be ameliorated include sensory neuropathy (*e.g.*, paresthesia, hypesthesia in distal limbs), autonomic neuropathy (*e.g.*, gastrointestinal dysfunction, such as gastric ulcer, or orthostatic hypotension), motor neuropathy, seizures, dementia, myelopathy, polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, vitreous opacities, renal insufficiency, nephropathy, substantially reduced mBMI (modified Body Mass Index), cranial nerve dysfunction, and corneal lattice dystrophy. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. RNAi gents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In

certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes), the retina or parts of the retina (*e.g.*, retinal pigment epithelium), the central nervous system or parts of the central nervous system (*e.g.*, ventricles or choroid plexus), or the pancreas or certain cells or parts of the pancreas. In some embodiments, a "sample derived from a subject" refers tocerebrospinal fluid obtained from the subject. In preferred embodiments, a "sample derived from a subject" refers to blood or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue (or subcomponents thereof) or retinal tissue (or subcomponents thereof) derived from the subject.

II. RNAi Agents

The present invention provides RNAi agents with superior gene silencing activity. It is shown herein and in Provisional Application No. 61/561,710 (to which the present application claims priority) that a superior result may be obtained by introducing one or more motifs of three identical modifications on three consecutive nucleotides into a sense strand and/or antisense strand of a RNAi agent, particularly at or near the cleavage site. The sense strand and antisense strand of the RNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense and/or antisense strand. The RNAi agent also optionally conjugates with a GalNAc derivative ligand, for instance on the sense strand. The resulting RNAi agents present superior gene silencing activity.

The inventors surprisingly discovered that when the sense strand and antisense strand of the RNAi agent are completely modified, having one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a RNAi agent superiorly enhanced the gene silencing acitivity of the RNAi agent.

Accordingly, the invention provides RNAi agents, e.g., double stranded RNAi agents, capable of inhibiting the expression of a target gene (*i.e.*, a TTR gene) *in vivo*. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the

RNAi agent can range from 12-30 nucleotides in length. For example, each strand can be between 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length, 19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as an "RNAi agent." The duplex region of an RNAi agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be between 14-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17 - 23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19- 21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, and 27.

In one embodiment, the RNAi agent may contain one or more overhang regions and/or capping groups of RNAi agent at 3'-end, or 5'-end or both ends of a strand. The overhang can be 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be other sequence. The first and second strands can also be joined, *e.g.*, by additional bases to form a hairpin, or by other non-base linkers.

The RNAi agents provided by the present invention include agents with chemical modifications as disclosed, for example, in U.S. Provisional Application No. 61/561,710, filed on November 18, 2011, International Application No. PCT/US2011/051597, filed on September 15, 2010, and PCT Publication WO 2009/073809, the entire contents of each of which are incorporated herein by reference.

In one embodiment, the nucleotides in the overhang region of the RNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2-F, 2'-O-methyl, thymidine (T), 2`-O-methoxyethyl-5-methyluridine (Teo), 2`-O-methoxyethyladenosine (Aeo), 2`-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof. For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be other sequence.

The 5'- or 3'- overhangs at the sense strand, antisense strand or both strands of the RNAi agent may be phosphorylated. In some embodiments, the overhang region contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In one embodiment, the overhang is present at the 3'-end of the sense strand, antisense strand or both strands. In one embodiment, this 3'-overhang is present in the antisense strand. In one embodiment, this 3'-overhang is present in the sense strand.

The RNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang is located at the 3'-terminal end of the sense strand or, alternatively, at the 3'-terminal end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or vice versa. Generally, the antisense strand of the RNAi has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While the Applicants are not bound by theory, the theoretical mechanism is that the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In one embodiment, the RNAi agent is a double ended bluntmer of 19 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7,8,9 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent is a double ended bluntmer of 20 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8,9,10 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent is a double ended bluntmer of 21 nt in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5'end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end.

In one embodiment, the RNAi agent comprises a 21 nucleotides (nt) sense strand and a 23 nucleotides (nt) antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9,10,11 from the 5'end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5'end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nt overhang. Preferably, the 2 nt overhang is at the 3'-end of the antisense. Optionally, the RNAi agent further comprises a ligand (preferably $GalNAc_3$).

In one embodiment, the RNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1- 23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense strand when sense and antisense strands are aligned for maximum

complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In one embodiment, the RNAi agent comprises sense and antisense strands, wherein the RNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11,12,13 from the 5' end; wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region which is at least 25 nucleotides in length, and the second strand is sufficiently complemenatary to a target mRNA along at least 19 nt of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein dicer cleavage of the RNAi agent preferentially results in an siRNA comprising the 3' end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the RNAi agent further comprises a ligand.

In one embodiment, the sense strand of the RNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In one embodiment, the antisense strand of the RNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand

For RNAi agent having a duplex region of 17-23 nt in length, the cleavage site of the antisense strand is typically around the 10, 11 and 12 positions from the 5'-end. Thus, the motifs of three identical modifications may occur at the 9, 10, 11 positions; 10, 11, 12 positions; 11, 12, 13 positions; 12, 13, 14 positions; or 13, 14, 15 positions of the

antisense strand, the count starting from the 1st nucleotide from the 5'-end of the antisense strand, or, the count starting from the 1st paired nucleotide within the duplex region from the 5'- end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the RNAi from the 5'-end.

The sense strand of the RNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, *i.e.*, at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In one embodiment, the sense strand of the RNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif should occur at or near the cleavage site of the strand and the other motifs may be wing modifications. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adajacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other than the chemistry of the motifs are distinct from each other and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the RNAi agent may contain at least two motifs of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense

strand may also contain one or more wing modifications in an alignment similar to the wing modifications that is present on the sense strand.

In one embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end or both ends of the strand.

In another embodiment, the wing modification on the sense strand or antisense strand of the RNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end or both ends of the strand.

When the sense strand and the antisense strand of the RNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two or three nucleotides.

When the sense strand and the antisense strand of the RNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two or three nucleotides in the duplex region.

In one embodiment, every nucleotide in the sense strand and antisense strand of the RNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens and/or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, *e.g.*, of the 2′ hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, *e.g.*, a modification of a base, or a phosphate moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3' or 5'

terminal position, may only occur in a terminal region, *e.g.,* at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of a RNA or may only occur in a single strand region of a RNA. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both termini, may only occur in a terminal region, *e.g.*, at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at termini. The 5' end or ends can be phosphorylated.

It may be possible, *e.g.*, to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, *e.g.*, in a 5' or 3' overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3' or 5' overhang may be modified, *e.g.*, with a modification described herein. Modifications can include, *e.g.*, the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, *e.g.*, the use of deoxyribonucleotides, , 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase , and modifications in the phosphate group, *e.g.*, phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In one embodiment, each residue of the sense strand and antisense strand is independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'- O-methyl, 2'-O-allyl, 2'-C- allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'- O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'- O-methyl or 2'-fluoro modifications, or others.

In one embodiment, the N_a and/or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The

alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB...," "AABBAABBAABB...," "AABAABAABAAB...," "AAABAAABAAAB...," "AAABBBAAABBB...," or "ABCABCABCABC...," etc.

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, *i.e.*, modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB...", "ACACAC..." "BDBDBD..." or "CDCDCD...," etc.

In one embodiment, the RNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and vice versa. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5'-3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5'-3'of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5'-3' of the strand and the alternating motif in the antisenese strand may start with "BBAABBAA" from 5'-3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In one embodiment, the RNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, *i.e.*, the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'- O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand and/or antisense strand interrupts the initial modification pattern present in the sense strand and/or antisense strand. This interruption of the modification pattern of the sense and/or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense and/or antisense strand surprisingly enhances the gene silencing acitivty to the target gene.

In one embodiment, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is "...N_aYYYN_b...," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Altnernatively, N_a and/or N_b may be present or absent when there is a wing modification present.

The RNAi agent may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand or antisense strand or both in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand.

In one embodiment, the RNAi comprises the phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paried nucleotide next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mistmatch can occur in the overhang region or the duplex region. The base pair can be ranked on the basis of their propensity to promote dissociation or melting (*e.g.*, on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, *e.g.*, non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In one embodiment, the RNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'- end of the antisense strand can be chosen independently from the group of: A:U, G:U, I:C, and mismatched pairs, *e.g.*, non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In one embodiment, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from the group consisting of A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2 or 3 base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'- end of the antisense strand is an AU base pair.

In one embodiment, the sense strand sequence may be represented by formula (I):

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

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

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

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In one embodiment, the N_a and/or N_b comprise modifications of alternating pattern.

In one embodiment, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the RNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (*e.g.*: can occur at positions 6, 7, 8, 7, 8, 9, 8, 9, 10, 9, 10, 11, 10, 11,12 or 11, 12, 13) of - the sense strand, the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

5' n_p-N_a-YYY-N_b-ZZZ-N_a-n_q 3' (Ia);

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

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

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

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

When the sense strand is represented as formula (Ic), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

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

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

5' $n_{q'}$-N_a'-(Z'Z'Z')$_k$-N_b'-Y'Y'Y'-N_b'-(X'X'X')$_l$-N'$_a$-n_p' 3' (II)

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

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

each n_p' and n_q' independently represent an overhang nucleotide;

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

and

X′X′X′, Y′Y′Y′ and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, the N_a' and/or N_b' comprise modifications of alternating pattern.

The Y′Y′Y′ motif occurs at or near the cleavage site of the antisense strand. For example, when the RNAi agent has a duplex region of 17-23 nt in length, the Y′Y′Y′ motif can occur at positions 9, 10, 11;10, 11, 12; 11, 12, 13; 12, 13, 14 ; or 13, 14, 15 of the antisense strand, with the count starting from the 1st nucleotide, from the 5'-end; or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end. Preferably, the Y′Y′Y′ motif occurs at positions 11, 12, 13.

In one embodiment, Y′Y′Y′ motif is all 2'-OMe modified nucleotides.

In one embodiment, k is 1 and l is 0, or k is 0 and l is 1, or both k and l are 1.

The antisense strand can therefore be represented by the following formulas:

5' n_q'-N_a′-Z′Z′Z′-N_b′-Y′Y′Y′-N_a′-n_p' 3' (IIa);

5' n_q'-N_a′-Y′Y′Y′-N_b′-X′X′X′-n_p' 3' (IIb); or

5' n_q'-N_a′- Z′Z′Z′-N_b′-Y′Y′Y′-N_b′- X′X′X′-N_a′-n_p' 3' (IIc).

When the antisense strand is represented by formula (IIa), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Preferably, N_b is 0, 1, 2, 3, 4, 5 or 6.

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

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, 2'-deoxy or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X′, Y′ and Z′, in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In one embodiment, the sense strand of the RNAi agent may contain YYY motif occurring at 9, 10 and 11 positions of the strand when the duplex region is 21 nt, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In one embodiment the antisense strand may contain Y′Y′Y′ motif occurring at positions 11, 12, 13 of the strand, the count starting from the 1st nucleotide from the 5'-end, or optionally, the count starting at the 1st paired nucleotide within the duplex region, from the 5'- end; and Y′ represents 2'-O-methyl modification. The antisense strand may additionally contain X′X′X′ motif or Z′Z′Z′ motifs as wing modifications at the opposite end of the duplex region; and X′X′X′ and Z′Z′Z′ each independently represents a 2'-OMe modification or 2'-F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib) and (Ic) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb) and (IIc), respectively.

Accordingly, the RNAi agents of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):

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

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

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p′, q, and q′ are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

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

wherein

each n_p', n_p, n_q', and n_q independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X′X′X′, Y′Y′Y′, and Z′Z′Z′ each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 1. In another embodiment, k is 1 and l is 0; k is 0 and l is 1; or both k and l are 1.

Exemplary combinations of the sense strand and antisense strand forming a RNAi duplex include the formulas below:

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

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

(IIIa)

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

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

(IIIb)

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

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

(IIIc)

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

When the RNAi agent is represented as formula (IIIb), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the RNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2 or 0modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b and N_b' independently comprises modifications of alternating pattern.

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

When the RNAi agent is represented by formula (III), (IIIa), (IIIb) or (IIIc), at least one of the Y nucleotides may form a base pair with one of the Y′ nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y′ nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y′ nucleotides.

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

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

In one embodiment, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, and/or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In one embodiment, the RNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb) or (IIIc), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprise a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, the RNAi agent is a multimer containing three, four, five, six or more duplexes represented by formula (III), (IIIa), (IIIb) or (IIIc), wherein the duplexes are connected by a linker. The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

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

Various publications describe multimeric RNAi agents . Such publications include WO2007/091269, US Patent No. 7858769, WO2010/141511, WO2007/117686, WO2009/014887 and WO2011/031520 the entire contents of which are hereby incorporated herein by reference.

The RNAi agent that contains conjugations of one or more carbohydrate moieties to a RNAi agent can optimize one or more properties of the RNAi agent. In many cases, the carbohydrate moiety will be attached to a modified subunit of the RNAi agent. For example, the ribose sugar of one or more ribonucleotide subunits of a dsRNA agent can be replaced with another moiety, *e.g.*, a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, *i.e.*, all ring atoms are carbon atoms, or a heterocyclic ring system, *i.e.*, one or more ring atoms may be a heteroatom, *e.g.*, nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, *e.g.* fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, *e.g.* a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into

the backbone, *e.g.*, the phosphate, or modified phosphate, *e.g.*, sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, *e.g.*, a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, *e.g.*, a carbohydrate, *e.g.* monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide and polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, *e.g.*, an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, *e.g.*, a ligand to the constituent ring.

The RNAi agents may be conjugated to a ligand via a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl and and decalin; preferably, the acyclic group is selected from serinol backbone or diethanolamine backbone.

In certain specific embodiments, the RNAi agent of the invention is an agent selected from the group of agents listed in Table 1 and consisting of D1000, D1001, D1002, D1003, D1004, D1005, D1006, D1007, D1008, D1009, D1010, D1011, D1012, D1013, D1014, D1015, D1016, D1017, D1018, D1019, D1020, D1021, D1022, D1023, D1024, D1025, D1026, D1027, D1028, D1029, D1030, D1031, D1032, D1033, D1034, D1035, D1036, D1037, D1038, D1039, D1040, D1041, D1042, D1043, D1044, D1045, D1046, D1047, D1048, D1049, D1050, D1051, D1052, D1053, D1054, D1055, D1056, D1057, D1058, D1059, D1060, D1061, D1062, D1063, D1064, D1065, D1066, D1067, D1068, D1069, D1070, D1071, D1072, D1073, D1074, D1075, D1076, D1077, D1078, D1079, D1080, D1081, D1082, D1083, D1084, D1085, D1086, D1087, D1088, D1089, D1090, D1091, D1092, D1093, D1094, D1095, D1096, D1097, D1098, D1099, D1100, D1101, D1102, D1103, D1104, D1105, D1106, D1107, D1108, D1109, D1110, D1111, D1112, D1113, D1114, D1115, D1116, D1117, D1118, D1119, D1120, D1121, D1122, D1123, D1124, D1125, D1126, D1127, D1128, D1129, D1130, D1131, D1132, D1133,

D1134, D1135, D1136, D1137, D1138, D1139, D1140, D1141, D1142, D1143, D1144, D1145, D1146, D1147, D1148, D1149, D1150, D1151, D1152, D1153, D1154, D1155, D1156, D1157, D1158, D1159, D1160, D1161, D1162, D1163, D1164, D1165, D1166, D1167, D1168, D1169, D1170, D1171, D1172, D1173, D1174, D1175, D1176, D1177, D1178, D1179, D1180, D1181, D1182, D1183, D1184, D1185, D1186, D1187, D1188, D1189, D1190, D1191, D1192, D1193, D1194, D1195, D1196, D1197, D1198, D1199, D1200, D1201, D1202, D1203, D1204, D1205, D1206, D1207, D1208, D1209, D1210, D1211, D1212, D1213, D1214, D1215, D1216, D1217, D1218, D1219, D1220, D1221, D1222, D1223, D1224, D1225, D1226, D1227, D1228, D1229, D1230, D1231, D1232, D1233, D1234, D1235, D1236, D1237, D1238, D1239, D1240, D1241, D1242, D1243, D1244, D1245, D1246, D1247, D1248, D1249, D1250, D1251, D1252, D1253, D1254, D1255, D1256, D1257, D1258, D1259, D1260, D1261, D1262, D1263, D1264, D1265, D1266, D1267, D1268, D1269, D1270, D1271, D1272, D1273, D1274, D1275, D1276, D1277, D1278, D1279, D1280, D1281, D1282, D1283, D1284, D1285, D1286, D1287, D1288, D1289, D1290, D1291, D1292, D1293, D1294, D1295, D1296, D1297, D1298, D1299, D1300, D1301, D1302, D1303, D1304, D1305, D1306, D1307, D1308, D1309, D1310, D1311, D1312, D1313, D1314, D1315, D1316, D1317, D1318, D1319, D1320, D1321, D1322, D1323, D1324, D1325, D1326, D1327, D1328, D1329, D1330, D1331, D1332, D1333, D1334, D1335, D1336, D1337, D1338, D1339, D1340, D1341, D1342, D1343, D1344, D1345, D1346, D1347, D1348, D1349, D1350, D1351, D1352, D1353, D1354, D1355, D1356, D1357, D1358, D1359, D1360, D1361, D1362, D1363, D1364, D1365, D1366, D1367, D1368, D1369, D1370, D1371, D1372, D1373, D1374, D1375, D1376, D1377, D1378, D1379, D1380, D1381, D1382, D1383, D1384, D1385, D1386, D1387, D1388, D1389, D1390, D1391, D1392, D1393, D1394, D1395, D1396, D1397, D1398, D1399, D1400, D1401, D1402, D1403, D1404, D1405, D1406, D1407, D1408, D1409, D1410, D1411, D1412, D1413, D1414, D1415, D1416, D1417, D1418, D1419, D1420, D1421, D1422, D1423, D1424, D1425, D1426, D1427, D1428, D1429, D1430, D1431, D1432, D1433, D1434, D1435, D1436, D1437, D1438, D1439, D1440, D1441, D1442, D1443, D1444, D1445, D1446, D1447, D1448, D1449, D1450, D1451, D1452, D1453, D1454, D1455, D1456, D1457, D1458, D1459, D1460, D1461, D1462, D1463, D1464, D1465, D1466, D1467, D1468, D1469, D1470, D1471, D1472, D1473, D1474,

D1475, D1476, D1477, D1478, D1479, D1480, D1481, D1482, D1483, D1484, D1485, D1486, D1487, D1488, D1489, D1490, D1491, D1492, D1493, D1494, D1495, D1496, D1497, D1498, D1499, . D1500, D1501, D1502, D1503, D1504, D1505, D1506, D1507, D1508, D1509, D1510, D1511, D1512, D1513, D1514, D1515, D1516, D1517, D1518, D1519, D1520, D1521, D1522, D1523, D1524, D1525, D1526, D1527, D1528, D1529, D1530, D1531, D1532, D1533, D1534, D1535, D1536, D1537, D1538, D1539, D1540, D1541, D1542, D1543, D1544, D1545, D1546, D1547, D1548, D1549, D1550, D1551, D1552, D1553, D1554, D1555, D1556, D1557, D1558, D1559, D1560, D1561, D1562, D1563, D1564, D1565, D1566, D1567, D1568, D1569, D1570, D1571, D1572, D1573, D1574, D1575, D1576, D1577, D1578, D1579, D1580, D1581, D1582, D1583, D1584, D1585, D1586, D1587, D1588, D1589, D1590, D1591, D1592, D1593, D1594, D1595, D1596, D1597, D1598, D1599, D1600, D1601, D1602, D1603, D1604, D1605, D1606, D1607, D1608, D1609, D1610, D1611, D1612, D1613, D1614, D1615, D1616, D1617, D1618, D1619, D1620, D1621, D1622, D1623, D1624, D1625, D1626, D1627, D1628, D1629, D1630, D1631, D1632, D1633, D1634, D1635, D1636, D1637, D1638, D1639, D1640, D1641, D1642, D1643, D1644, D1645, D1646, D1647, D1648, D1649, D1650, D1651, D1652, D1653, D1654, D1655, D1656, D1657, D1658, D1659, D1660, D1661, D1662, D1663, D1664, D1665, D1666, D1667, D1668, D1669, D1670, D1671, D1672, D1673, D1674, D1675, D1676, D1677, D1678, D1679, D1680, D1681, D1682, D1683, D1684, D1685, D1686, D1687, D1688, D1689, D1690, D1691, D1692, D1693, D1694, D1695, D1696, D1697, D1698, D1699, D1700, D1701, D1702, D1703, D1704, D1705, D1706, D1707, D1708, D1709, D1710, D1711, D1712, D1713, D1714, D1715, D1716, D1717, D1718, D1719, D1720, D1721, D1722, D1723, D1724, D1725, D1726, D1727, D1728, D1729, D1730, D1731, D1732, D1733, D1734, D1735, D1736, D1737, D1738, D1739, D1740, D1741, D1742, D1743, D1744, D1745, D1746, D1747, D1748, D1749, D1750, D1751, D1752, D1753, D1754, D1755, D1756, D1757, D1758, D1759, D1760, D1761, D1762, D1763, D1764, D1765, D1766, D1767, D1768, D1769, D1770, D1771, D1772, D1773, D1774, D1775, D1776, D1777, D1778, D1779, D1780, D1781, D1782, D1783, D1784, D1785, D1786, D1787, D1788, D1789, D1790, D1791, D1792, D1793, D1794, D1795, D1796, D1797, D1798, D1799, D1800, D1801, D1802, D1803, D1804, D1805, D1806, D1807, D1808, D1809, D1810, D1811, D1812, D1813, D1814,

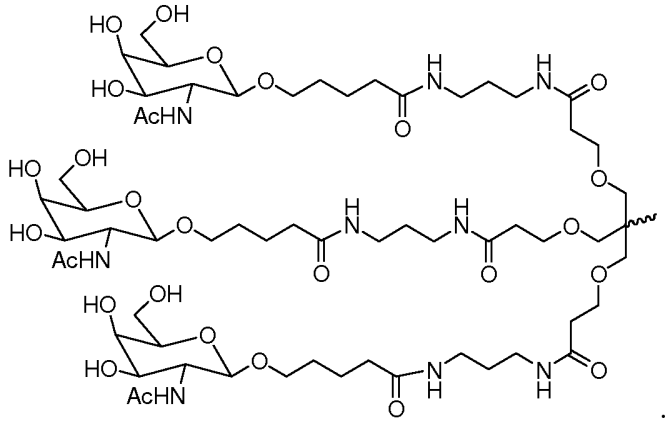
D1815, D1816, D1817, D1818, D1819, D1820, D1821, D1822, D1823, D1824, D1825, D1826, D1827, D1828, D1829, D1830, D1831, D1832, D1833, D1834, D1835, D1836, D1837, D1838, D1839, D1840, D1841, D1842, D1843, D1844, D1845, D1846, D1847, D1848, D1849, D1850, D1851, D1852, D1853, D1854, D1855, D1856, D1857, D1858, D1859, D1860, D1861, D1862, D1863, D1864, D1865, D1866, D1867, D1868, D1869, D1870, D1871, D1872, D1873, D1874, D1875, D1876, D1877, D1878, D1879, D1880, D1881, D1882, D1883, D1884, D1885, D1886, D1887, D1888, D1889, D1890, D1891, D1892, D1893, D1894, D1895, D1896, D1897, D1898, D1899, D1900, D1901, D1902, D1903, D1904, D1905, D1906, D1907, D1908, D1909, D1910, D1911, D1912, D1913, D1914, D1915, D1916, D1917, D1918, D1919, D1920, D1921, D1922, D1923, D1924, D1925, D1926, D1927, D1928, D1929, D1930, D1931, D1932, D1933, D1934, D1935, D1936, D1937, D1938, D1939, D1940, D1941, D1942, D1943, D1944, D1945, D1946, D1947, D1948, D1949, D1950, D1951, D1952, D1953, D1954, D1955, D1956, D1957, D1958, D1959, D1960, D1961, D1962, D1963, D1964, D1965, D1966, D1967, D1968, D1969, D1970, D1971, D1972, D1973, D1974, D1975, D1976, D1977, D1978, D1979, D1980, D1981, D1982, D1983, D1984, D1985, D1986, D1987, D1988, D1989, D1990, D1991, D1992, D1993, D1994, D1995, D1996, D1997, D1998, D1999, D2000, D2001, D2002, D2003, D2004, D2005, D2006, D2007, D2008, D2009, D2010, D2011, D2012, D2013, D2014, D2015, D2016, D2017, D2018, D2019, D2020, D2021, D2022, D2023, D2024, D2025, D2026, D2027, D2028, D2029, D2030, D2031, D2032, D2033, D2034, D2035, D2036, D2037, D2038, D2039, D2040, D2041, D2042, D2043, D2044, D2045, D2046, D2047, D2048, D2049, D2050, D2051, D2052, D2053, D2054, D2055, D2056, D2057, D2058, D2059, D2060, D2061, D2062, D2063, D2064, D2065, D2066, D2067, D2068, D2069, D2070, D2071, D2072, D2073, D2074, D2075, D2076, D2077, D2078, D2079, D2080, D2081, D2082, D2083, D2084, D2085, D2086, D2087, D2088, D2089, D2090 and D2091.

These agents may further comprise a ligand, such as a GalNAc ligand.

Ligands

The RNAi agents of the invention, e.g., double stranded RNAi agents, may optionally be conjugated to one or more ligands. The ligand can be attached to the sense

strand, antisense strand or both strands, at the 3'-end, 5'-end or both ends. For instance, the ligand may be conjugated to the sense strand. In preferred embodiments, the ligand is conjgated to the 3'-end of the sense strand. In one preferred embodiment, the ligand is a GalNAc ligand. In particularly preferred embodiments, the ligand is $GalNAc_3$:



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A wide variety of entities can be coupled to the RNAi agents of the present invention. Preferred moieties are ligands, which are coupled, preferably covalently, either directly or indirectly via an intervening tether.

In preferred embodiments, a ligand alters the distribution, targeting or lifetime of the molecule into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, *e.g.*, molecule, cell or cell type, compartment, receptor *e.g.*, a cellular or organ compartment, tissue, organ or region of the body, as, *e.g.*, compared to a species absent such a ligand. Ligands providing enhanced affinity for a selected target are also termed targeting ligands.

Some ligands can have endosomolytic properties. The endosomolytic ligands promote the lysis of the endosome and/or transport of the composition of the invention, or its components, from the endosome to the cytoplasm of the cell. The endosomolytic ligand may be a polyanionic peptide or peptidomimetic which shows pH-dependent membrane activity and fusogenicity. In one embodiment, the endosomolytic ligand assumes its active conformation at endosomal pH. The "active" conformation is that conformation in which the endosomolytic ligand promotes lysis of the endosome and/or transport of the composition of the invention, or its components, from the endosome to the cytoplasm of the cell. Exemplary endosomolytic ligands include the GALA peptide

(Subbarao *et al.*, *Biochemistry*, 1987, 26: 2964-2972), the EALA peptide (Vogel *et al.*, *J. Am. Chem. Soc.*, 1996, 118: 1581-1586), and their derivatives (Turk *et al.*, *Biochem. Biophys. Acta*, 2002, 1559: 56-68). In one embodiment, the endosomolytic component may contain a chemical group (*e.g.*, an amino acid) which will undergo a change in charge or protonation in response to a change in pH. The endosomolytic component may be linear or branched.

Ligands can improve transport, hybridization, and specificity properties and may also improve nuclease resistance of the resultant natural or modified oligoribonucleotide, or a polymeric molecule comprising any combination of monomers described herein and/or natural or modified ribonucleotides.

Ligands in general can include therapeutic modifiers, *e.g.*, for enhancing uptake; diagnostic compounds or reporter groups *e.g.*, for monitoring distribution; cross-linking agents; and nuclease-resistance conferring moieties. General examples include lipids, steroids, vitamins, sugars, proteins, peptides, polyamines, and peptide mimics.

Ligands can include a naturally occurring substance, such as a protein (*e.g.*, human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); a carbohydrate (*e.g.*, a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, *e.g.*, a synthetic polyamino acid, an oligonucleotide (*e.g.*, an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, *e.g.*, a cell or tissue targeting agent, *e.g.*, a lectin, glycoprotein, lipid or protein, *e.g.*, an antibody, that binds to a specified

cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

Other examples of ligands include dyes, intercalating agents (*e.g.*, acridines), cross-linkers (*e.g.*, psoralene, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (*e.g.*, phenazine, dihydrophenazine), artificial endonucleases or a chelator (*e.g.*, EDTA), lipophilic molecules, *e.g.*, cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O(hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine)and peptide conjugates (*e.g.*, antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (*e.g.*, PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (*e.g.*, biotin), transport/absorption facilitators (*e.g.*, aspirin, vitamin E, folic acid), synthetic ribonucleases (*e.g.*, imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, *e.g.*, glycoproteins, or peptides, *e.g.*, molecules having a specific affinity for a co-ligand, or antibodies *e.g.*, an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Ligands may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, or aptamers. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, *e.g.*, a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, *e.g.*, by disrupting the cell's microtubules, microfilaments, and/or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

The ligand can increase the uptake of the oligonucleotide into the cell by, for example, activating an inflammatory response. Exemplary ligands that would have such an effect include tumor necrosis factor alpha (TNFalpha), interleukin-1 beta, or gamma interferon.

In one aspect, the ligand is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, *e.g.*, human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, *e.g.*, a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, and/or (c) can be used to adjust binding to a serum protein, *e.g.*, HSA.

A lipid based ligand can be used to modulate, *e.g.*, control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In a preferred embodiment, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In another preferred embodiment, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of or in addition to the lipid based ligand.

In another aspect, the ligand is a moiety, *e.g.*, a vitamin, which is taken up by a target cell, *e.g.*, a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, *e.g.*, of the malignant or non-malignant type, *e.g.*, cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include B vitamins, *e.g.*, folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by cancer cells. Also included are HAS, low density lipoprotein (LDL) and high-density lipoprotein (HDL).

In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long. A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (*e.g.*, consisting primarily of Tyr, Trp or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:4). An RFGF analogue (*e.g.*, amino acid sequence AALLPVLLAAP) (SEQ ID NO:5) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ) (SEQ ID NO:6) and the Drosophila Antennapedia protein (RQIKIWFQNRRMKWKK) (SEQ ID NO:7) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a

peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam *et al.*, *Nature*, 354:82-84, 1991). Preferably the peptide or peptidomimetic tethered to an iRNA agent via an incorporated monomer unit is a cell targeting peptide such as an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized.An RGD peptide moiety can be used to target a tumor cell, such as an endothelial tumor cell or a breast cancer tumor cell (Zitzmann *et al.*, *Cancer Res.*, 62:5139-43, 2002). An RGD peptide can facilitate targeting of an iRNA agent to tumors of a variety of other tissues, including the lung, kidney, spleen, or liver (Aoki *et al.*, *Cancer Gene Therapy* 8:783-787, 2001). Preferably, the RGD peptide will facilitate targeting of an iRNA agent to the kidney. The RGD peptide can be linear or cyclic, and can be modified, *e.g.*, glycosylated or methylated to facilitate targeting to specific tissues. For example, a glycosylated RGD peptide can deliver an iRNA agent to a tumor cell expressing $\alpha_V\beta_3$ (Haubner *et al.*, *Jour. Nucl. Med.*, 42:326-336, 2001). Peptides that target markers enriched in proliferating cells can be used. For example, RGD containing peptides and peptidomimetics can target cancer cells, in particular cells that exhibit an integrin. Thus, one could use RGD peptides, cyclic peptides containing RGD, RGD peptides that include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Generally, such ligands can be used to control proliferating cells and angiogeneis. Preferred conjugates of this type of ligand target PECAM-1, VEGF, or other cancer gene, *e.g.*, a cancer gene described herein.

A "cell permeation peptide" is capable of permeating a cell, *e.g.*, a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (*e.g.*, LL-37 or Ceropin P1), a disulfide bond-containing peptide (*e.g.*, α -defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (*e.g.*, PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic

peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., Nucl. Acids Res. 31:2717-2724, 2003).

In one embodiment, a targeting peptide can be an amphipathic α-helical peptide. Exemplary amphipathic α-helical peptides include, but are not limited to, cecropins, lycotoxins, paradaxins, buforin, CPF, bombinin-like peptide (BLP), cathelicidins, ceratotoxins, S. clava peptides, hagfish intestinal antimicrobial peptides (HFIAPs), magainines, brevinins-2, dermaseptins, melittins, pleurocidin, H_2A peptides, Xenopus peptides, esculentinis-1, and caerins. A number of factors will preferably be considered to maintain the integrity of helix stability. For example, a maximum number of helix stabilization residues will be utilized (*e.g.*, leu, ala, or lys), and a minimum number helix destabilization residues will be utilized (*e.g.*, proline, or cyclic monomeric units. The capping residue will be considered (for example Gly is an exemplary N-capping residue and/or C-terminal amidation can be used to provide an extra H-bond to stabilize the helix. Formation of salt bridges between residues with opposite charges, separated by i ± 3, or i ± 4 positions can provide stability. For example, cationic residues such as lysine, arginine, homo-arginine, ornithine or histidine can form salt bridges with the anionic residues glutamate or aspartate.

Peptide and peptidomimetic ligands include those having naturally occurring or modified peptides, *e.g.*, D or L peptides; α, β, or γ peptides; N-methyl peptides; azapeptides; peptides having one or more amide, *i.e.*, peptide, linkages replaced with one or more urea, thiourea, carbamate, or sulfonyl urea linkages; or cyclic peptides.

The targeting ligand can be any ligand that is capable of targeting a specific receptor. Examples are: folate, GalNAc, galactose, mannose, mannose-6P, clusters of sugars such as GalNAc cluster, mannose cluster, galactose cluster, or an apatamer. A cluster is a combination of two or more sugar units. The targeting ligands also include integrin receptor ligands, Chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL and HDL ligands. The ligands can also be based on nucleic acid, *e.g.*, an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein.

Endosomal release agents include imidazoles, poly or oligoimidazoles, PEIs, peptides, fusogenic peptides, polycaboxylates, polyacations, masked oligo or poly cations or anions, acetals, polyacetals, ketals/polyketyals, orthoesters, polymers with masked or unmasked cationic or anionic charges, dendrimers with masked or unmasked cationic or anionic charges.

PK modulator stands for pharmacokinetic modulator. PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins etc. Examplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin etc. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, *e.g.*, oligonucleotides of about 5 bases, 10 bases, 15 bases or 20 bases, comprising multiple phosphorothioate linkages in the backbaone are also amenable to the present invention as ligands (*e.g.*, as PK modulating ligands).

In addition, aptamers that bind serum components (*e.g.*, serum proteins) are also amenable to the present invention as PK modulating ligands.

Other ligand conjugates amenable to the invention are described in U.S. Patent Applications USSN: 10/916,185, filed August 10, 2004; USSN: 10/946,873, filed September 21, 2004; USSN: 10/833,934, filed August 3, 2007; USSN: 11/115,989 filed April 27, 2005 and USSN: 11/944,227 filed November 21, 2007, which are incorporated by reference in their entireties for all purposes.

When two or more ligands are present, the ligands can all have same properties, all have different properties or some ligands have the same properties while others have different properties. For example, a ligand can have targeting properties, have endosomolytic activity or have PK modulating properties. In a preferred embodiment, all the ligands have different properties.

Ligands can be coupled to the oligonucleotides at various places, for example, 3'-end, 5'-end, and/or at an internal position. In preferred embodiments, the ligand is attached to the oligonucleotides *via* an intervening tether, *e.g.*, a carrier described herein. The ligand or tethered ligand may be present on a monomer when the monomer is

incorporated into the growing strand. In some embodiments, the ligand may be incorporated via coupling to a "precursor" monomer after the "precursor" monomer has been incorporated into the growing strand. For example, a monomer having, *e.g.*, an amino-terminated tether (*i.e.*, having no associated ligand), *e.g.*, TAP-$(CH_2)_nNH_2$ may be incorporated into a growing oligonucelotide strand. In a subsequent operation, *i.e.*, after incorporation of the precursor monomer into the strand, a ligand having an electrophilic group, *e.g.*, a pentafluorophenyl ester or aldehyde group, can subsequently be attached to the precursor monomer by coupling the electrophilic group of the ligand with the terminal nucleophilic group of the precursor monomer's tether.

In another example, a monomer having a chemical group suitable for taking part in Click Chemistry reaction may be incorporated, *e.g.*, an azide or alkyne terminated tether/linker. In a subsequent operation, *i.e.*, after incorporation of the precursor monomer into the strand, a ligand having complementary chemical group, *e.g.* an alkyne or azide can be attached to the precursor monomer by coupling the alkyne and the azide together.

For double- stranded oligonucleotides, ligands can be attached to one or both strands. In some embodiments, a double-stranded iRNA agent contains a ligand conjugated to the sense strand. In other embodiments, a double-stranded iRNA agent contains a ligand conjugated to the antisense strand.

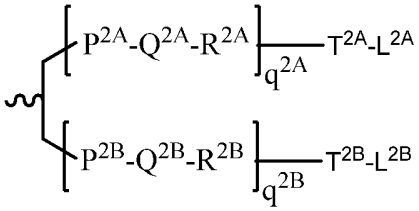
In some embodiments, ligand can be conjugated to nucleobases, sugar moieties, or internucleosidic linkages of nucleic acid molecules. Conjugation to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In some embodiments, the 2-, 6-, 7-, or 8-positions of a purine nucleobase are attached to a conjugate moiety. Conjugation to pyrimidine nucleobases or derivatives thereof can also occur at any position. In some embodiments, the 2-, 5-, and 6-positions of a pyrimidine nucleobase can be substituted with a conjugate moiety. Conjugation to sugar moieties of nucleosides can occur at any carbon atom. Example carbon atoms of a sugar moiety that can be attached to a conjugate moiety include the 2', 3', and 5' carbon atoms. The 1' position can also be attached to a conjugate moiety, such as in an abasic residue. Internucleosidic linkages can also bear conjugate moieties. For phosphorus-containing linkages (*e.g.*, phosphodiester, phosphorothioate,

phosphorodithiotate, phosphoroamidate, and the like), the conjugate moiety can be attached directly to the phosphorus atom or to an O, N, or S atom bound to the phosphorus atom. For amine- or amide-containing internucleosidic linkages (e.g., PNA), the conjugate moiety can be attached to the nitrogen atom of the amine or amide or to an adjacent carbon atom.

Any suitable ligand in the field of RNA interference may be used, although the ligand is typically a carbohydrate *e.g.* monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, polysaccharide.

Linkers that conjugate the ligand to the nucleic acid include those discussed above. For example, the ligand can be one or more GalNAc (*N*-acetylglucosamine) derivatives attached through a bivalent or trivalent branched linker.

In one embodiment, the dsRNA of the invention is conjugated to a bivalent and trivalent branched linkers include the structures shown in any of formula (IV) – (VII):



Formula (IV)

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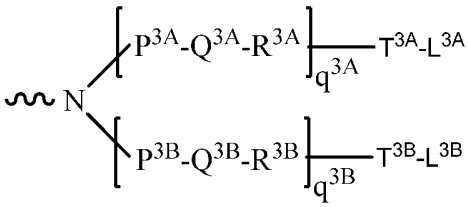

Formula (V)

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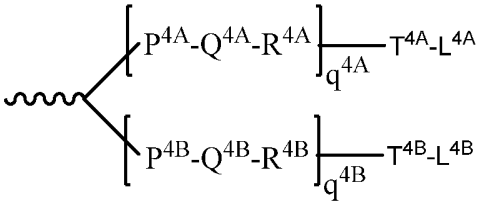
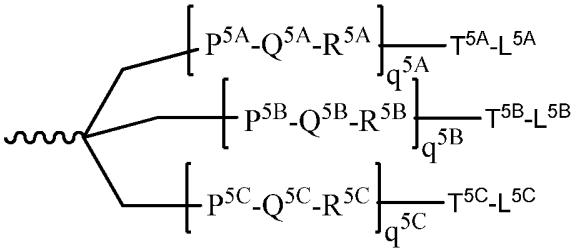


Formula (VI)

, or



Formula (VII)

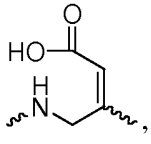
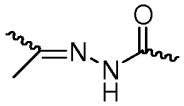
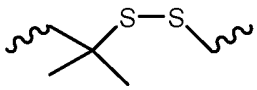
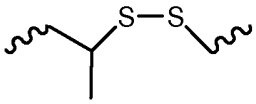
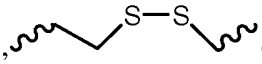
;

wherein:

q^{2A}, q^{2B}, q^{3A}, q^{3B}, $q4^{A}$, q^{4B}, q^{5A}, q^{5B} and q^{5C} represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

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

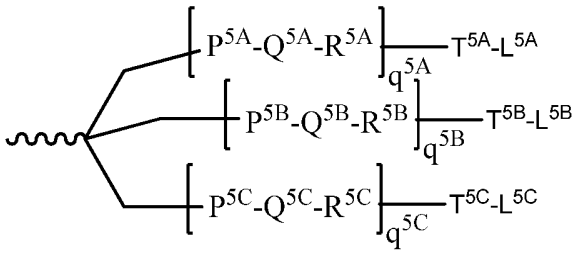
Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherin one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R''), C≡C or C(O);

R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, -C(O)-$CH(R^a)$-NH-, CO, CH=N-O,  ,  ,  ,  ,  or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B} and L^{5C} represent the ligand; *i.e.* each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and

R^a is H or amino acid side chain.

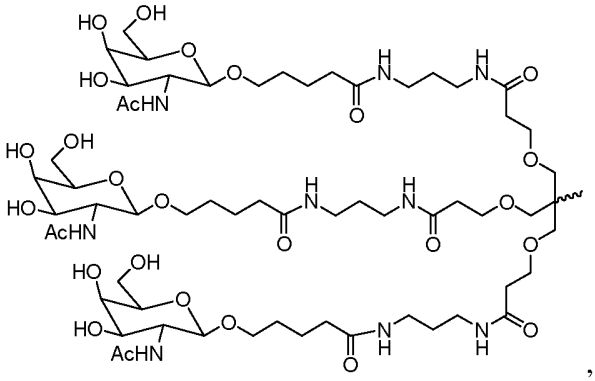
Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (VII):



Formula (VII)

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

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the following compounds:



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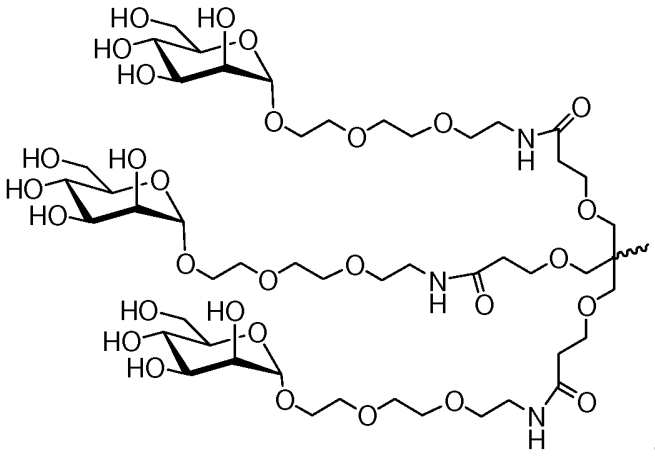

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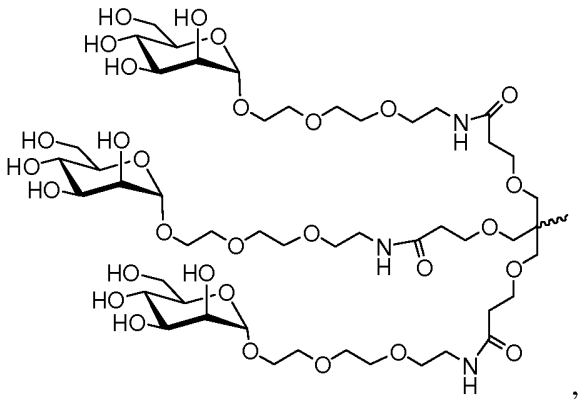

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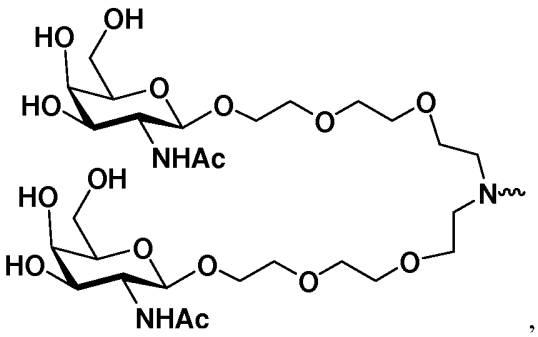

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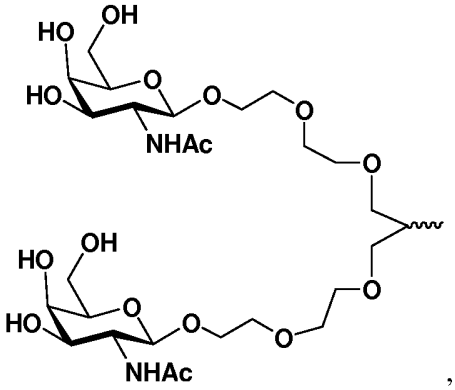

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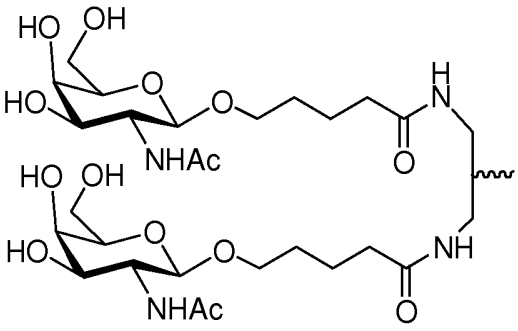
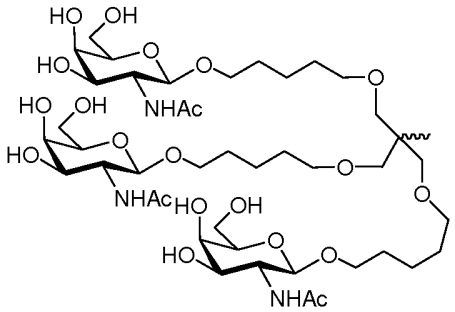
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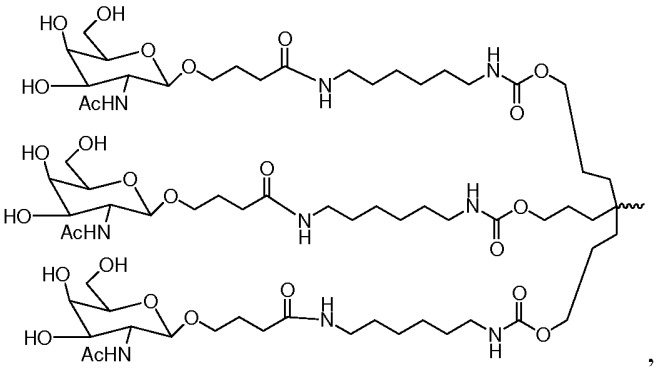
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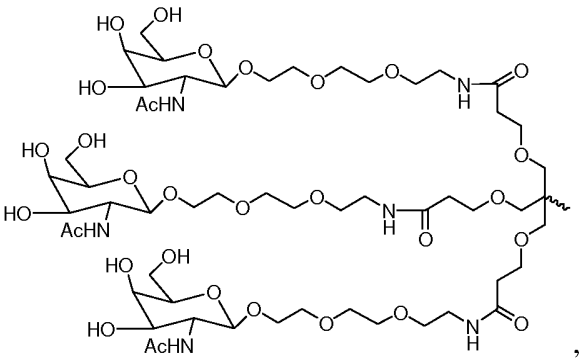
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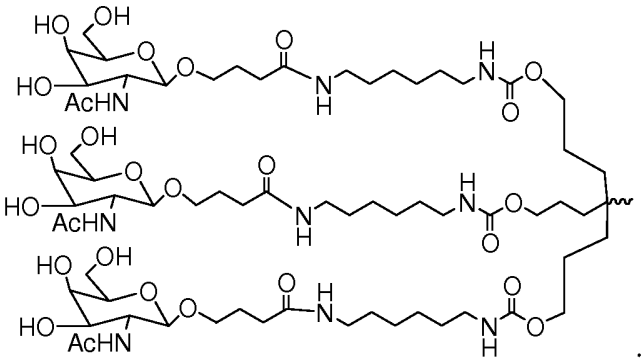
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In other embodiments, the RNAi agent of the invention is an agent selected from the group consisting of AD-45163, AD-45165, AD-51544, AD-51545, AD-51546, and AD-51547.

III. Pharmaceutical Compositions

The RNAi agents of the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals. The pharmaceutical compositions comprising RNAi agents of the invention may be, for example, solutions with or without a buffer, or compositions containing pharmaceutically acceptable carriers. Such compositions include, for example, aqueous or crystalline compositions, liposomal formulations, micellar formulations, emulsions, and gene therapy vectors.

In the methods of the invention, the RNAi agent may be administered in a solution. A free RNAi agent may be administered in an unbuffered solution, e.g., in saline or in water. Alternatively, the free siRNA may also be administred in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In a preferred embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the RNAi agent can be adjusted such that it is suitable for administering to a subject.

In some embodiments, the buffer solution further comprises an agent for controlling the osmolarity of the solution, such that the osmolarity is kept at a desired value, *e.g.*, at the physiologic values of the human plasma. Solutes which can be added to the buffer solution to control the osmolarity include, but are not limited to, proteins, peptides, amino acids, non-metabolized polymers, vitamins, ions, sugars, metabolites, organic acids, lipids, or salts. In some embodiments, the agent for controlling the osmolarity of the solution is a salt. In certain embodiments, the agent for controlling the osmolarity of the solution is sodium chloride or potassium chloride.

In other embodiments, the RNAi agent is formulated as a composition that includes one or more RNAi agents and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and

all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

In one embodiment, the RNAi agent preparation includes at least a second therapeutic agent (*e.g.*, an agent other than an RNA or a DNA). For example, an RNAi agent composition for the treatment of a TTR-associated disease, *e.g.*, a transthyretin-related hereditary amyloidosis (familial amyloid polyneuropathy, FAP), may include a known drug for the amelioration of FAP, *e.g.*, Tafamidis (INN, or Fx-1006A or Vyndaqel).

A formulated RNAi agent composition can assume a variety of states. In some examples, the composition is at least partially crystalline, uniformly crystalline, and/or anhydrous (*e.g.*, it contains less than 80, 50, 30, 20, or 10% of water). In another example, the RNAi agent is in an aqueous phase, *e.g.*, in a solution that includes water.

The aqueous phase or the crystalline compositions can be incorporated into a delivery vehicle, *e.g.*, a liposome (particularly for the aqueous phase) or a particle (*e.g.*, a microparticle as can be appropriate for a crystalline composition). Generally, the RNAi agent composition is formulated in a manner that is compatible with the intended method of administration, as described herein. For example, in particular embodiments the composition is prepared by at least one of the following methods: spray drying, lyophilization, vacuum drying, evaporation, fluid bed drying, or a combination of these techniques; or sonication with a lipid, freeze-drying, condensation and other self-assembly.

An RNAi agent preparation can be formulated in combination with another agent, *e.g.*, another therapeutic agent or an agent that stabilizes RNAi agent, *e.g.*, a protein that complexes with the RNAi agent to form an iRNP. Still other agents include chelators, *e.g.*, EDTA (*e.g.*, to remove divalent cations such as Mg^{2+}), salts, RNAse inhibitors (*e.g.*, a broad specificity RNAse inhibitor such as RNAsin) and so forth.

In one embodiment, the RNAi agent preparation includes another siRNA compound, *e.g.*, a second RNAi agent that can mediate RNAi with respect to a second gene, or with respect to the same gene. Still other preparation can include at least 3, 5, ten, twenty, fifty, or a hundred or more different RNAi agent species. Such RNAi agents can mediate RNAi with respect to a similar number of different genes.

The iRNA agents of the invention may be formulated for pharmaceutical use. Pharmaceutically acceptable compositions comprise a therapeutically-or prophylactically effective amount of one or more of the the dsRNA agents in any of the preceding embodiments, taken alone or formulated together with one or more pharmaceutically acceptable carriers (additives), excipient and/or diluents.

Methods of preparing pharmaceutical compositions of the invention include the step of bringing into association an RNAi agent of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the compositionsare prepared by uniformly and intimately bringing into association an RNAi agent of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

The pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; (3) topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; (5) sublingually; (6) ocularly; (7) transdermally; or (8) nasally. Delivery using subcutaneous or intravenous methods can be particularly advantageous.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and

animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the compositionand not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceuticalcompositions.

The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of RNAi agent which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, and the particular mode of administration. The RNAi agent which can be combined with a carrier material to produce a single dosage form will generally be that amount of the RNAi agent which produces a desired effect, e.g., therapeutic or prophylactic effect. Generally, out of one

hundred per cent, this amount will range from about 0.1 per cent to about ninety-nine percent of RNAi agent, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

In certain embodiments, a composition of the present invention comprises an excipient selected from the group consisting of cyclodextrins, celluloses, liposomes, micelle forming agents, *e.g.*, bile acids, and polymeric carriers, *e.g.*, polyesters and polyanhydrides; and an RNAi agent of the present invention. In certain embodiments, an aforementioned composition renders orally bioavailable an RNAi agent of the present invention.

In some cases, in order to prolong the effect of an RNAi agent, it is desirable to slow the absorption of the agent from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the RNAi agent then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered RNAi agent may be accomplished by dissolving or suspending the agent in an oil vehicle.

Liposomes

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

liposomes are also specifically targeted, *e.g.*, to direct the RNAi agent to particular cell types.

A liposome containing an RNAi agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The RNAi agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the RNAi agent and condense around the RNAi agent to form a liposome. After condensation, the detergent is removed, *e.g.,* by dialysis, to yield a liposomal preparation of RNAi agent.

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

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

775:169, 1984). These methods are readily adapted to packaging RNAi agent preparations into liposomes.

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

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

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

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

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated RNAi agents

in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

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

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

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

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

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

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome II (glyceryl distearate/ cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into

the dermis of mouse skin. Such formulations with RNAi agent are useful for treating a dermatological disorder.

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

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

Surfactants

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes (see above). RNAi agent (or a precursor, *e.g.*, a larger dsiRNA which can be processed into a siRNA, or a DNA which encodes a siRNA or precursor) compositions can include a surfactant. In one embodiment, the siRNA is formulated as an emulsion that includes a surfactant. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in "Pharmaceutical Dosage Forms," Marcel Dekker, Inc., New York, NY, 1988, p. 285).

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

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

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

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

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

Micelles and other Membranous Formulations

The RNAi agents of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the

hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compound. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

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

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

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, *i.e.,* there is one

phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, *e.g.,* through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

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

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

Particles

In another embodiment, an RNAi agent of the invention may be incorporated into a particle, *e.g.*, a microparticle. Microparticles can be produced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

IV. Methods For Inhibiting TTR Expression

The present invention also provides methods of inhibiting expression of a transthyretin (TTR) in a cell. The methods include contacting a cell with an RNAi agent, e.g., double stranded RNAi agent, in an amount effective to inhibit expression of TTR in the cell, thereby inhibiting expression of TTR in the cell.

Contacting of a cell with an RNAi agent, e.g., a double stranded RNAi agent, may be done *in vitro* or *in vivo*. Contacting a cell *in vivo* with the RNAi agent includes contacting a cell or group of cells within a subject, *e.g.*, a human subject, with the RNAi agent. Combinations of *in vitro* and *in vivo* methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, *e.g.*, a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest, *e.g.*, the liver of a subject.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

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

"Inhibiting expression of a TTR gene" includes any level of inhibition of a TTR gene, *e.g.*, at least partial suppression of the expression of a TTR gene. The expression of the TTR gene may be assessed based on the level, or the change in the level, of any variable associated with TTR gene expression, *e.g.*, TTR mRNA level, TTR protein level, or the number or extent of amyloid deposits. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with TTR expression compared with a control level. The control level may be any type of control level that is utilized in the art, *e.g.*, a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, *e.g.*, buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of a TTR gene is inhibited by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%,at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%. at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%.

Inhibition of the expression of a TTR gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which a TTR gene is transcribed and which has or have been treated (*e.g.*, by contacting the cell or cells with an RNAi agent of the invention, or by administering an RNAi agent of the invention to a subject in which the cells are or were present) such that the expression of a TTR gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s)). In preferred embodiments, the inhibition is assessed by expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \bullet 100\%$$

Alternatively, inhibition of the expression of a TTR gene may be assessed in terms of a reduction of a parameter that is functionally linked to TTR gene expression, *e.g.*, TTR protein expression, retinol binding protein level, vitamin A level, or presence of amyloid deposits comprising TTR. TTR gene silencing may be determined in any cell expressing TTR, either constitutively or by genomic engineering, and by any assay known in the art. The liver is the major site of TTR expression. Other significant sites of expression include the choroid plexus, retina and pancreas.

Inhibition of the expression of a TTR protein may be manifested by a reduction in the level of the TTR protein that is expressed by a cell or group of cells (*e.g.*, the level of protein expressed in a sample derived from a subject). As explained above for the assessment of mRNA suppression, the inhibiton of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of a TTR gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (*e.g.*, a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of TTR mRNA that is expressed by a cell or group of cells, or the level of circulating TTR mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of TTR in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, *e.g.*, mRNA of the TTR gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy RNA preparation kits (Qiagen) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays (Melton *et al.*, *Nuc. Acids Res.* 12:7035), Northern blotting, *in situ* hybridization, and microarray analysis. Circulating TTR mRNA may be detected using methods the described in PCT/US2012/043584, the entire contents of which are hereby incorporated herein by reference.

In one embodiment, the level of expression of TTR is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific TTR. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to TTR mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of TTR mRNA.

An alternative method for determining the level of expression of TTR in a sample involves the process of nucleic acid amplification and/or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, *e.g.*, by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of TTR is determined by quantitative fluorogenic RT-PCR (*i.e.*, the TaqMan™ System).

The expression levels of TTR mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as Northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of TTR expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

The level of TTR protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, Western blotting, radioimmunoassay (RIA),

enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention can be monitored by detecting or monitoring a reduction in an amyloid TTR deposit. Reducing an amyloid TTR deposit, as used herein, includes any decrease in the size, number, or severity of TTR deposits, or to a prevention or reduction in the formation of TTR deposits, within an organ or area of a subject, as may be assessed *in vitro* or *in vivo* using any method known in the art. For example, some methods of assessing amyloid deposits are described in Gertz, M.A. & Rajukumar, S.V. (Editors) (2010), *Amyloidosis: Diagnosis and Treatment*, New York: Humana Press. Methods of assessing amyloid deposits may include biochemical analyses, as well as visual or computerized assessment of amyloid deposits, as made visible, *e.g.*, using immunohistochemical staining, fluorescent labeling, light microscopy, electron microscopy, fluorescence microscopy, or other types of microscopy. Invasive or noninvasive imaging modalities, including, *e.g.*, CT, PET, or NMR/MRI imaging may be employed to assess amyloid deposits.

The methods of the invention may reduce TTR deposits in any number of tissues or regions of the body including but not limited to the heart, liver, spleen, esophagus, stomach, intestine (ileum, duodenum and colon), brain, sciatic nerve, dorsal root ganglion, kidney and retina.

The term "sample" as used herein refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, lymph, urine, cerebrospinal fluid, saliva, ocular fluids, and the like. Tissue samples may include samples from tissues, organs or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organis. In certain embodiments, samples may be derived from the liver (*e.g.*, whole liver or certain segments of liver or certain types of cells in the liver, such as, *e.g.*, hepatocytes), the retina or parts of the retina (*e.g.*, retinal pigment epithelium), the central nervous system or parts of the central nervous system (*e.g.*, ventricles or choroid plexus), or the pancreas or certain cells or parts of the pancreas. In preferred embodiments, a "sample derived

from a subject" refers to blood or plasma drawn from the subject. In further embodiments, a "sample derived from a subject" refers to liver tissue or retinal tissue derived from the subject.

In some embodiments of the methods of the invention, the RNAi agent is administered to a subject such that the RNAi agent is delivered to a specific site within the subject. The inhibition of expression of TTR may be assessed using measurements of the level or change in the level of TTR mRNA or TTR protein in a sample derived from fluid or tissue from the specific site within the subject. In preferred embodiments, the site is selected from the group consisting of liver, choroid plexus, retina, and pancreas. The site may also be a subsection or subgroup of cells from any one of the aforementioned sites (*e.g.*, hepatocytes or retinal pigment epithelium). The site may also include cells that express a particular type of receptor (*e.g.*, hepatocytes that express the asialogycloprotein receptor).

V. Methods for Treating or Preventing a TTR-Associated Disease

The present invention also provides methods for treating or preventing a TTR-associated disease in a subject. The methods include administering to the subject a therapeutically effective amount or prophylactically effective amount of an RNAi agent of the invention.

As used herein, a "subject" includes either a human or a non-human animal, preferably a vertebrate, and more preferably a mammal. A subject may include a transgenic organism. Most preferably, the subject is a human, such as a human suffering from or predisposed to developing a TTR-associated disease.

In some embodiments, the subject is suffering from a TTR-associated disease. In other embodiments, the subject is a subject at risk for developing a TTR-associated disease, *e.g.*, a subject with a TTR gene mutation that is associated with the development of a TTR associated disease, a subject with a family history of TTR-associated disease, or a subject who has signs or symptoms suggesting the development of TTR amyloidosis.

A "TTR-associated disease," as used herein, includes any disease caused by or associated with the formation of amyloid deposits in which the fibril precurosors consist

of variant or wild-type TTR protein. Mutant and wild-type TTR give rise to various forms of amyloid deposition (amyloidosis). Amyloidosis involves the formation and aggregation of misfolded proteins, resulting in extracellular deposits that impair organ function. Climical syndromes associated with TTR aggregation include, for example, senile systemic amyloidosis (SSA); systemic familial amyloidosis; familial amyloidotic polyneuropathy (FAP); familial amyloidotic cardiomyopathy (FAC); and leptomeningeal amyloidosis, also known as leptomeningeal or meningocerebrovascular amyloidosis, central nervous system (CNS) amyloidosis, or amyloidosis VII form.

In some embodiments of the methods of the invention, RNAi agents of the invention are administered to subjects suffering from familial amyloidotic cardiomyopathy (FAC) and senile systemic amyloidosis (SSA). Normal-sequence TTR causes cardiac amyloidosis in people who are elderly and is termed senile systemic amyloidosis (SSA) (also called senile cardiac amyloidosis (SCA) or cardiac amyloidosis). SSA often is accompanied by microscopic deposits in many other organs. TTR mutations accelerate the process of TTR amyloid formation and are the most important risk factor for the development of clinically significant TTR amyloidosis (also called ATTR (amyloidosis-transthyretin type)). More than 85 amyloidogenic TTR variants are known to cause systemic familial amyloidosis.

In some embodiments of the methods of the invention, RNAi agents of the invention are administered to subjects suffering from transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP). Such subjects may suffer from ocular manifestations, such as vitreous opacity and glaucoma. It is known to one of skill in the art that amyloidogenic transthyretin (ATTR) synthesized by retinal pigment epithelium (RPE) plays important roles in the progression of ocular amyloidosis. Previous studies have shown that panretinal laser photocoagulation, which reduced the RPE cells, prevented the progression of amyloid deposition in the vitreous, indicating that the effective suppression of ATTR expression in RPE may become a novel therapy for ocular amyloidosis (see, *e.g.*, Kawaji, T., et al., Ophthalmology. (2010) 117: 552-555). The methods of the invention are useful for treatment of ocular manifestations of TTR related FAP, *e.g.*, ocular amyloidosis. The RNAi agent can be delivered in a manner suitable for targeting a particular tissue, such as the eye. Modes of ocular delivery

include retrobulbar, subcutaneous eyelid, subconjunctival, subtenon, anterior chamber or intravitreous injection (or internal injection or infusion). Specific formulations for ocular delivery include eye drops or ointments.

Another TTR-associated disease is hyperthyroxinemia, also known as "dystransthyretinemic hyperthyroxinemia" or "dysprealbuminemic hyperthyroxinemia". This type of hyperthyroxinemia may be secondary to an increased association of thyroxine with TTR due to a mutant TTR molecule with increased affinity for thyroxine. See, *e.g.*, Moses *et al.* (1982) *J. Clin. Invest.*, 86, 2025-2033.

The RNAi agents of the invention may be administered to a subject using any mode of administration known in the art, including, but not limited to subcutaneous, intravenous, intramuscular, intraocular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof. In preferred embodiments, the agents are administered subcutaneously.

In some embodiments, the administration is via a depot injection. A depot injection may release the RNAi agent in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, *e.g.*, a desired inhibition of TTR, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the RNAi agent to the liver.

Other modes of administration include epidural, intracerebral, intracerebroventricular, nasal administration, intraarterial, intracardiac, intraosseous infusion, intrathecal, and intravitreal, and pulmonary. The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the

area to be treated. The route and site of administration may be chosen to enhance targeting.

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

In some embodiments, the RNAi agent is administered to a subject in a therapeutically or prophylactically effective amount.

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

"Prophylactically effective amount," as used herein, is intended to include the amount of an RNAi agent that, when administered to a subject who does not yet experience or display symptoms of a TTR-associated disease, but who may be predisposed to the disease, is sufficient to prevent or ameliorate the disease or one or more symptoms of the disease. Symptoms that may be ameliorated include sensory neuropathy (*e.g.*, paresthesia, hypesthesia in distal limbs), autonomic neuropathy (*e.g.*, gastrointestinal dysfunction, such as gastric ulcer, or orthostatic hypotension), motor neuropathy, seizures, dementia, myelopathy, polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, vitreous opacities, renal insufficiency, nephropathy, substantially reduced mBMI (modified Body Mass Index), cranial nerve dysfunction, and corneal lattice dystrophy. Ameliorating the disease includes slowing the course of the disease or reducing the severity of later-developing disease. The "prophylactically effective amount" may vary depending on the RNAi agent, how the

agent is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of an RNAi agent that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. RNAi agents employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" also include an amount that provides a benefit in the treatment, prevention, or management of pathological processes or symptom(s) of pathological processes mediated by TTR expression. Symptoms of TTR amyloidosis include sensory neuropathy (*e.g.* paresthesia, hypesthesia in distal limbs), autonomic neuropathy (*e.g.*, gastrointestinal dysfunction, such as gastric ulcer, or orthostatic hypotension), motor neuropathy, seizures, dementia, myelopathy, polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, vitreous opacities, renal insufficiency, nephropathy, substantially reduced mBMI (modified Body Mass Index), cranial nerve dysfunction, and corneal lattice dystrophy.

The dose of an RNAi agent that is administered to a subject may be tailored to balance the risks and benefits of a particular dose, for example, to achieve a desired level of TTR gene suppression (as assessed, *e.g.*, based on TTR mRNA suppression, TTR protein expression, or a reduction in an amyloid deposit, as defined above) or a desired therapeutic or prophylactic effect, while at the same time avoiding undesirable side effects.

In one embodiment, the RNAi agent is administered at a dose of between about 0.25 mg/kg to about 50 mg/kg, *e.g.*, between about 0.25 mg/kg to about 0.5 mg/kg, between about 0.25 mg/kg to about 1 mg/kg, between about 0.25 mg/kg to about 5 mg/kg, between about 0.25 mg/kg to about 10 mg/kg, between about 1 mg/kg to about 10 mg/kg, between about 5 mg/kg to about 15 mg/kg, between about 10 mg/kg to about 20 mg/kg, between about 15 mg/kg to about 25 mg/kg, between about 20 mg/kg to about

30 mg/kg, between about 25 mg/kg to about 35 mg/kg, or between about 40 mg/kg to about 50 mg/kg.

In some embodiments, the RNAi agent is administered at a dose of about 0.25 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 21 mg/kg, about 22 mg/kg, about 23 mg/kg, about 24 mg/kg, about 25 mg/kg, about 26 mg/kg, about 27 mg/kg, about 28 mg/kg, about 29 mg/kg, 30 mg/kg, about 31 mg/kg, about 32 mg/kg, about 33 mg/kg, about 34 mg/kg, about 35 mg/kg, about 36 mg/kg, about 37 mg/kg, about 38 mg/kg, about 39 mg/kg, about 40 mg/kg, about 41 mg/kg, about 42 mg/kg, about 43 mg/kg, about 44 mg/kg, about 45 mg/kg, about 46 mg/kg, about 47 mg/kg, about 48 mg/kg, about 49 mg/kg or about 50 mg/kg.

In some embodiments, the RNAi agent is administered in two or more doses. If desired to facilitate repeated or frequent infusions, implantation of a delivery device, *e.g.*, a pump, semi-permanent stent (*e.g.*, intravenous, intraperitoneal, intracisternal or intracapsular), or reservoir may be advisable. In some embodiments, the number or amount of subsequent doses is dependent on the achievement of a desired effect, *e.g.*, the suppression of a TTR gene, or the achievement of a therapeutic or prophylactic effect, *e.g.*, reducing an amyloid deposit or reducing a symptom of a TTR-associated disease. In some embodiments, the RNAi agent is administered according to a schedule. For example, the RNAi agent may be administered twice per week, three times per week, four times per week, or five times per week. In some embodiments, the schedule involves regularly spaced administrations, *e.g.*, hourly, every four hours, every six hours, every eight hours, every twelve hours, daily, every 2 days, every 3 days, every 4 days, every 5 days, weekly, biweekly, or monthly. In other embodiments, the schedule involves closely spaced administrations followed by a longer period of time during which the agent is not administered. For example, the schedule may involve an initial set of doses that are administered in a relatively short period of time (*e.g.*, about every 6 hours, about every 12 hours, about every 24 hours, about every 48 hours, or about every

72 hours) followed by a longer time period (*e.g.*, about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 5 weeks, about 6 weeks, about 7 weeks, or about 8 weeks) during which the RNAi agent is not administered. In one embodiment, the RNAi agent is initially administered hourly and is later administered at a longer interval (*e.g.*, daily, weekly, biweekly, or monthly). In another embodiment, the RNAi agent is initially administered daily and is later administered at a longer interval (*e.g.*, weekly, biweekly, or monthly). In certain embodiments, the longer interval increases over time or is determined based on the achievement of a desired effect. In a specific embodiment, the RNAi agent is administered once daily during a first week, followed by weekly dosing starting on the eighth day of administration. In another specific embodiment, the RNAi agent is administered every other day during a first week followed by weekly dosing starting on the eighth day of administration.

Any of these schedules may optionally be repeated for one or more iterations. The number of iterations may depend on the achievement of a desired effect, *e.g.*, the suppression of a TTR gene, retinol binding protein level, vitamin A level, and/or the achievement of a therapeutic or prophylactic effect, *e.g.*, reducing an amyloid deposit or reducing a symptom of a TTR-associated disease.

In some embodiments, the RNAi agent is administered with other therapeutic agents or other therapeutic regimens. For example, other agents or other therapeutic regimens suitable for treating a TTR-associated disease may include a liver transplant, which can reduce mutant TTR levels in the body; Tafamidis (Vyndaqel), which kinetically stabilizes the TTR tetramer preventing tetramer dissociation required for TTR amyloidogenesis; and diuretics, which may be employed, for example, to reduce edema in TTR amyloidosis with cardiac involvement.

In one embodiment, a subject is administered an initial dose and one or more maintenance doses of an RNAi agent. The maintenance dose or doses can be the same or lower than the initial dose, *e.g.*, one-half of the initial dose. A maintenance regimen can include treating the subject with a dose or doses ranging from 0.01 μg to 15 mg/kg of body weight per day, *e.g.*, 10 mg/kg, 1 mg/kg, 0.1 mg/kg, 0.01 mg/kg, 0.001 mg/kg, or 0.00001 mg/kg of bodyweight per day. The maintenance doses are, for example, administered no more than once every 2 days, once every 5 days, once every 7 days,

once every 10 days, once every 14 days, once every 21 days, or once every 30 days. Further, the treatment regimen may last for a period of time which will vary depending upon the nature of the particular disease, its severity and the overall condition of the patient. In certain embodiments the dosage may be delivered no more than once per day, *e.g.*, no more than once per 24, 36, 48, or more hours, *e.g.*, no more than once every 5 or 8 days. Following treatment, the patient can be monitored for changes in his/her condition. The dosage of the RNAi agent may either be increased in the event the patient does not respond significantly to current dosage levels, or the dose may be decreased if an alleviation of the symptoms of the disease state is observed, if the disease state has been ablated, or if undesired side-effects are observed.

VI. Kits

The present invention also provides kits for performing any of the methods of the invention. Such kits include one or more RNAi agent(s) and instructions for use, *e.g.*, instructions for inhibiting expression of a TTR in a cell by contacting the cell with the RNAi agent(s) in an amount effective to inhibit expression of the TTR. The kits may optionally further comprise means for contacting the cell with the RNAi agent (*e.g.*, an injection device), or means for measuring the inhibition of TTR (*e.g.*, means for measuring the inhibition of TTR mRNA or TTR protein). Such means for measuring the inhibition of TTR may comprise a means for obtaining a sample from a subject, such as, *e.g.*, a plasma sample. The kits of the invention may optionally further comprise means for administering the RNAi agent(s) to a subject or means for determining the therapeutically effective or prophylactically effective amount.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references and published patents and patent applications cited throughout the application are hereby incorporated herein by reference.

EXAMPLES

Example 1: Inhibition of TTR with TTR-GalNAc conjugates

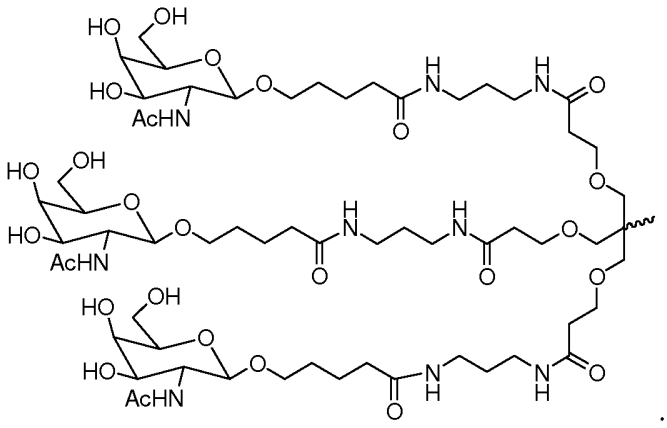
A single dose of the TTR RNAi agent AD-43527 was administered to mice subcutaneously and TTR mRNA levels were determined 72 hours post administration.

The mouse/rat cross-reactive GalNAc-conjugate, AD-43527, was chosen for *in vivo* evaluation in WT C57BL/6 mice for silencing of TTR mRNA in liver. The sequence of each strand of AD-43527 is shown below.

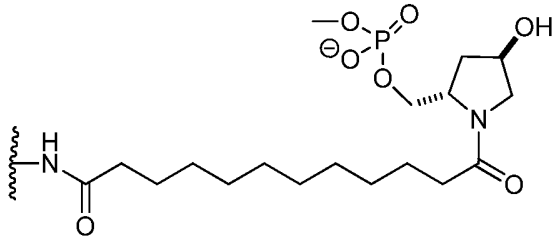
Strand: s= sense; as= antisense

Duplex #	Strand	Oligo #	Sequence 5′ to 3′
AD-43527	s	A-89592	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfaAfL96 (SEQ ID NO: 8)
	as	A-83989	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu (SEQ ID NO: 9
			L96 = GalNAc3; lowercase nts (a,u,g,c) are 2′-O-methyl nucleotides, Nf (*i.e.*, Af) is a 2′-fluoro nucleotide

The ligand used was $GalNAc_3$:

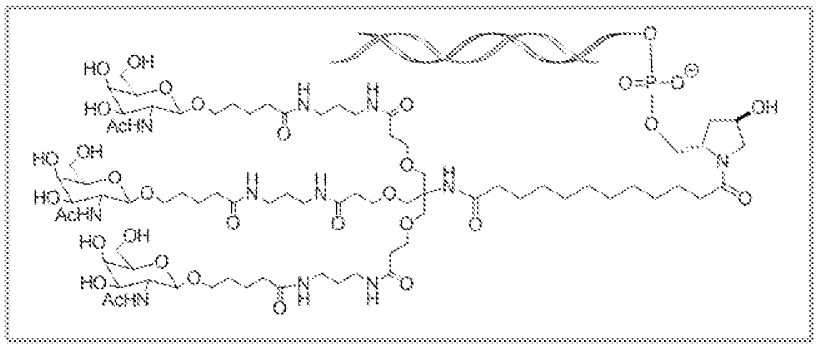


This GalNAc3 ligand was conjugated to the 3'-end of the sense strand using the linker and tether as shown below:



,

The structure of the resulting $GalNAc_3$ conjugated sense strand is shown in the following schematic:



Additional RNAi agents that target TTR and have the following sequences and modifications were synthesized and assayed.

Mouse/rat cross reactive TTR RNAi agents

Duplex	Sense strand 5'-3'	Antisense strand 5'-3'
AD-43528	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfaAfQ11L96 (SEQ ID NO: 10)	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu (SEQ ID NO: 11)

Human/cyno cross reactive TTR RNAi agents; parent duplex is AD-18328 [having a sense strand 5'-3' sequence of GuAAccAAGAGuAuuccAudTdT (SEQ ID NO: 12) and antisense strand 5' to 3' sequence of AUGGAAuACUCUUGGUuACdTdT (SEQ ID NO: 13) with the following modifications: alternating 2'F/2'OMe w/2 PS on AS.

Duplex	Sense strand 5'-3'	Antisense strand 5'-3'
AD-45163	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfaUfL96 (SEQ ID NO: 14)	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa (SEQ ID NO: 16)
AD-45164	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfaUfQ11L96 (SEQ ID NO: 15)	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa (SEQ ID NO: 17)

L96 = $GalNAc_3$; lowercase nts (a,u,g,c) are 2'-O-methyl nucleotides, Nf (*i.e.*, Af) is a 2'-fluoro nucleotide; Q11 is cholesterol; s is phosphorothioate.

AD-43527 was administered to female C57BL/6 mice (6-10 weeks, 5 per group) via subcutaneous injection at a dose volume of 10μl/g at a dose of 30, 15, 7.5, 3.5, 1.75 or 0.5 mg/kg of AD-43527. Control animals received PBS by subcutaneous injection at the same dose volume.

After approximately seventy two hours, mice were anesthetized with 200 μl of ketamine, and then exsanguinated by severing the right caudal artery. Liver tissue was collected, flash-frozen and stored at -80°C until processing.

Efficacy of treatment was evaluated by measurement of TTR mRNA in the liver at 72 hours post-dose. TTR liver mRNA levels were assayed utilizing the Branched DNA assays- QuantiGene 1.0 (Panomics). Briefly, mouse liver samples were ground and tissue lysates were prepared. Liver lysis mixture (a mixture of 1 volume of lysis mixture, 2 volume of nuclease-free water and 10μl of Proteinase-K/ml for a final concentration of 20mg/ml) was incubated at 65 °C for 35 minutes. 5μl of liver lysate and 95μl of working probe set (TTR probe for gene target and GAPDH for endogenous control) were added into the Capture Plate. Capture Plates were incubated at 53 °C ±1 °C (aprx. 16-20hrs). The next day, the Capture Plates were washed 3 times with 1X Wash Buffer (nuclease-free water, Buffer Component 1 and Wash Buffer Component 2), then dried by centrifuging for 1 minute at 240g. 100μl of Amplifier Probe mix per well was added into the Capture Plate, which was sealed with aluminum foil and incubated for 1 hour at 46°C ±1°C. Following a 1 hour incubation, the wash step was repeated, then 100μl of Label Probe mix per well was added. Capture plates were incubated at 46 °C ±1 °C for 1 hour. The plates were then washed with 1X Wash Buffer, dried and 100μl substrate per well was added into the Capture Plates. Capture Plates were incubated for 30 minutes at 46°C followed by incubation for 30 minutes at room temperature. Plates were read using the SpectraMax Luminometer following incubation. bDNA data were analyzed by subtracting the average background from each duplicate sample, averaging the resultant duplicate GAPDH (control probe) and TTR (experimental probe) values, and then computing the ratio: (experimental probe-background)/(control probe-background). The average TTR mRNA level was calculated for each group and normalized to the PBS group average to give relative TTR mRNA as a % of the PBS control group.

The results are shown in Figure 1. The GalNAc conjugated RNAi agent targeting TTR had an ED_{50} of approximately 5 mg/kg for TTR mRNA knockdown. These results demonstrate that GalNAc conjugated RNAi agents that target TTR are effective at inhibiting expression of TTR mRNA.

Example 2: Inhibition of TTR with TTR-GalNAc conjugates is durable

Mice were administered a subcutaneous dose (either 7.5 or 30.0 mg/kg) of AD-43527, a GalNAc conjugated RNAi agent that targets TTR. The TTR mRNA levels in the liver were evaluated at 1, 3, 5, 7, 10, 13, 15, 19, 26, 33, and 41 days post treatment using the method described in Example 1.

The results are shown in Figure 2. At day 19, administration of 30.0 mg/kg GalNAc conjugated RNAi agents still showed about 50% silencing. Full recovery of expression occurred at day 41.

These results demonstrated that the inhibition provided by GalNAc conjugated siRNA targeting TTR is durable, lasting up to 3, 5, 7, 10, 13, 15, 19, 26 or 33 days post treatment.

Example 3. RNA Synthesis and Duplex Annealing

1. Oligonucleotide Synthesis

Oligonucleotides were synthesized on an AKTAoligopilot synthesizer or an ABI 394 synthsizer. Commercially available controlled pore glass solid support (dT-CPG, 500Å, Prime Synthesis) and RNA phosphoramidites with standard protecting groups, 5'-*O*-dimethoxytrityl N6-benzoyl-2'-*t*-butyldimethylsilyl-adenosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-*t*-butyldimethylsilyl-cytidine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, 5'-*O*-dimethoxytrityl-N2--isobutryl-2'-*t*-butyldimethylsilyl-guanosine-3'-*O*-N,N'-diisopropyl-2-cyanoethylphosphoramidite, and 5'-*O*-dimethoxytrityl-2'-*t*-butyldimethylsilyl-uridine-3'-O-N,N'-diisopropyl-2-cyanoethylphosphoramidite (Pierce Nucleic Acids Technologies) were used for the oligonucleotide synthesis unless otherwise specified. The 2'-F phosphoramidites, 5'-*O*-dimethoxytrityl-N4-acetyl-2'-fluro-cytidine-3'-*O*-

N,N'-diisopropyl-2-cyanoethyl-phosphoramidite and 5'-*O*-dimethoxytrityl-2'-fluro-uridine-3'-O-N,N'-diisopropyl-2-cyanoethyl-phosphoramidite were purchased from (Promega). All phosphoramidites were used at a concentration of 0.2M in acetonitrile (CH_3CN) except for guanosine which was used at 0.2M concentration in 10% THF/ANC (v/v). Coupling/recycling time of 16 minutes was used. The activator was 5-ethyl thiotetrazole (0.75M, American International Chemicals), for the PO-oxidation Iodine/Water/Pyridine was used and the PS-oxidation PADS (2 %) in 2,6-lutidine/ACN (1:1 v/v) was used.

Ligand conjugated strands were synthesized using a solid support containing the corresponding ligand. For example, the introduction of a carbohydrate moiety/ligand (for e.g., GalNAc) at the 3'-end of a sequence was achieved by starting the synthesis with the corresponding carbohydrate solid support. Similarly a cholesterol moiety at the 3'-end was introduced by starting the synthesis on the cholesterol support. In general, the ligand moiety was tethered to *trans*-4-hydroxyprolinol via a tether of choice as described in the previous examples to obtain a hydroxyprolinol-ligand moiety. The hydroxyprolinol-ligand moiety was then coupled to a solid support via a succinate linker or was converted to phosphoramidite via standard phosphitylation conditions to obtain the desired carbohydrate conjugate building blocks. Fluorophore labeled siRNAs were synthesized from the corresponding phosphoramidite or solid support, purchased from Biosearch Technologies. The oleyl lithocholic $(GalNAc)_3$ polymer support made in house at a loading of 38.6 μmol/gram. The Mannose $(Man)_3$ polymer support was also made in house at a loading of 42.0 μmol/gram.

Conjugation of the ligand of choice at the desired position, for example at the 5'-end of the sequence, was achieved by coupling of the corresponding phosphoramidite to the growing chain under standard phosphoramidite coupling conditions unless otherwise specified. An extended 15 minute coupling of 0.1M solution of phosphoramidite in anhydrous CH_3CN in the presence of 5-(ethylthio)-1*H*-tetrazole activator to a solid bound oligonucleotide. Oxidation of the internucleotide phosphite to the phosphate was carried out using standard iodine-water as reported in Beaucage, S.L. (2008) Solid-phase synthesis of siRNA oligonucleotides. *Curr. Opin. Drug Discov. Devel.*, 11, 203–216; Mueller, S., Wolf, J. and Ivanov, S.A. (2004) Current Strategies for the Synthesis

of RNA. *Curr. Org. Synth.*, 1, 293–307; Xia, J., Noronha, A., Toudjarska, I., Li, F., Akinc, A., Braich, R., Frank-Kamenetsky, M., Rajeev, K.G., Egli, M. and Manoharan, M. (2006) Gene Silencing Activity of siRNAs with a Ribo-difluorotoluyl Nucleotide. *ACS Chem. Biol.*, 1, 176–183 or by treatment with *tert*-butyl hydroperoxide/acetonitrile/water (10: 87: 3) with a 10 minute oxidation wait time conjugated oligonucleotide. Phosphorothioate was introduced by the oxidation of phosphite to phosphorothioate by using a sulfur transfer reagent such as DDTT (purchased from AM Chemicals), PADS and or Beaucage reagent The cholesterol phosphoramidite was synthesized in house, and used at a concentration of 0.1 M in dichloromethane. Coupling time for the cholesterol phosphoramidite was 16 minutes.

2. Deprotection- I (Nucleobase Deprotection)

After completion of synthesis, the support was transferred to a 100 ml glass bottle (VWR). The oligonucleotide was cleaved from the support with simultaneous deprotection of base and phosphate groups with 80 mL of a mixture of ethanolic ammonia [ammonia: ethanol (3:1)] for 6.5h at 55°C. The bottle was cooled briefly on ice and then the ethanolic ammonia mixture was filtered into a new 250 ml bottle. The CPG was washed with 2 x 40 mL portions of ethanol/water (1:1 v/v). The volume of the mixture was then reduced to ~ 30 ml by roto-vap. The mixture was then frozen on dry ice and dried under vacuum on a speed vac.

3. Deprotection-II (Removal of 2' TBDMS group)

The dried residue was resuspended in 26 ml of triethylamine, triethylamine trihydrofluoride (TEA.3HF) or pyridine-HF and DMSO (3:4:6) and heated at 60°C for 90 minutes to remove the *tert*-butyldimethylsilyl (TBDMS) groups at the 2' position. The reaction was then quenched with 50 ml of 20mM sodium acetate and pH adjusted to 6.5, and stored in freezer until purification.

4. Analysis

The oligonucleotides were analyzed by high-performance liquid chromatography (HPLC) prior to purification and selection of buffer and column depends on nature of the sequence and or conjugated ligand.

5. HPLC Purification

The ligand conjugated oligonucleotides were purified by reverse phase preparative HPLC. The unconjugated oligonucleotides were purified by anion-exchange HPLC on a TSK gel column packed in house. The buffers were 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN (buffer A) and 20 mM sodium phosphate (pH 8.5) in 10% CH_3CN, 1M NaBr (buffer B). Fractions containing full-length oligonucleotides were pooled, desalted, and lyophilized. Approximately 0.15 OD of desalted oligonucleotidess were diluted in water to 150 µl and then pipetted in special vials for CGE and LC/MS analysis. Compounds were finally analyzed by LC-ESMS and CGE.

6. RNAi Agent preparation

For the preparation of an RNAi agent, equimolar amounts of sense and antisense strand were heated in 1xPBS at 95°C for 5 minutes and slowly cooled to room temperature. The integrity of the duplex was confirmed by HPLC analysis. Table 1 below reflects the RNAi agents which target human or rodent TTR mRNA.

Table 1: RNAi Agents and Results of *In Vitro* Screening

Duplex ID	S ID	SEQ ID NO:	Sense strand (S)	AS ID	SEQ ID NO:	Antisense strand (AS)	% of mRNA remained conc. of siRNA			IC50 (nM)
							1 nM	0.1 nM	0.01 nM	
D1000	S1000	18	AfuGfuAfaCfcAfAfGfaGfuAfuUfcCfasu	AS1000	1110	AfUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.1	0.47	0.006
D1001	S1001	19	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1001	1111	aUfsgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.10	0.49	0.0065
D1002	S1002	20	AfuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1002	1112	aUfgGfAfAfuAfcUfcuuGfgsUfuAfcAfusGfsa	0.04	0.10	0.46	0.0068
D1003	S1003	21	AfuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1003	1113	aUfgGfAfAfuAfcUfcuuGfgUfsuAfcAfusGfsa	0.05	0.12	0.56	0.0073
D1004	S1004	22	aUGuaACccAGagUAuuCCasu	AS1004	1114	AUggAAuaCUcuUGguUAcaUsGsa	0.07	0.13	0.44	0.008
D1005	S1005	23	AfuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1005	1115	aUfgGfAfAfuAfcUfcuuGfgsUfsuAfcAfusGfsa	0.06	0.11	0.53	0.0093
D1006	S1006	24	AfuGfuAfAfccAfAfGfaGfuAfuUfcCfasUf	AS1006	1116	aUfgGfaAfuAfcUfcuuGfGfuuAfcAfusGfsa	0.05	0.16	0.55	0.0095
D1007	S1007	25	AfuGfuAfAfCfcAfAfGfaGfuAfuUfcCfasUf	AS1007	1117	aUfgGfaAfuAfcUfcuuGfguuAfcAfusGfsa	0.05	0.14	0.48	0.0098
D1008	S1008	26	auguaaccaadGadGudAudAcdGasu	AS1008	1118	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.07	0.11	0.33	0.010
D1009	S1009	27	UfgGfGfAfuUfuCfAfUfgUfaAfcCfAfAfgsAf	AS1009	1119	uCfuugGfuUfaCfaugAfaAfuccCfasUfsc	0.03	0.14	0.56	0.0101
D1010	S1010	28	UfgGfgauUfuCfAfUfgUfaAfcCfaAfgsAf	AS1010	1120	uCfuUfgGfuUfaCfaugAfaAfUfCfcCfasUfsc	0.03	0.14	0.65	0.0101
D1011	S1011	29	aUfGfuAfAfccAfAfGfaGfuAfuUfcCfasUf	AS1011	1121	aUfgGfaAfuAfcUfcuuGfGfuuAfcaUfsgsa	0.06	0.10	0.55	0.011
D1012	S1012	30	UfgGfgAfuUfuCfAfUfgUfaacCfaAfgsAf	AS1012	1122	uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc	0.04	0.13	0.54	0.0114
D1013	S1013	31	auguaaccaadGadGudAudAcdGasu	AS1013	1123	aUfgGfaAfuAfcUfcUfugdGudTadCadTsgsa	0.11	0.19	0.49	0.011
D1014	S1014	32	AfuGfuaaCfcAfAfGfaGfuAfuUfcCfasUf	AS1014	1124	aUfgGfaAfuAfcUfcuuGfgUfUfAfcAfusGfsa	0.04	0.16	0.59	0.013
D1015	S1015	33	AfuguAfaccAfaGfdAGfdTAdTudCcdAsu	AS1015	1125	dAUdGgdAadTAfdCUfcUfuGfgUfuAfcAfusGfsa	0.07	0.15	0.51	0.013
D1016	S1016	34	auGfuAfaCfcAfAfGfaGfuAfuUfcCfasUf	AS1016	1126	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfUfsGfsa	0.05	0.14	0.64	0.013
D1017	S1017	35	UfGfggAfuUfuCfAfUfgUfAfAfcCfaAfgsAf	AS1017	1127	uCfuUfgGfuuaCfaugAfaAfuCfCfcasUfsc	0.09	0.41	0.74	0.0133
D1018	S1018	36	AfuguAfaCfcAfAfGfaGfuAfuUfcCfasUf	AS1018	1128	aUfgGfaAfuAfcUfcuuGfgUfuAfCfAfusGfsa	0.03	0.14	0.61	0.014
D1019	S1019	37	AfuGfuAfaccAfAfGfaGfuAfuUfcCfasUf	AS1019	1129	aUfgGfaAfuAfcUfcuuGfGfUfuAfcAfusGfsa	0.02	0.2	0.7	0.014
D1020	S1020	38	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1020	1130	asUfsgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.04	0.16	0.67	0.0156

D1021	S1021	39	aUfguAfAfccAfAfgagUfaUfuCfcasUf	AS1021	1131	aUfGfgAfaUfaCfUfCfuuGfGfuuAfCfaUfsgsa	0.11	0.24	0.64	0.016
D1022	S1022	40	dTdGggdAdTuudCdAugdTdAacdCdAagsdA	AS1022	1132	udCdTugdGdTuadCdAugdAdAaudCdCcasdTsc	0.08	0.27	0.64	0.0161
D1023	S1023	41	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1023	1133	aUfgsGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.19	0.63	0.0163
D1024	S1024	42	UfgGfgAfuUfuCfAfUfguaAfcCfaAfgsAf	AS1024	1134	uCfuUfgGfuUfAfCfaugAfaAfuCfcCfasUfsc	0.05	0.25	0.69	0.0164
D1025	S1025	43	UfgGfgAfuUfuCfAfUfgUfAfAfcCfaAfgsAf	AS1025	1135	uCfuUfgGfuuaCfaugAfaAfuCfcCfasUfsc	0.04	0.18	0.75	0.0166
D1026	S1026	44	UfgGfgAfuUfuCfAfUfgUfaAfcCfaAfgsAf	AS1026	1136	uCfuUfgGfuUfaCfaugAfaAfuCfcCfasUfsc	0.04	0.19	0.66	0.0178
D1027	S1027	45	UfgGfgAfuUfuCfAfUfgUfaAfccaAfgsAf	AS1027	1137	uCfuUfGfGfuUfaCfaugAfaAfuCfcCfasUfsc	0.04	0.19	0.69	0.018
D1028	S1028	46	dAdTgudAdAccdAdAgadGdTaudTdCcasdT	AS1028	1138	adTdGgadAdTacdTdCuudGdGuudAdCausdGsa	0.15	0.29	0.72	0.018
D1029	S1029	47	AdTGdTAdACdCAdAGdAGdTAdTUdCCdAsU	AS1029	1139	dAUdGGdAAdTAdCUdCUdTGdGUdTAdCAdTsGsdA	0.1	0.27	0.61	0.018
D1030	S1030	48	UfgGfGfAfuuuCfAfUfgUfaAfcCfaAfgsAf	AS1030	1140	uCfuUfgGfuUfaCfaugAfAfAfuccCfasUfsc	0.04	0.21	0.64	0.0187
D1031	S1031	49	AfuGfuAfAfccAfAfGfAfGfuAfuuccAfsu	AS1031	1141	AfUfGfGfAfAfuAfCfCfUfuGfGfuuAfcAfusGfsa	0.06	0.15	0.62	0.019
D1032	S1032	50	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1032	1142	asUfgGfAfAfuAfcUfcuuGfgUfsuAfcAfusGfsa	0.09	0.34	0.78	0.021
D1033	S1033	51	UfgGfgAfuUfuCfaUfGfUfaacCfaAfgsAf	AS1033	1143	uCfuUfgGfUfUfacaUfgAfaAfuCfcCfasUfsc	0.06	0.26	0.57	0.0212
D1034	S1034	52	AfuGfuAfAfccAfaGfaGfuAfuUfcCfasUf	AS1034	1144	aUfgGfaAfuAfcUfcUfuGfGfuuAfcAfusGfsa	0.11	0.39	0.82	0.0216
D1035	S1035	53	UfgGfgAfuuuCfAfUfgUfaAfcCfaAfgsAf	AS1035	1145	uCfuUfgGfuUfaCfaugAfAfAfuCfcCfasUfsc	0.04	0.16	0.56	0.0222
D1036	S1036	54	UfgGfGfAfuUfuCfaUfgUfaAfcCfAfAfgsAf	AS1036	1146	uCfuugGfuUfaCfaUfgAfaAfucccCfasUfsc	0.06	0.31	0.78	0.0234
D1037	S1037	55	UfgGfGfAfuUfuCfAfUfgUfaAfcCfaAfgsAf	AS1037	1147	uCfuUfgGfuUfaCfaugAfaAfucccCfasUfsc	0.03	0.14	0.62	0.0235
D1038	S1038	56	UfGfggAfUfuuCfAfugUfAfacCfAfagsAf	AS1038	1148	uCfUfugGfUfuaCfAfugAfAfauCfCfcasUfsc	0.09	0.39	0.78	0.0239
D1039	S1039	57	AfuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1039	1149	aUfgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.14	0.59	0.025
D1040	S1040	58	AfuGfuAfaCfcAfAfGfaGfuAfuUfccasUf	AS1040	1150	aUfGfGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.13	0.56	0.025
D1041	S1041	59	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1041	1151	asUfgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.06	0.27	0.79	0.0252
D1042	S1042	60	UfgGfgAfuuuCfAfUfgUfAfAfcCfaAfgsAf	AS1042	1152	uCfuUfgGfuuaCfaugAfAfAfuCfccCfasUfsc	0.05	0.27	0.67	0.0259
D1043	S1043	61	AfuGfuAfaCfcAfAfGfaGfuauUfcCfasUf	AS1043	1153	aUfgGfaAfUfAfcUfcuuGfgUfuAfcAfusGfsa	0.02	0.16	0.63	0.027
D1044	S1044	62	AfsuGfuAfaCfcAfAfGfaGfuAfuucCfasUf	AS1044	1154	asUfgGfAfAfuAfcUfcuuGfgsUfsuAfcAfusGfsa	0.06	0.30	0.81	0.0271
D1045	S1045	63	aUfguAfAfccAfAfgaGfGfauUfCfcasUf	AS1045	1155	aUfGfgaAfUfacUfCfuuGfGfuuAfCfaUfsgsa	0.12	0.29	0.8	0.028
D1046	S1046	64	AfuGfuAfaCfcAfAfGfaguAfuUfcCfasUf	AS1046	1156	aUfgGfaAfuAfCfUfcuuGfgUfuAfcAfusGfsa	0.03	0.15	0.59	0.030
D1047	S1047	65	UfgGfGfAfuUfuCfaUfgUfAfAfcCfaAfgsAf	AS1047	1157	uCfuUfgGfuuaCfaUfgAfaAfucccCfasUfsc	0.08	0.44	0.83	0.0324

D1048	S1048	66	AfuGfuAfaCfcAfAfGfaGfuAfuUfcCfasUf	AS1048	1158	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.07	0.23	0.67	0.036
D1049	S1049	67	AfuGfuAfAfccAfAfGfAfGfuAfuuccAfsu	AS1049	1159	AfUfGfGfAfAfuAfCfUfCfUfUfGfGfUfuAfCfAfusGfsa	0.08	0.23	0.73	0.037
D1050	S1050	68	UfgGfgAfuuuCfaUfgUfaAfcCfAfAfgsAf	AS1050	1160	uCfuugGfuUfaCfaUfgAfAfAfuCfcCfasUfsc	0.06	0.29	0.78	0.0372
D1051	S1051	69	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1051	1161	aUfgGfaAfudAcdTcdTudGgdTuAfcAfusgsa	0.12	0.41	0.86	0.040
D1052	S1052	70	AfuguAfaccAfaGfdAGfdTAdTUdCcdAsu	AS1052	1162	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.22	0.72	0.042
D1053	S1053	71	AfuguAfaccAfaGfdAGfdTAdTUdCcdAsu	AS1053	1163	dAUdGGdAAfuAfcUfcUfuGfGfUfuAfCfAfusGfsa	0.09	0.31	0.69	0.044
D1054	S1054	72	AfuGfuAfaCfcAfaGfadGdTAfuUfcdCdAsUf	AS1054	1164	adTdGGfaAfudAdCUfcUfuGfgUfuAfcAfusGfsa	0.1	0.45	0.75	0.047
D1055	S1055	73	AfuguAfaccAfaGfaGfdTAdTUdCcdAsu	AS1055	1165	dAUdGGdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.12	0.26	0.7	0.049
D1056	S1056	74	AuGuAaCcAaGaGuAuUcCasU	AS1056	1166	aUgGaAuAcUcUuuGgUuuAcAusGsa	0.08	0.24	0.65	0.050
D1057	S1057	75	AfuguAfaccAfagaGfuauUfccasUf	AS1057	1167	aUfGfGfaAfUfAfcUfCfUfuGfGfUfuAfCfAfusGfsa	0.14	0.42	0.62	0.051
D1058	S1058	76	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1058	1168	aUfgGfaAfudAcdTcdTudGgdTuAfcAfusGfsa	0.12	0.36	0.86	0.053
D1059	S1059	77	AfuguAfaccAfaGfdAGfdTAdTUdCcdAsu	AS1059	1169	dAUdGGdAadTAfdCUfcUfuGfgUfuAfcAfusGfsa	0.09	0.27	0.7	0.054
D1060	S1060	78	adTgudAdAccdAdAgagdTadTudCcasdT	AS1060	1170	adTdGgdAadTadCdTdCuudGdGuudAdCadTsgsa	0.11	0.37	0.66	0.056
D1061	S1061	79	AfuGfuAfaCfcAfaGfdAdGuAfuUfcdCdAsUf	AS1061	1171	adTdGGfaAfuAfdCdTcUfuGfgUfuAfcAfusGfsa	0.1	0.31	0.77	0.059
D1062	S1062	80	AfuguAfaccAfaGfdAGfdTAdTUdCcdAsu	AS1062	1172	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.27	0.65	0.059
D1063	S1063	81	adTdGuadAdCcccdAdGagdTdAuudCdCasu	AS1063	1173	dAdTggdAdAuadCdTcudTdGgudTdAcadTsdGsa	0.12	0.44	0.82	0.064
D1064	S1064	82	AfuGfuAfaCfcAfaGfaGfdTdAuUfcdCdAsUf	AS1064	1174	adTdGGfaAfdTdAcUfcUfuGfgUfuAfcAfusGfsa	0.12	0.32	0.83	0.064
D1065	S1065	83	AfuguAfaccAfaGfaGfdTAdTudCcdAsu	AS1065	1175	dAUdGgdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.13	0.34	0.72	0.066
D1066	S1066	84	AfuGfuAfaCfcAfaGfaGfudAdTUfcdCdAsUf	AS1066	1176	adTdGGfadAdTAfcUfcUfuGfgUfuAfcAfusGfsa	0.11	0.33	0.72	0.067
D1067	S1067	85	AfuguAfaccAfaGfaGfdTAdTUdCcdAsu	AS1067	1177	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.11	0.37	0.62	0.070
D1068	S1068	86	AfuguAfaccAfaGfaGfdTAdTUdCcdAsu	AS1068	1178	dAUdGGdAAuAfcUfcUfuGfGfUfuAfCfAfusGfsa	0.16	0.33	0.64	0.072
D1069	S1069	87	aUfGfuaAfCfccAfGfagUfAfuuCfCfasu	AS1069	1179	AfUfggAfAfuaCfUfcuUfGfguUfAfcaUfsGfsa	0.14	0.43	0.73	0.074
D1070	S1070	88	AfuGfuAfaCfCfAfaGfaguAfuUfcCfasUf	AS1070	1180	aUfgGfaAfuAfCfUfcUfuggUfuAfcAfusGfsa	0.08	0.42	0.94	0.075
D1071	S1071	89	UfgGfgAfuuuCfaUfgUfaAfcCfaAfgsAf	AS1071	1181	uCfuUfgGfuUfaCfaUfgAfAfAfuCfcCfasUfsc	0.14	0.28	0.83	0.0797
D1072	S1072	90	AfuGfuAfaCfcAfaGfAfGfuauUfcCfasUf	AS1072	1182	aUfgGfaAfUfAfcucUfuGfgUfuAfcAfusGfsa	0.05	0.26	0.8	0.082
D1073	S1073	91	AfuGfuAfaCfcAfaGfadGdTdAdTUfcCfasUf	AS1073	1183	aUfgGfadAdTdAdCUfcUfuGfgUfuAfcAfusGfsa	0.12	0.41	0.73	0.083
D1074	S1074	92	AfUfguAfAfccAfAfgaGfUfauUfCfcasUf	AS1074	1184	aUfGfgaAfUfacUfCfuuGfGfuuAfCfausGfsa	0.14	0.44	0.75	0.086

D1075	S1075	93	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1075	1185	aUfgGfdAdAdTdAcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.41	0.72	0.088
D1076	S1076	94	AfuGfuAfaCfcAfaGfaGfudAdTdTdCCfasUf	AS1076	1186	aUfgdGdAdAdTAfcUfcUfuGfgUfuAfcAfusGfsa	0.15	0.45	0.86	0.088
D1077	S1077	95	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasu	AS1077	1187	AfUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.08	0.46	0.95	0.092
D1078	S1078	96	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1078	1188	dAUdGGdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.09	0.32	0.76	0.093
D1079	S1079	97	AfuguAfaccAfaGfaGfdTadTudCcdAsu	AS1079	1189	dAudGgdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.14	0.38	0.76	0.095
D1080	S1080	98	AfuGfuAfaCfcAfaGfAfGfuAfuucCfasUf	AS1080	1190	aUfgGfAfAfuAfcucUfuGfgUfuAfcAfusGfsa	0.05	0.42	0.86	0.099
D1081	S1081	99	AfuGfuAfaCfcAfaGfaGfuAfuUfdCdCdAsdT	AS1081	1191	dAdTdGdGaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.17	0.47	0.9	0.105
D1082	S1082	100	AfuGfuAfaccagaguAfuUfcCfasUf	AS1082	1192	aUfgGfaAfudACfudCUfudGGfudTAfcAfusgsa	0.12	0.44	0.83	0.106
D1083	S1083	101	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1083	1193	adTdGGfaAfdTdAcUfcUfuGfgUfuAfcAfusGfsa	0.11	0.34	0.74	0.109
D1084	S1084	102	AfuGfuAfAfCfcAfaGfaGfuauUfcCfasUf	AS1084	1194	aUfgGfaAfUfAfcUfcUfuGfguuAfcAfusGfsa	0.1	0.45	0.93	0.117
D1085	S1085	103	AfuGfUfAfaCfcAfaGfaGfuauUfcCfasUf	AS1085	1195	aUfgGfaAfUfAfcUfcUfuGfgUfuacAfusGfsa	0.07	0.42	0.78	0.120
D1086	S1086	104	aUfguAfAfccAfAfgaGfuAfuUfcCfasUf	AS1086	1196	aUfgGfaAfuAfcUfCfuuGfGfuuuAfCfaUfsgsa	0.17	0.45	0.83	0.1197
D1087	S1087	105	AfuGfuAfaCfcAfaGfaGfUfAfuUfcCfasu	AS1087	1197	AfUfgGfaAfuacUfcUfuGfgUfuAfcAfusGfsa	0.05	0.3	0.7	0.120
D1088	S1088	106	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1088	1198	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusgsa	0.11	0.46	0.8	0.120
D1089	S1089	107	AfuGfuAfaCfcAfaGfaGfUfAfuUfcCfasUf	AS1089	1199	aUfgGfaAfuacUfcUfuGfgUfuAfcAfusGfsa	0.14	0.49	0.85	0.122
D1090	S1090	108	AfuGfuAfaCfcAfaGfaGfuauUfcCfasUf	AS1090	1200	aUfgGfaAfUfAfcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.41	0.85	0.125
D1091	S1091	109	AfuguAfaccAfaGfaGfdTAdTudCcdAsu	AS1091	1201	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.16	0.38	0.77	0.125
D1092	S1092	110	AfuGfuAfaCfcAfaGfAfGfuAfuUfcCfasu	AS1092	1202	AfUfgGfaAfuAfcucUfuGfgUfuAfcAfusGfsa	0.05	0.31	0.93	0.126
D1093	S1093	111	auGfuAfaCfcAfaGfAfGfuAfuUfcCfasUf	AS1093	1203	aUfgGfaAfuAfcucUfuGfgUfuAfcAfUfsGfsa	0.06	0.33	0.9	0.135
D1094	S1094	112	AfuGfuAfaCfcAfaGfaGfUfAfuUfccasUf	AS1094	1204	aUfGfGfaAfuacUfcUfuGfgUfuAfcAfusGfsa	0.07	0.39	0.85	0.142
D1095	S1095	113	AfuGfuAfaCfcAfaGfAfGfuAfuUfcCfasUf	AS1095	1205	aUfgGfaAfuAfcucUfuGfgUfuAfcAfusGfsa	0.09	0.39	0.76	0.146
D1096	S1096	114	AfuGfuAfaCfcAfaGfaGfUfAfuucCfasUf	AS1096	1206	aUfgGfAfAfuacUfcUfuGfgUfuAfcAfusGfsa	0.06	0.38	0.85	0.147
D1097	S1097	115	AfuGfUfAfaCfcAfaGfaGfuAfuucCfasUf	AS1097	1207	aUfgGfAfAfuAfcUfcUfuGfgUfuacAfusGfsa	0.12	0.47	0.87	0.147
D1098	S1098	116	AfuGfuAfaCfcAfaGfaGfuAfUfUfccasUf	AS1098	1208	aUfGfGfaauAfcUfcUfuGfgUfuAfcAfusGfsa	0.06	0.42	0.85	0.151
D1099	S1099	117	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1099	1209	dAUdGGdAadTAfdCUfcUfuGfgUfuAfcAfusGfsa	0.16	0.41	0.85	0.152
D1100	S1100	118	AfuguAfaccAfaGfaGfuAfuUfcCfasUf	AS1100	1210	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.15	0.48	0.72	0.152
D1101	S1101	119	AfuGfuAfaCfcAfaGfAfGfuAfuUfccasUf	AS1101	1211	aUfGfGfaAfuAfcucUfuGfgUfuAfcAfusGfsa	0.06	0.38	0.94	0.158

D1102	S1102	120	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1102	1212	aUfgGfaAfuAfdCuCfdTuGfdGuUfacAfusGfsa	0.21	0.45	0.89	0.162
D1103	S1103	121	AfuGfuaaCfCfAfaGfaGfuAfuUfcCfasUf	AS1103	1213	aUfgGfaAfuAfcUfcUfuggUfUfAfcAfusGfsa	0.14	0.49	0.95	0.163
D1104	S1104	122	AfuGfuAfaccAfaGfaGfUfAfuUfcCfasUf	AS1104	1214	aUfgGfaAfuacUfcUfuGfGfUfuAfcAfusGfsa	0.06	0.36	0.92	0.163
D1105	S1105	123	AfuGfuAfaCfcAfaGfaGfuAfuucCfasUf	AS1105	1215	aUfgGfAfAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.45	0.84	0.167
D1106	S1106	124	AfuGfuaaCfcAfaGfAfGfuAfuUfcCfasUf	AS1106	1216	aUfgGfaAfuAfcucUfuGfgUfUfAfcAfusGfsa	0.09	0.43	0.91	0.170
D1107	S1107	125	AfuGfuAfaccAfaGfAfGfuAfuUfcCfasUf	AS1107	1217	aUfgGfaAfuAfcucUfuGfGfUfuAfcAfusGfsa	0.09	0.46	1	0.171
D1108	S1108	126	AfuguAfaccAfaGfaGfdTadTudCcdAsu	AS1108	1218	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.11	0.39	0.71	0.176
D1109	S1109	127	AfuGfUfAfaCfcAfaGfaGfuAfuUfccasUf	AS1109	1219	aUfGfGfaAfuAfcUfcUfuGfgUfuacAfusGfsa	0.1	0.43	0.9	0.180
D1110	S1110	128	AfuGfuAfaCfcAfaGfaguAfUfUfcCfasUf	AS1110	1220	aUfgGfaauAfCfUfcUfuGfgUfuAfcAfusGfsa	0.06	0.42	0.88	0.182
D1111	S1111	129	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1111	1221	dAUdGGdAAuAfcUfcUfuGfGfUfuAfCfAfusGfsa	0.18	0.49	0.79	0.183
D1112	S1112	130	AfuGfUfAfaccAfaGfaGfuAfuUfcCfasUf	AS1112	1222	aUfgGfaAfuAfcUfcUfuGfGfUfuacAfusGfsa	0.14	0.48	0.85	0.195
D1113	S1113	131	AfuGfuAfaCfcAfaGfaguAfuUfcCfasUf	AS1113	1223	aUfgGfaAfuAfCfUfcUfuGfgUfuAfcAfusGfsa	0.09	0.41	0.85	0.201
D1114	S1114	132	auGfuAfaCfcAfaGfaGfUfAfuUfcCfasUf	AS1114	1224	aUfgGfaAfuacUfcUfuGfgUfuAfcAfUfsGfsa	0.05	0.44	0.94	0.201
D1115	S1115	133	AfuguAfaCfcAfaGfaGfUfAfuUfcCfasUf	AS1115	1225	aUfgGfaAfuacUfcUfuGfgUfuAfCfAfusGfsa	0.08	0.41	0.96	0.204
D1116	S1116	134	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1116	1226	adTdGGfadAdTAfcUfcUfuGfgUfuAfcAfusGfsa	0.15	0.47	0.79	0.208
D1117	S1117	135	AfuGfuaaCfcAfaGfaGfUfAfuUfcCfasUf	AS1117	1227	aUfgGfaAfuacUfcUfuGfgUfUfAfcAfusGfsa	0.08	0.42	0.92	0.224
D1118	S1118	136	auguaaccaagaguauuccasu	AS1118	1228	AfUfGfGfAfAfUfAfCfUfCfUfUfGfGfUfUfAfCfAfUfsgsa	0.19	0.5	0.87	0.303
D1119	S1119	137	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1119	1229	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.14	0.55	0.89	
D1120	S1120	138	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1120	1230	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.19	0.63	0.72	
D1121	S1121	139	AfuGfuAfaccAfaGfaGfuAfuUfcCfasUf	AS1121	1231	aUfgGfaAfuAfcUfcUfuGfGfUfuAfcAfusGfsa	0.14	0.61	0.91	
D1122	S1122	140	AfUfGfuAfaCfcAfaGfaGfuAfuUfccasUf	AS1122	1232	aUfGfGfaAfuAfcUfcUfuGfgUfuAfcausGfsa	0.14	0.54	0.95	
D1123	S1123	141	auGfuAfAfCfcAfaGfaGfuAfuUfcCfasUf	AS1123	1233	aUfgGfaAfuAfcUfcUfuGfguuAfcAfUfsGfsa	0.13	0.61	0.97	
D1124	S1124	142	AfuGfuAfaCfcAfaGfaGfuAfUfUfcCfasUf	AS1124	1234	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfsa	0.14	0.56	0.94	
D1125	S1125	143	AfuGfuAfaCfcaaGfaGfuAfuUfcCfasUf	AS1125	1235	aUfgGfaAfuAfcUfcUfUfGfgUfuAfcAfusGfsa	0.21	0.74	0.95	
D1126	S1126	144	AfUfGfuAfaCfcAfaGfaGfuAfuucCfasUf	AS1126	1236	aUfgGfAfAfuAfcUfcUfuGfgUfuAfcausGfsa	0.2	0.69	0.91	
D1127	S1127	145	AfuguAfAfCfcAfaGfaGfuAfuUfcCfasUf	AS1127	1237	aUfgGfaAfuAfcUfcUfuGfguuAfCfAfusGfsa	0.17	0.7	0.96	
D1128	S1128	146	AfUfGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1128	1238	aUfgGfaAfuAfcUfcUfuGfgUfuAfcausGfsa	0.19	0.62	0.85	

D1129	S1129	147	AfuGfuAfaCfcAfaGfaGfuAfuUfcfCfasUf	AS1129	1239	aUfggaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.23	0.76	0.98		
D1130	S1130	148	AfuGfuAfaCfcAfagaGfuAfuUfccCfasUf	AS1130	1240	aUfgGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.21	0.64	0.9		
D1131	S1131	149	AfuGfuAfAfCfcaaGfaGfuAfuUfcCfasUf	AS1131	1241	aUfgGfaAfuAfcUfcUfUfGfguuAfcAfusGfsa	0.17	0.7	1.01		
D1132	S1132	150	AfuGfUfAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1132	1242	aUfgGfaAfuAfcUfcUfuGfgUfuacAfusGfsa	0.17	0.58	0.87		
D1133	S1133	151	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfAfsUf	AS1133	1243	augGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.33	0.89	1.05		
D1134	S1134	152	AfUfGfuAfaCfcAfaGfaguAfuUfcCfasUf	AS1134	1244	aUfgGfaAfuAfCfUfcUfuGfgUfuAfcausGfsa	0.16	0.64	0.96		
D1135	S1135	153	AfuGfUfAfaCfcAfaGfaguAfuUfcCfasUf	AS1135	1245	aUfgGfaAfuAfCfUfcUfuGfgUfuacAfusGfsa	0.12	0.53	0.96		
D1136	S1136	154	AfuGfuAfAfCfcAfagaGfuAfuUfcCfasUf	AS1136	1246	aUfgGfaAfuAfcUfCfUfuGfguuAfcAfusGfsa	0.16	0.58	0.98		
D1137	S1137	155	AfuGfuAfAfCfcAfaGfaGfuAfuUfcCfasUf	AS1137	1247	aUfgGfaAfuAfcUfcUfuGfguuAfcAfusGfsa	0.16	0.6	0.91		
D1138	S1138	156	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1138	1248	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsAf	0.1	0.54	0.91		
D1139	S1139	157	AfUfGfuAfaCfcAfagaGfuAfuUfcCfasUf	AS1139	1249	aUfgGfaAfuAfcUfCfUfuGfgUfuAfcausGfsa	0.24	0.68	0.98		
D1140	S1140	158	AfuGfUfAfaCfcAfagaGfuAfuUfcCfasUf	AS1140	1250	aUfgGfaAfuAfcUfCfUfuGfgUfuacAfusGfsa	0.13	0.75	0.9		
D1141	S1141	159	AfuGfuAfAfCfcAfaGfaguAfuUfcCfasUf	AS1141	1251	aUfgGfaAfuAfCfUfcUfuGfguuAfcAfusGfsa	0.15	0.52	1.05		
D1142	S1142	160	AfuGfuAfaCfCfAfaGfaGfuAfuUfcCfasUf	AS1142	1252	aUfgGfaAfuAfcUfcUfuggUfuAfcAfusGfsa	0.16	0.66	0.89		
D1143	S1143	161	auGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1143	1253	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfUfsGfsa	0.12	0.51	0.89		
D1144	S1144	162	AfUfGfuAfaCfcaaGfaGfuAfuUfcCfasUf	AS1144	1254	aUfgGfaAfuAfcUfcUfUfGfgUfuAfcausGfsa	0.25	0.71	0.95		
D1145	S1145	163	AfuGfUfAfaCfcaaGfaGfuAfuUfcCfasUf	AS1145	1255	aUfgGfaAfuAfcUfcUfUfGfgUfuacAfusGfsa	0.17	0.74	0.98		
D1146	S1146	164	AfuguAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1146	1256	aUfgGfaAfuAfcUfcUfuGfgUfuAfCfAfusGfsa	0.11	0.51	0.86		
D1147	S1147	165	AfuGfuAfaCfcAfaGfaGfuAfuUfccasUf	AS1147	1257	aUfGfGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.1	0.52	0.83		
D1148	S1148	166	AfUfGfuAfaccAfaGfaGfuAfuUfcCfasUf	AS1148	1258	aUfgGfaAfuAfcUfcUfuGfGfGfUfuAfcausGfsa	0.14	0.63	0.98		
D1149	S1149	167	AfuGfuAfAfCfcAfaGfaGfuAfuucCfasUf	AS1149	1259	aUfgGfAfAfuAfcUfcUfuGfguuAfcAfusGfsa	0.13	0.58	0.88		
D1150	S1150	168	AfuGfuaaCfcAfaGfaGfuAfuUfcCfasUf	AS1150	1260	aUfgGfaAfuAfcUfcUfuGfgUfUfAfcAfusGfsa	0.15	0.62	0.94		
D1151	S1151	169	AfUfGfuaaCfcAfaGfaGfuAfuUfcCfasUf	AS1151	1261	aUfgGfaAfuAfcUfcUfuGfgUfUfAfcausGfsa	0.18	0.73	0.94		
D1152	S1152	170	auGfUfAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1152	1262	aUfgGfaAfuAfcUfcUfuGfgUfuacAfUfsGfsa	0.13	0.53	0.97		
D1153	S1153	171	AfuGfuAfAfCfcAfaGfaGfuAfuUfccasUf	AS1153	1263	aUfGfGfaAfuAfcUfcUfuGfguuAfcAfusGfsa	0.13	0.53	0.98		
D1154	S1154	172	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfgsAf	AS1154	1264	uCfuUfgGfuUfaCfaUfgAfaAfuCfcCfasUfsc	0.09	0.5	0.78		
D1155	S1155	173	UfgGfGfAfuuuCfaUfgUfAfAfcCfaAfgsAf	AS1155	1265	uCfuUfgGfuuaCfaUfgAfAfAfuccCfasUfsc	0.13	0.62	0.89		

D1156	S1156	174	UfgGfgAfuuuCfaUfGfUfaAfcCfaAfgsAf	AS1156	1266	uCfuUfgGfuUfacaUfgAfAfAfuCfcCfasUfsc	0.12	0.65	0.85		
D1157	S1157	175	UfgGfgAfuUfuCfaUfgUfAfAfcCfaAfgsAf	AS1157	1267	uCfuUfgGfuuaCfaUfgAfaAfuCfcCfasUfsc	0.11	0.54	0.85		
D1158	S1158	176	UfgGfgAfuuuCfaUfgUfAfAfcCfaAfgsAf	AS1158	1268	uCfuUfgGfuuaCfaUfgAfAfAfuCfcCfasUfsc	0.13	0.53	0.8		
D1159	S1159	177	UfGfggAfUfuUfcAfuGfuAfAfccAfAfgsAf	AS1159	1269	uCfuuGfGfuuAfcAfuGaAfauCfCfcasUfsc	0.59	0.89	0.81		
D1160	S1160	178	UfGfggAfUfuuCfaUfgUfAfAfcCfaAfgsAf	AS1160	1270	uCfuUfgGfuuaCfaUfgAfAfauCfCfcasUfsc	0.16	0.72	0.9		
D1161	S1161	179	UfgGfgAfuUfucaUfGfUfaAfcCfaAfgsAf	AS1161	1271	uCfuUfgGfuUfacaUfGfAfaAfuCfcCfasUfsc	0.27	0.69	0.86		
D1162	S1162	180	AfuGfuAfaCfcaaGfaGfUfAfuUfcCfasUf	AS1162	1272	aUfgGfaAfuacUfCfUfUfGfgUfuAfcAfusGfsa	0.12	0.6	0.95		
D1163	S1163	181	AfuGfuAfaccAfaGfaGfuAfUfUfcCfasUf	AS1163	1273	aUfgGfaauAfCfUfCfUfuGfGfUfuAfcAfusGfsa	0.05	0.56	1.02		
D1164	S1164	182	AfuGfuAfaCfcAfagaGfUfAfuUfcCfasUf	AS1164	1274	aUfgGfaAfuacUfCfUfuGfgUfuAfcAfusGfsa	0.13	0.55	1		
D1165	S1165	183	AfuGfuAfaCfcaaGfaGfuAfUfUfcCfasUf	AS1165	1275	aUfgGfaauAfCfUfCfUfGfgUfuAfcAfusGfsa	0.09	0.6	0.97		
D1166	S1166	184	AfuguAfaCfCfAfaGfaGfuAfuUfcCfasUf	AS1166	1276	aUfgGfaAfuAfcUfCfUfuggUfuAfCfAfusGfsa	0.15	0.59	0.91		
D1167	S1167	185	AfuGfuAfaCfcAfagaGfuAfUfUfcCfasUf	AS1167	1277	aUfgGfaauAfCfUfCfUfuGfgUfuAfcAfusGfsa	0.11	0.59	1		
D1168	S1168	186	AfuGfuAfaCfCfAfagaGfuAfuUfcCfasUf	AS1168	1278	aUfgGfaAfuAfcUfCfUfuggUfuAfcAfusGfsa	0.13	0.57	0.94		
D1169	S1169	187	auGfuAfaCfcAfaGfaGfuAfUfUfcCfasUf	AS1169	1279	aUfgGfaauAfCfUfCfUfuGfgUfuAfcAfUfsGfsa	0.08	0.5	0.9		
D1170	S1170	188	AfuguAfaCfcAfaGfaGfuAfUfUfcCfasUf	AS1170	1280	aUfgGfaauAfCfUfCfUfuGfgUfuAfCfAfusGfsa	0.06	0.53	0.91		
D1171	S1171	189	auGfuAfaCfcAfaGfaGfuAfuUfCfCfasUf	AS1171	1281	aUfggaAfuAfcUfCfUfuGfgUfuAfcAfUfsGfsa	0.07	0.56	0.89		
D1172	S1172	190	AfuGfuAfaCfCfAfaGfaGfuAfuucCfasUf	AS1172	1282	aUfgGfAfAfuAfcUfcUfuggUfuAfcAfusGfsa	0.13	0.59	0.98		
D1173	S1173	191	AfuGfuAfaCfcaaGfAfGfuAfuUfcCfasUf	AS1173	1283	aUfgGfaAfuAfcucUfUfGfgUfuAfcAfusGfsa	0.2	0.65	1.03		
D1174	S1174	192	AfuGfuaaCfcAfaGfaGfuAfUfUfcCfasUf	AS1174	1284	aUfgGfaauAfCfUfCfUfuGfgUfUfAfcAfusGfsa	0.07	0.51	0.95		
D1175	S1175	193	AfuguAfaCfcAfaGfaGfuAfuUfCfCfasUf	AS1175	1285	aUfggaAfuAfcUfCfUfuGfgUfuAfCfAfusGfsa	0.2	0.53	0.76		
D1176	S1176	194	auGfuAfaCfcAfaGfaGfuAfuUfcCfAfsUf	AS1176	1286	augGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.74	0.98	0.81		
D1177	S1177	195	AfuGfuAfaCfcAfaGfaGfuAfuucCfAfsUf	AS1177	1287	augGfAfAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.43	0.64	0.88		
D1178	S1178	196	auguaaccAfaGfaGfuAfuUfcCfasUf	AS1178	1288	aUfgGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.17	0.49	0.81		
D1179	S1179	197	AfuGfuaaCfcAfaGfaGfuAfuUfCfCfasUf	AS1179	1289	aUfggaAfuAfcUfCfUfuGfgUfUfAfcAfusGfsa	0.22	0.65	0.73		
D1180	S1180	198	AfuguAfaCfcAfaGfaGfuAfuUfcCfAfsUf	AS1180	1290	augGfaAfuAfcUfCfUfuGfgUfuAfcAfUfsGfsa	0.6	1.09	0.8		
D1181	S1181	199	auGfuAfaCfcAfaGfaGfuAfuUfccasu	AS1181	1291	aUfgGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.3	0.78	0.78		
D1182	S1182	200	auguaaccaaGfaGfuAfuUfcCfasUf	AS1182	1292	aUfgGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.35	0.73	0.84		

D1183	S1183	201	AfuGfuAfaccAfaGfaGfuAfuUfCfCfasUf	AS1183	1293	aUfggaAfuAfcUfcUfuGfGfUfuAfcAfusGfsa	0.19	0.6	0.94	
D1184	S1184	202	AfuGfuaaCfcAfaGfaGfuAfuUfccCfAfsUf	AS1184	1294	augGfaAfuAfcUfcUfuGfgUfuAfCfAfusGfsa	0.61	1.08	0.8	
D1185	S1185	203	auGfuAfaCfcAfaGfaGfuAfuuccasu	AS1185	1295	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.16	0.52	0.72	
D1186	S1186	204	auguaaccaagaGfuAfuUfcCfasUf	AS1186	1296	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.2	0.53	0.74	
D1187	S1187	205	AfuGfuAfaCfcaaGfaGfuAfuUfCfCfasUf	AS1187	1297	aUfggaAfuAfcUfcUfUfGfgUfuAfcAfusGfsa	0.34	0.66	0.85	
D1188	S1188	206	AfuGfuAfaccAfaGfaGfuAfuUfccCfAfsUf	AS1188	1298	augGfaAfuAfcUfcUfuGfgUfUfAfcAfusGfsa	0.61	0.98	1.02	
D1189	S1189	207	AfuGfuAfaCfcAfaGfaGfuAfuuccasu	AS1189	1299	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.3	0.73	0.85	
D1190	S1190	208	auguaaccaagaguauuccasu	AS1190	1300	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.28	0.69	0.78	
D1191	S1191	209	AfuGfuAfaCfcAfaGfaGfuAfuUfccCfasUf	AS1191	1301	aUfgGfaAfuAfcUfcUfugdGudTadCadTsgsa	0.33	0.88	0.64	
D1192	S1192	210	AfuGfuAfaCfcAfagaGfuAfuUfCfCfasUf	AS1192	1302	aUfggaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.31	0.64	0.83	
D1193	S1193	211	AfuGfuAfaCfcaaGfaGfuAfuUfccCfAfsUf	AS1193	1303	augGfaAfuAfcUfcUfuGfGfUfuAfcAfusGfsa	0.64	0.82	0.92	
D1194	S1194	212	AfuGfuAfaCfcAfaGfaGfuauuccasu	AS1194	1304	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.21	0.62	0.77	
D1195	S1195	213	AfuGfuAfaCfcAfaGfaGfuAfuUfccCfasUf	AS1195	1305	aUfgGfaAfuAfcUfcUfuGfGfUfuAfCfAfusGfsa	0.17	0.7	0.95	
D1196	S1196	214	AfuGfuAfaCfcAfaGfaguAfuUfCfCfasUf	AS1196	1306	aUfggaAfuAfCfUfcUfuGfgUfuAfcAfusGfsa	0.19	0.71	0.65	
D1197	S1197	215	AfuGfuAfaCfcAfagaGfuAfuUfccCfAfsUf	AS1197	1307	augGfaAfuAfcUfcUfUfGfgUfuAfcAfusGfsa	0.64	0.82	0.93	
D1198	S1198	216	auguAfaCfcAfaGfaGfuAfuUfccasu	AS1198	1308	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.19	0.65	0.72	
D1199	S1199	217	AfuGfuAfaCfcAfaGfaGfuauUfCfCfasUf	AS1199	1309	aUfggaAfUfAfcUfcUfuGfgUfuAfcAfusGfsa	0.15	0.52	0.64	
D1200	S1200	218	AfuGfuAfaCfcAfaGfaguAfuUfccCfAfsUf	AS1200	1310	augGfaAfuAfcUfCfUfuGfgUfuAfcAfusGfsa	0.48	0.74	0.92	
D1201	S1201	219	auguAfaCfcAfaGfaGfuAfuUfccCfasu	AS1201	1311	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.17	0.71	0.77	
D1202	S1202	220	AfuGfuAfaCfcAfaGfaGfuauUfccCfAfsUf	AS1202	1312	augGfaAfuAfCfUfcUfuGfgUfuAfcAfusGfsa	0.43	0.69	0.85	
D1203	S1203	221	auguaaCfcAfaGfaGfuAfuUfcCfasUf	AS1203	1313	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.14	0.61	0.76	
D1204	S1204	222	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1204	1314	adTdGGfaAfudAdCUfcUfuGfgUfuAfcAfusGfsa	0.16	0.56	0.89	
D1205	S1205	223	AfuGfuAfaCfcAfaGfaGfdTdAdTdTcCfasUf	AS1205	1315	aUfgGfdAdAdTdAcUfcUfuGfgUfuAfcAfusGfsa	0.13	0.57	0.9	
D1206	S1206	224	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1206	1316	adTdGdGdAAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.29	0.73	0.89	
D1207	S1207	225	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1207	1317	adTdGGfaAfuAfdCdTcUfuGfgUfuAfcAfusGfsa	0.16	0.56	0.78	
D1208	S1208	226	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1208	1318	aUfdGdGdAdAuAfcUfcUfuGfgUfuAfcAfusGfsa	0.22	0.67	0.89	
D1209	S1209	227	AfuguAfaccAfaGfaGfuAfuUfcCfasUf	AS1209	1319	aUfgGfaAfuAfcUfcUfuGfGfUfuAfCfAfusGfsa	0.14	0.55	0.78	

D1210	S1210	228	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1210	1320	aUfgdGdAdAdTAfcUfcUfuGfgUfuAfcAfusGfsa	0.14	0.5	0.84	
D1211	S1211	229	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1211	1321	aUfgGfadAdTdAdCUfcUfuGfgUfuAfcAfusGfsa	0.14	0.59	0.72	
D1212	S1212	230	auguaaccaaGfaGfuAfuUfcCfasUf	AS1212	1322	aUfgGfaAfuAfcUfcUfugdGudTadCadTsgsa	0.21	0.74	0.77	
D1213	S1213	231	AfuGfuAfaCfcAfaGfaGfuAfudTdCdCdAsUf	AS1213	1323	adTdGdGdAAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.15	0.53	0.91	
D1214	S1214	232	aUfgUfaAfcCfaAfgAfgUfaUfuCfcAfsu	AS1214	1324	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.12	0.71	0.87	
D1215	S1215	233	AfuGfuAfaCfcAfaGfaGfuAfdTdTdCdCasUf	AS1215	1325	aUfdGdGdAdAuAfcUfcUfuGfgUfuAfcAfusGfsa	0.18	0.67	0.97	
D1216	S1216	234	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1216	1326	aUfgGfaAfuacucuuggUfuAfcAfusgsa	0.36	0.87	1.07	
D1217	S1217	235	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1217	1327	aUfgGfaAfuAfCfUfCfUfuGfGfGfuuAfcAfusgsa	0.37	0.73	1.03	
D1218	S1218	236	AfUfguAfAfccAfAfgaGfUfauUfCfcasUf	AS1218	1328	aUfGfgaAfUfacUfCfuuGfGfGfuuAfCfausGfsa	0.23	0.42	0.84	
D1219	S1219	237	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1219	1329	aUfgGfaAfuaCfUfcUfUfgGfuuAfcAfusgsa	0.43	0.71	1.03	
D1220	S1220	238	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1220	1330	aUfgGfaAfuAfcUfCfUfuGfGfGfuuAfcAfusgsa	0.37	0.63	0.99	
D1221	S1221	239	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1221	1331	aUfgGfaAfuAfcUfCfUfuGfGfGfuUfacAfusgsa	0.29	0.84	0.88	
D1222	S1222	240	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1222	1332	aUfgGfaAfuaCfuCfuUfgGfuuAfcAfusgsa	0.31	0.8	0.99	
D1223	S1223	241	auGfuAfAfccAfaGfagUfaUfUfcCfasUf	AS1223	1333	aUfgGfaaUfaCfUfcUfuGfGfGfuuAfcAfAfsgsa	0.09	0.52	0.82	
D1224	S1224	242	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1224	1334	aUfgGfaAfuadCudCudTgdGuuAfcAfusgsa	0.22	0.79	1	
D1225	S1225	243	auGfuaAfccAfagAfguAfuuCfcasUf	AS1225	1335	aUfGfgAfAfuAfCfuCfUfuGfGfGfuUfAfcAfUfsGfsa	0.31	0.76	0.84	
D1226	S1226	244	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1226	1336	aUfgGfaAfuadCUfcdTUfgdGuuAfcAfusgsa	0.26	0.64	0.87	
D1227	S1227	245	augUfaacCfaagAfguaUfuccAfsu	AS1227	1337	aUfgGfAfaUfAfCfuCfUfUfgGfUfUfaCfAfUfsGfsa	0.33	0.79	0.81	
D1228	S1228	246	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1228	1338	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.464	0.932	0.978	
D1229	S1229	247	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1229	1339	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.453	1.047	1.178	
D1230	S1230	248	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1230	1340	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.831	0.967	1.151	
D1231	S1231	249	auGfuAfAfCfcAfaGfaGfuAfuUfcCfasu	AS1231	1341	AfUfgGfaAfuAfcUfcUfuGfguuAfcAfUfsGfsa	0.09	0.5	1.07	
D1232	S1232	250	AfuGfuAfaCfCfAfaGfaGfuAfuUfcCfasu	AS1232	1342	AfUfgGfaAfuAfcUfcUfuggUfuAfcAfusGfsa	0.11	0.54	1.1	
D1233	S1233	251	AfuGfuAfaCfcAfaGfaGfuAfuUfCfCfasu	AS1233	1343	AfUfggaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.19	0.61	0.74	
D1234	S1234	252	aUfgUfaAfcCfaAfgAfgUfaUfuCfcAfsu	AS1234	1344	AfuGfgAfaUfaCfuCfuUfgGfuUfaCfaUfsgsAf	0.22	0.61	0.98	
D1235	S1235	253	aUfgUfaAfcCfaAfgAfgUfaUfuCfcAfsu	AS1235	1345	AfuGfgAfaUfaCfuCfuUfgGfuUfaCfaUfsgsAf	0.27	0.69	0.92	
D1236	S1236	254	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1236	1346	AfuGfgAfaUfaCfuCfuUfgGfuUfaCfaUfsgsAf	0.54	1.08	0.8	

D1237	S1237	255	augUfaAfccaAfgAfguaUfuCfcasu	AS1237	1347	AfUfGfgAfaUfAfCfuCfuUfGfGfuUfaCfAfUfsgsa	0.29	0.61	0.79		
D1238	S1238	256	AfugUfaAfccaAfgAfguaUfuCfcasu	AS1238	1348	AfUfGfgAfaUfAfCfuCfuUfGfGfuUfaCfAfusgsa	0.31	0.6	0.88		
D1239	S1239	257	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1239	1349	dAUdGGdAauAfcUfcUfuGfgUfuAfcAfusGfsa	0.2	0.67	0.85		
D1240	S1240	258	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1240	1350	dAUdGgdAauAfcUfcUfuGfgUfuAfcAfusGfsa	0.23	0.58	0.68		
D1241	S1241	259	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1241	1351	dAudGgdAauAfcUfcUfuGfgUfuAfcAfusGfsa	0.25	0.65	0.78		
D1242	S1242	260	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1242	1352	dAUdGgdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.18	0.64	0.84		
D1243	S1243	261	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1243	1353	dAUdGGdAAfuAfcUfcUfuGfGfGfUfuAfCfAfusGfsa	0.19	0.72	0.87		
D1244	S1244	262	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1244	1354	dAUdGgdAadTAfdCUfcUfuGfgUfuAfcAfusGfsa	0.16	0.55	0.8		
D1245	S1245	263	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1245	1355	dAUdGGdAAuAfcUfcUfuGfgUfuAfcAfusGfsa	0.22	0.51	0.9		
D1246	S1246	264	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1246	1356	dAudGgdAadTAfcUfcUfuGfgUfuAfcAfusGfsa	0.27	0.78	0.66		
D1247	S1247	265	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1247	1357	dAdTdGdGaAfuAfcUfcUfuGfgUfuAfcAfusGfsa	0.16	0.57	0.97		
D1248	S1248	266	AfacaAfuguUfcUfuGfdCUdCudAudAsa	AS1248	1358	dTUdAudAgdAGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.09	0.36	0.0047	
D1249	S1249	267	AfaCfaGfuGfuUfcUfuGfCfCfUfcUfaUfasa	AS1249	1359	UfUfaUfaGfagcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.10	0.47	0.005	
D1250	S1250	268	AfaCfaGfuGfuUfcUfugcUfcUfAfUfasAf	AS1250	1360	uUfauaGfaGfCfAfaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.55	0.005	
D1251	S1251	269	AfaCfaGfuGfuUfcUfuGfcucUfAfUfasAf	AS1251	1361	uUfauaGfAfGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.49	0.006	
D1252	S1252	270	cAGuGuucuuGcucuAuAAdTdT	AS1252	1362	UuAuAGAGcAAGAAcACUGdTdT				0.006	
D1253	S1253	271	AfaCfaGfuGfuUfcUfugcUfCfUfaUfasAf	AS1253	1363	uUfaUfagaGfCfAfaGfaAfcAfcUfgUfusUfsu	0.05	0.12	0.43	0.006	
D1254	S1254	272	AfaCfaGfuGfuUfCfUfuGfcUfcUfaUfasa	AS1254	1364	UfUfaUfaGfaGfcAfagaAfcAfcUfgUfusUfsu	0.06	0.13	0.39	0.006	
D1255	S1255	273	AfaCfaGfuGfuUfcUfuGfcUfCfUfaUfasa	AS1255	1365	UfUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.17	0.48	0.007	
D1256	S1256	274	AfaCfaGfuGfuUfCfUfGfcUfcUfaUfasa	AS1256	1366	UfUfaUfaGfaGfcaaGfaAfcAfcUfgUfusUfsu	0.08	0.14	0.40	0.007	
D1257	S1257	275	AfaCfaGfuGfuUfcUfuGfcUfCfUfaUfasAf	AS1257	1367	uUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsUf	0.07	0.12	0.40	0.007	
D1258	S1258	276	AfaCfaguGfuUfCfUfuGfcUfcUfaUfasAf	AS1258	1368	uUfaUfaGfaGfcAfagaAfcAfCfUfgUfusUfsu	0.08	0.13	0.41	0.007	
D1259	S1259	277	AfaCfAfGfuGfuUfcUfuGfcucUfaUfasAf	AS1259	1369	uUfaUfaGfAfGfcAfaGfaAfcAfcugUfusUfsu	0.05	0.11	0.35	0.008	
D1260	S1260	278	AfacaGfuGfuUfCfUfuGfcUfcUfaUfasAf	AS1260	1370	uUfaUfaGfaGfcAfagaAfcAfcUfGfUfusUfsu	0.06	0.12	0.40	0.008	
D1261	S1261	279	AfacaGfuGfuUfcUfuGfCfUfCfUfaUfasAf	AS1261	1371	uUfaUfagaGfcAfaGfaAfcAfcUfGfUfusUfsu	0.06	0.13	0.42	0.008	
D1262	S1262	280	AfaCfaGfuGfuUfcUfuGfcucUfaUfasAf	AS1262	1372	uUfaUfaGfAfGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.37	0.008	
D1263	S1263	281	cAGuGuucuuGcucuAuAAdTdT	AS1263	1373	UuAuAGAGcAAGAAcACUGdTdT				0.008	

D1264	S1264	282	AfaCfaGfuGfuUfcUfuGfCfUfcUfauasAf	AS1264	1374	uUfAfUfaGfagcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.12	0.50	0.008
D1265	S1265	283	AfaCfaGfuguUfCfUfuGfcUfcUfaUfasAf	AS1265	1375	uUfaUfaGfaGfcAfagaAfCfAfcUfgUfusUfsu	0.12	0.13	0.48	0.009
D1266	S1266	284	AfacaGfuGfuUfcUfuGfcUfcUfAfUfasAf	AS1266	1376	uUfauaGfaGfcAfaGfaAfcAfcUfGfUfusUfsu	0.07	0.15	0.51	0.009
D1267	S1267	285	AfacaAfuguUfcUfuGfdCudCudAudAsa	AS1267	1377	dTudAudAgdAGfcAfaGfaAfcAfcAfgUfusUfsu	0.06	0.14	0.48	0.0088
D1268	S1268	286	AfaCfaGfuGfuUfCfUfuGfcucUfaUfasAf	AS1268	1378	uUfaUfaGfAfGfcAfagaAfcAfcUfgUfusUfsu	0.05	0.09	0.35	0.009
D1269	S1269	287	cAGuGuucuuGcucuAuAAdTdT	AS1269	1379	UuAuAGAGcAAGAAcACUGdTdT				0.009
D1270	S1270	288	aaCfaGfuGfuUfcUfuGfcUfCfUfaUfasAf	AS1270	1380	uUfaUfagaGfcAfaGfaAfcAfcUfgUfUfsUfsu	0.07	0.14	0.49	0.009
D1271	S1271	289	AfaCfaGfUfGfuUfcUfuGfcucUfaUfasAf	AS1271	1381	uUfaUfaGfAfGfcAfaGfaAfcacUfgUfusUfsu	0.06	0.10	0.36	0.009
D1272	S1272	290	cAGuGuucuuGcucuAuAAdTdT	AS1272	1382	UuAuAGAGcAAGAAcACUGdTdT				0.009
D1273	S1273	291	AfaCfaGfUfGfuUfcUfuGfcUfcUfaUfasAf	AS1273	1383	uUfaUfaGfaGfcAfaGfaAfcacUfgUfusUfsUf	0.06	0.13	0.51	0.009
D1274	S1274	292	AfaCfaGfuGfuUfCfUfuGfcUfcuaUfasAf	AS1274	1384	uUfaUfAfGfaGfcAfagaAfcAfcUfgUfusUfsu	0.06	0.12	0.46	0.010
D1275	S1275	293	cAGuGuucuuGcucuAuAAdTdT	AS1275	1385	UuAuAGAGcAAGAAcACUGdTdT				0.010
D1276	S1276	294	AfaCfaGfuGfuUfCfUfuGfcUfcUfauasAf	AS1276	1386	uUfAfUfaGfaGfcAfagaAfcAfcUfgUfusUfsu	0.06	0.14	0.47	0.010
D1277	S1277	295	AfaCfaguGfuUfcUfuGfcUfCfUfaUfasAf	AS1277	1387	uUfaUfagaGfcAfaGfaAfcAfCfUfgUfusUfsu	0.07	0.15	0.50	0.010
D1278	S1278	296	AfaCfaGfuGfuUfCfUfugcUfcUfaUfasAf	AS1278	1388	uUfaUfaGfaGfCfAfagaAfcAfcUfgUfusUfsu	0.06	0.13	0.43	0.010
D1279	S1279	297	cAGuGuucuuGcucuAuAAdTdT	AS1279	1389	UuAuAGAGcAAGAAcACUGdTdT				0.010
D1280	S1280	298	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1280	1390	UfUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.06	0.14	0.45	0.010
D1281	S1281	299	AfaCfAfGfuGfuUfcUfuGfcUfcUfaUfasa	AS1281	1391	UfUfaUfaGfaGfcAfaGfaAfcAfcugUfusUfsu	0.07	0.18	0.46	0.011
D1282	S1282	300	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1282	1392	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.55	0.011
D1283	S1283	301	AfaCfaGfuGfuUfcUfuGfcucUfaUfasAf	AS1283	1393	uUfaUfaGfAfGfcAfaGfaAfcAfcUfgUfususu	0.07	0.12	0.45	0.011
D1284	S1284	302	AfacaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1284	1394	uUfaUfaGfaGfcAfaGfaAfcAfcUfGfUfusUfsu	0.06	0.13	0.48	0.011
D1285	S1285	303	AfAfCfaGfuGfuUfcUfuGfcucUfaUfasAf	AS1285	1395	uUfaUfaGfAfGfcAfaGfaAfcAfcUfguusUfsu	0.06	0.11	0.40	0.011
D1286	S1286	304	AfaCfAfGfuGfuUfcUfuGfcUfcUfauasAf	AS1286	1396	uUfAfUfaGfaGfcAfaGfaAfcAfcugUfusUfsu	0.06	0.16	0.47	0.011
D1287	S1287	305	AfaCfaGfuGfuUfcUfugcUfcUfaUfasAf	AS1287	1397	uUfaUfaGfaGfCfAfaGfaAfcAfcUfgUfususu	0.07	0.19	0.46	0.012
D1288	S1288	306	AfaCfaGfuGfuUfcUfugcUfcUfaUfasAf	AS1288	1398	uUfaUfaGfaGfCfAfaGfaAfcAfcUfgUfusUfsu	0.06	0.17	0.46	0.012
D1289	S1289	307	AfaCfaGfuGfuUfcUfUfGfcucUfaUfasAf	AS1289	1399	uUfaUfaGfAfGfcaaGfaAfcAfcUfgUfusUfsu	0.05	0.09	0.31	**0.012**
D1290	S1290	308	AfAfCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1290	1400	UfUfaUfaGfaGfcAfaGfaAfcAfcUfguusUfsu	0.06	0.16	0.49	0.013

D1291	S1291	309	AfaCfaGfuGfuUfCfUfuGfcUfcUfaUfasAf	AS1291	1401	uUfaUfaGfaGfcAfagaAfcAfcUfgUfusUfsUf	0.06	0.11	0.32	0.013
D1292	S1292	310	AfaCfAfGfuGfuUfcUfugcUfcUfaUfasAf	AS1292	1402	uUfaUfaGfaGfCfAfaGfaAfcAfcugUfusUfsu	0.06	0.14	0.44	0.013
D1293	S1293	311	AfaCfaGfUfGfuUfcUfuGfcUfcUfaUfasa	AS1293	1403	UfUfaUfaGfaGfcAfaGfaAfcacUfgUfusUfsu	0.07	0.16	0.39	0.013
D1294	S1294	312	AfaCfAfGfuGfuUfcUfuGfcUfcuaUfasAf	AS1294	1404	uUfaUfAfGfaGfcAfaGfaAfcAfcugUfusUfsu	0.07	0.18	0.41	0.014
D1295	S1295	313	AfaCfaGfUfGfuUfcUfuGfcUfcuaUfasAf	AS1295	1405	uUfaUfAfGfaGfcAfaGfaAfcacUfgUfusUfsu	0.07	0.18	0.47	0.014
D1296	S1296	314	adAdCagdTdGuudCdTugdCdTcudAdTasa	AS1296	1406	dTdTaudAdGagdCdAagdAdAcadCdTgudTsdTsu	0.12	0.21	0.68	0.0146
D1297	S1297	315	AfacaGfUfGfuUfcUfuGfcUfcUfaUfasAf	AS1297	1407	uUfaUfaGfaGfcAfaGfaAfcacUfGfUfusUfsu	0.06	0.15	0.50	0.016
D1298	S1298	316	AfaCfaGfUfGfuUfcUfuGfcUfcUfauasAf	AS1298	1408	uUfAfUfaGfaGfcAfaGfaAfcacUfgUfusUfsu	0.08	0.17	0.50	0.016
D1299	S1299	317	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1299	1409	uUfaUfaGfaGfcAfaGfaAfcAfCfUfgUfususu	0.07	0.16	0.50	0.018
D1300	S1300	318	AfaCfaGfuGfuUfcUfUfGfcUfcUfauasAf	AS1300	1410	uUfAfUfaGfaGfcaaGfaAfcAfcUfgUfusUfsu	0.06	0.12	0.43	0.020
D1301	S1301	319	AfaCfaGfUfGfuUfcUfugcUfcUfaUfasAf	AS1301	1411	uUfaUfaGfaGfCfAfaGfaAfcacUfgUfusUfsu	0.07	0.17	0.45	0.021
D1302	S1302	320	AfaCfaGfuguUfcUfUfGfcUfcUfaUfasAf	AS1302	1412	uUfaUfaGfaGfcaaGfaAfCfAfcUfgUfusUfsu	0.06	0.14	0.49	0.021
D1303	S1303	321	AfAfCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1303	1413	uUfaUfaGfaGfcAfaGfaAfcAfCfUfguusUfsu	0.07	0.24	0.51	0.022
D1304	S1304	322	AfaCfaGfuGfuucUfuGfcUfcUfaUfasAf	AS1304	1414	uUfaUfaGfaGfcAfaGfAfAfcAfcUfgUfususu	0.09	0.27	0.47	0.033
D1305	S1305	323	aadCdAgudGdTucdTdTgcdTdCuadTdAsa	AS1305	1415	udTadTdAgadGdCaadGdAacdAdCugdTdTsusu	0.19	0.36	0.86	0.045
D1306	S1306	324	AfacaGfuguUfcUfuGfdCUdCUdAudAsa	AS1306	1416	dTUdAUdAGfaGfcAfaGfaAfCfAfcUfGfUfusUfsu	0.08	0.22	0.61	
D1307	S1307	325	AfacaGfuguUfcUfdTGfdCUdCUdAudAsa	AS1307	1417	dTUdAUdAGfaGfcAfaGfaAfCfAfcUfGfUfusUfsu	0.13	0.39	0.84	
D1308	S1308	326	AfacaGfuguUfcUfuGfdCUdCUdAudAsa	AS1308	1418	dTUdAUdAgdAGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.13	0.48	
D1309	S1309	327	AfacaGfuguUfcUfdTGfdCUdCUdAudAsa	AS1309	1419	dTUdAUdAgdAGfdCAfaGfaAfcAfcUfgUfusUfsu	0.07	0.13	0.58	
D1310	S1310	328	AfacaAfuguUfcUfdTGfdCUdCudAudAsa	AS1310	1420	dTUdAudAgdAGfdCAfaGfaAfcAfcAfgUfusUfsu	0.07	0.14	0.55	
D1311	S1311	329	AfaCfaAfuGfuUfcUfuGfcUfcUfdAdTdAsdA	AS1311	1421	dTdTdAdTaGfaGfcAfaGfaAfcAfcAfgUfusUfsu	0.10	0.30	0.66	
D1312	S1312	330	AfacaGfuguUfcUfuGfdCUdCUdAudAsa	AS1312	1422	dTUdAUdAgdAGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.13	0.48	
D1313	S1313	331	AfAfCfaGfuGfuucUfuGfcUfcUfaUfasAf	AS1313	1423	uUfaUfaGfaGfcAfaGfAfAfcAfcUfguusUfsu	0.14	0.38	0.74	
D1314	S1314	332	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1314	1424	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.19	0.54	
D1315	S1315	333	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1315	1425	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.55	
D1316	S1316	334	AfaCfaGfuGfuUfcUfuGfcUfcUfauasAf	AS1316	1426	uUfAfUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.16	0.53	
D1317	S1317	335	AfacaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1317	1427	uUfaUfaGfaGfcAfaGfaAfcAfcUfGfUfususu	0.07	0.16	0.55	

D1318	S1318	336	AfAfCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1318	1428	uUfaUfaGfaGfcAfaGfaAfCfAfcUfguusUfsu	0.10	0.32	0.61	
D1319	S1319	337	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1319	1429	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.08	0.16	0.53	
D1320	S1320	338	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1320	1430	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.08	0.16	0.61	
D1321	S1321	339	AfaCfaGfuGfuUfcUfuGfcUfCfUfaUfasAf	AS1321	1431	uUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.14	0.58	
D1322	S1322	340	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1322	1432	uUfaUfaGfaGfcAfAfGfaAfcAfcUfgUfusUfsu	0.15	0.49	0.84	
D1323	S1323	341	AfaCfaGfuGfuUfcUfuGfcUfcuaUfasAf	AS1323	1433	uUfaUfAfGfaGfcAfaGfaAfcAfcUfgUfususu	0.07	0.20	0.62	
D1324	S1324	342	AfAfCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1324	1434	uUfaUfaGfaGfcAfaGfaAfcAfcUfguusUfsu	0.08	0.25	0.78	
D1325	S1325	343	AfAfCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1325	1435	uUfaUfaGfaGfcAfaGfaAfcAfcUfguusUfsu	0.08	0.18	0.80	
D1326	S1326	344	AfaCfaGfuGfuUfcUfuGfcUfcUfAfUfasAf	AS1326	1436	uUfauaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.21	0.66	
D1327	S1327	345	AfaCfaGfuGfuucUfuGfcUfcUfaUfasAf	AS1327	1437	uUfaUfaGfaGfcAfaGfAfAfcAfcUfgUfusUfsu	0.10	0.31	0.70	
D1328	S1328	346	AfAfCfaGfuGfuUfcUfuGfcUfcUfauasAf	AS1328	1438	uUfAfUfaGfaGfcAfaGfaAfcAfcUfguusUfsu	0.07	0.15	0.55	
D1329	S1329	347	AfaCfAfGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1329	1439	uUfaUfaGfaGfcAfaGfaAfcAfcugUfusUfsu	0.08	0.19	0.71	
D1330	S1330	348	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfAfsAf	AS1330	1440	uuaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.27	0.76	
D1331	S1331	349	AfaCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1331	1441	uUfaUfaGfaGfcAfaGfaAfCfAfcUfgUfusUfsu	0.07	0.21	0.65	
D1332	S1332	350	AfAfCfaGfuGfuUfcUfuGfcUfcuaUfasAf	AS1332	1442	uUfaUfAfGfaGfcAfaGfaAfcAfcUfguusUfsu	0.07	0.17	0.53	
D1333	S1333	351	AfaCfaGfUfGfuUfcUfuGfcUfcUfaUfasAf	AS1333	1443	uUfaUfaGfaGfcAfaGfaAfcacUfgUfusUfsu	0.08	0.25	0.73	
D1334	S1334	352	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1334	1444	uUfaUfaGfaGfcAfaGfaAfcAfCfUfgUfusUfsu	0.07	0.18	0.54	
D1335	S1335	353	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1335	1445	uUfaUfaGfaGfcAfAfGfaAfcAfcUfgUfususu	0.14	0.38	0.57	
D1336	S1336	354	AfaCfaGfuGfUfcUfuGfcUfcUfaUfasAf	AS1336	1446	uUfaUfaGfaGfcAfaGfaacAfcUfgUfusUfsu	0.16	0.50	0.96	
D1337	S1337	355	AfaCfaGfuGfuUfcUfuGfcUfcUfauasAf	AS1337	1447	uUfAfUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.54	
D1338	S1338	356	AfAfCfaGfuGfuUfcUfugcUfcUfaUfasAf	AS1338	1448	uUfaUfaGfaGfCfAfaGfaAfcAfcUfguusUfsu	0.08	0.20	0.69	
D1339	S1339	357	AfaCfaGfuGfuUfCfUfuGfcUfcUfaUfasAf	AS1339	1449	uUfaUfaGfaGfcAfagaAfcAfcUfgUfusUfsu	0.07	0.16	0.55	
D1340	S1340	358	AfaCfaGfuGfuUfcUfuGfcUfcuaUfasAf	AS1340	1450	uUfaUfAfGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.17	0.57	
D1341	S1341	359	AfaCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1341	1451	uUfaUfaGfaGfcAfaGfaAfCfAfcUfgUfususu	0.08	0.22	0.63	
D1342	S1342	360	AfAfCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1342	1452	uUfaUfaGfaGfcAfAfGfaAfcAfcUfguusUfsu	0.21	0.56	0.86	
D1343	S1343	361	AfacaGfuGfUfcUfuGfcUfcUfaUfasAf	AS1343	1453	uUfaUfaGfaGfcAfaGfaacAfcUfGfUfusUfsu	0.14	0.37	0.73	
D1344	S1344	362	AfaCfaGfuGfuucUfUfGfcUfcUfaUfasAf	AS1344	1454	uUfaUfaGfaGfcaaGfAfAfcAfcUfgUfusUfsu	0.08	0.20	0.66	

D1345	S1345	363	AfaCfAfGfuGfuUfcuuGfcUfcUfaUfasAf	AS1345	1455	uUfaUfaGfaGfcAfAfGfaAfcAfcugUfusUfsu	0.12	0.34	0.73		
D1346	S1346	364	AfaCfaGfuGfUfUfcUfuGfcUfcUfauasAf	AS1346	1456	uUfAfUfaGfaGfcAfaGfaacAfcUfgUfusUfsu	0.16	0.42	0.90		
D1347	S1347	365	AfaCfaGfuGfUfUfcUfuGfcUfcUfaUfasAf	AS1347	1457	uUfaUfaGfaGfcAfaGfaacAfcUfgUfusUfsUf	0.17	0.43	0.85		
D1348	S1348	366	AfaCfAfGfuGfuucUfuGfcUfcUfaUfasAf	AS1348	1458	uUfaUfaGfaGfcAfaGfAfAfcAfcugUfusUfsu	0.08	0.21	0.58		
D1349	S1349	367	AfaCfaGfuGfUfUfcUfuGfcUfcuaUfasAf	AS1349	1459	uUfaUfAfGfaGfcAfaGfaacAfcUfgUfusUfsu	0.21	0.39	0.88		
D1350	S1350	368	AfaCfaguGfuUfcUfUfGfcUfcUfaUfasAf	AS1350	1460	uUfaUfaGfaGfcaaGfaAfcAfCfUfgUfusUfsu	0.06	0.13	0.52		
D1351	S1351	369	AfaCfAfGfuguUfcUfuGfcUfcUfaUfasAf	AS1351	1461	uUfaUfaGfaGfcAfaGfaAfCfAfcugUfusUfsu	0.08	0.21	0.58		
D1352	S1352	370	AfaCfaGfUfGfuUfcuuGfcUfcUfaUfasAf	AS1352	1462	uUfaUfaGfaGfcAfAfGfaAfcacUfgUfusUfsu	0.18	0.49	0.84		
D1353	S1353	371	AfaCfaGfuGfUfUfcUfuGfcucUfaUfasAf	AS1353	1463	uUfaUfaGfAfGfcAfaGfaacAfcUfgUfusUfsu	0.11	0.25	0.68		
D1354	S1354	372	AfacaGfuGfuUfcUfUfGfcUfcUfaUfasAf	AS1354	1464	uUfaUfaGfaGfcaaGfaAfcAfcUfGfUfusUfsu	0.07	0.15	0.52		
D1355	S1355	373	AfaCfaGfUfGfuucUfuGfcUfcUfaUfasAf	AS1355	1465	uUfaUfaGfaGfcAfaGfAfAfcacUfgUfusUfsu	0.10	0.26	0.63		
D1356	S1356	374	AfaCfaGfuGfUfUfcUfugcUfcUfaUfasAf	AS1356	1466	uUfaUfaGfaGfCfAfaGfaacAfcUfgUfusUfsu	0.16	0.33	0.79		
D1357	S1357	375	AfaCfAfGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1357	1467	uUfaUfaGfaGfcAfaGfaAfcAfcugUfusUfsUf	0.09	0.19	0.51		
D1358	S1358	376	AfaCfaGfuGfUfUfcuuGfcUfcUfaUfasAf	AS1358	1468	uUfaUfaGfaGfcAfAfGfaacAfcUfgUfusUfsu	0.22	0.48	0.71		
D1359	S1359	377	AfaCfaGfuGfuUfcUfUfGfcUfcUfaUfasAf	AS1359	1469	uUfaUfaGfaGfcaaGfaAfcAfcUfgUfusUfsUf	0.10	0.17	0.61		
D1360	S1360	378	AfaCfaguGfUfUfcUfuGfcUfcUfaUfasAf	AS1360	1470	uUfaUfaGfaGfcAfaGfaacAfCfUfgUfusUfsu	0.14	0.40	0.87		
D1361	S1361	379	AfaCfaGfuGfuUfcUfUfGfcUfcuaUfasAf	AS1361	1471	uUfaUfAfGfaGfcaaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.52		
D1362	S1362	380	aaCfaGfuGfuUfcUfuGfCfUfcUfaUfasAf	AS1362	1472	uUfaUfaGfagcAfaGfaAfcAfcUfgUfUfsUfsu	0.10	0.28	0.81		
D1363	S1363	381	AfaCfaGfuGfuucUfuGfcUfcUfAfUfasAf	AS1363	1473	uUfauaGfaGfcAfaGfAfAfcAfcUfgUfusUfsu	0.06	0.16	0.68		
D1364	S1364	382	AfaCfaGfuGfuUfcUfugcUfcUfaUfAfsAf	AS1364	1474	uuaUfaGfaGfCfAfaGfaAfcAfcUfgUfusUfsu	0.09	0.26	0.67		
D1365	S1365	383	aacaguguucuugcucuauasa	AS1365	1475	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.20	0.59	0.95		
D1366	S1366	384	AfaCfaGfuGfuUfcUfuGfCfUfcUfauasAf	AS1366	1476	uUfAfUfaGfagcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.53		
D1367	S1367	385	AfaCfaGfuGfuUfcUfuGfCfUfcUfaUfasAf	AS1367	1477	uUfaUfaGfagcAfaGfaAfcAfcUfgUfusUfsUf	0.08	0.16	0.53		
D1368	S1368	386	AfaCfaGfuguUfcUfuGfcUfcUfAfUfasAf	AS1368	1478	uUfauaGfaGfcAfaGfaAfCfAfcUfgUfusUfsu	0.07	0.15	0.54		
D1369	S1369	387	AfaCfaGfuGfuUfcuuGfcUfcUfaUfAfsAf	AS1369	1479	uuaUfaGfaGfcAfAfGfaAfcAfcUfgUfusUfsu	0.23	0.56	0.89		
D1370	S1370	388	AfaCfaGfuGfuUfcUfuGfCfUfcuaUfasAf	AS1370	1480	uUfaUfAfGfagcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.12	0.55		
D1371	S1371	389	AfaCfaGfuGfuUfcUfuGfCfUfcuaUfasAf	AS1371	1481	uUfaUfAfGfagcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.18	0.58		

D1372	S1372	390	AfaCfaguGfuUfcUfuGfcUfcUfAfUfasAf	AS1372	1482	uUfauaGfaGfcAfaGfaAfcAfCfUfgUfusUfsu	0.06	0.15	0.56	
D1373	S1373	391	AfaCfaGfuGfuucUfuGfcUfcUfaUfAfsAf	AS1373	1483	uuaUfaGfaGfcAfaGfAfAfcAfcUfgUfusUfsu	0.21	0.51	0.89	
D1374	S1374	392	AfacaGfuguUfcUfuGfcUfcUfaUfasAf	AS1374	1484	uUfaUfaGfaGfcAfaGfaAfCfAfcUfGfUfusUfsu	0.08	0.21	0.64	
D1375	S1375	393	AfaCfaGfuGfuUfcuuGfCfUfcUfaUfasAf	AS1375	1485	uUfaUfaGfagcAfAfGfaAfcAfcUfgUfusUfsu	0.15	0.40	0.94	
D1376	S1376	394	AfaCfaGfuGfuUfcuuGfCfUfcUfaUfasAf	AS1376	1486	uUfaUfaGfagcAfAfGfaAfcAfcUfgUfusUfsu	0.13	0.40	0.96	
D1377	S1377	395	AfaCfaGfuGfuUfcUfuGfcUfCfUfauasAf	AS1377	1487	uUfAfUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.17	0.64	
D1378	S1378	396	AfaCfaGfuguUfcUfuGfcUfcUfaUfAfsAf	AS1378	1488	uuaUfaGfaGfcAfaGfaAfCfAfcUfgUfusUfsu	0.18	0.50	0.97	
D1379	S1379	397	AfaCfaGfuGfuucUfuGfCfUfcUfaUfasAf	AS1379	1489	uUfaUfaGfagcAfaGfAfAfcAfcUfgUfusUfsu	0.08	0.24	0.79	
D1380	S1380	398	aaCfaGfuGfuUfcUfuGfcUfcUfAfUfasAf	AS1380	1490	uUfauaGfaGfcAfaGfaAfcAfcUfgUfUfsUfsu	0.07	0.14	0.58	
D1381	S1381	399	AfaCfaguGfuUfcUfuGfcUfcUfaUfAfsAf	AS1381	1491	uuaUfaGfaGfcAfaGfaAfcAfCfUfgUfusUfsu	0.11	0.34	0.96	
D1382	S1382	400	AfaCfaGfuguUfcUfuGfCfUfcUfaUfasAf	AS1382	1492	uUfaUfaGfagcAfaGfaAfCfAfcUfgUfusUfsu	0.08	0.18	0.69	
D1383	S1383	401	AfaCfaGfuGfuUfcuuGfcUfCfUfaUfasAf	AS1383	1493	uUfaUfagaGfcAfAfGfaAfcAfcUfgUfusUfsu	0.14	0.38	0.85	
D1384	S1384	402	AfaCfaGfuGfuUfcUfuGfcUfcUfAfUfasAf	AS1384	1494	uUfauaGfaGfcAfaGfaAfcAfcUfgUfusUfsUf	0.07	0.16	0.54	
D1385	S1385	403	AfacaGfuGfuUfcUfuGfcUfcUfaUfAfsAf	AS1385	1495	uuaUfaGfaGfcAfaGfaAfcAfcUfGfUfusUfsu	0.08	0.20	0.75	
D1386	S1386	404	aacaguguucUfuGfcUcUaudAsa	AS1386	1496	uUfdAUdAGfaGfcAfaGfaadCadCudGdTdTsusu	0.25	0.56	0.90	
D1387	S1387	405	AfaCfaguGfuUfcUfuGfCfUfcUfaUfasAf	AS1387	1497	uUfaUfaGfagcAfaGfaAfcAfCfUfgUfusUfsu	0.08	0.19	0.70	
D1388	S1388	406	AfaCfaGfuGfuucUfuGfcUfCfUfaUfasAf	AS1388	1498	uUfaUfagaGfcAfaGfAfAfcAfcUfgUfusUfsu	0.08	0.14	0.60	
D1389	S1389	407	AfaCfaGfuGfuUfcUfuGfcUfcuaUfAfsAf	AS1389	1499	uuaUfAfGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.62	
D1390	S1390	408	aaCfaGfuGfuUfcUfuGfcUfcUfaUfAfsAf	AS1390	1500	uuaUfaGfaGfcAfaGfaAfcAfcUfgUfUfsUfsu	0.08	0.27	0.76	
D1391	S1391	409	aacaguguucdTudGcdTcdTadTasa	AS1391	1501	uUfdAUdAGfaGfcAfaGfaadCadCudGudTsusu	0.18	0.36	0.81	
D1392	S1392	410	AfacaGfuGfuUfcUfuGfCfUfcUfaUfasAf	AS1392	1502	uUfaUfaGfagcAfaGfaAfcAfcUfGfUfusUfsu	0.07	0.17	0.55	
D1393	S1393	411	AfaCfaGfuguUfcUfuGfcUfCfUfaUfasAf	AS1393	1503	uUfaUfagaGfcAfaGfaAfCfAfcUfgUfusUfsu	0.07	0.15	0.57	
D1394	S1394	412	AfaCfaGfuGfuUfcuuGfcUfcUfAfUfasAf	AS1394	1504	uUfauaGfaGfcAfAfGfaAfcAfcUfgUfusUfsu	0.26	0.68	1.06	
D1395	S1395	413	AfaCfaGfuGfuUfcUfuGfcucUfaUfAfsAf	AS1395	1505	uuaUfaGfAfGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.18	0.58	
D1396	S1396	414	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfAfsAf	AS1396	1506	uuaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsUf	0.09	0.27	0.73	
D1397	S1397	415	AfaCfaAfuGfuUfcUfuGfcdAdCdTdAUfasAf	AS1397	1507	uUfadTdAdGdAGfcAfaGfaGfcAfcAfgUfusUfsu	0.20	0.51	0.73	
D1398	S1398	416	AfacaGfuguUfcuuGfcucUfauasAf	AS1398	1508	uUfAfUfaGfAfGfcAfAfGfaAfCfAfcUfGfUfusUfsu	0.13	0.34	0.86	

D1399	S1399	417	dAacadGugudTcuudGcucdTauasdA	AS1399	1509	udTdAdTadGdAdGcdAdAdGadAdCdAcdTdGdTusdTsu	0.24	0.42	0.82	
D1400	S1400	418	AfaCfaAfuGfuUfcUfuGfdCdAdCdTaUfasAf	AS1400	1510	uUfaUfdAdGdAdGcAfaGfaGfcAfcAfgUfusUfsu	0.49	0.85	0.78	
D1401	S1401	419	AfaCfaAfuGfuUfcUfudGdCdAdCUfaUfasAf	AS1401	1511	uUfaUfadGdAdGdCAfaGfaGfcAfcAfgUfusUfsu	0.67	0.83	0.85	
D1402	S1402	420	aaCfAfguGfUfucUfUfgcUfCfuaUfAfsa	AS1402	1512	uUfaUfAfgaGfCfaaGfAfacAfCfugUfUfsusu	0.18	0.47	0.80	
D1403	S1403	421	AfaCfaAfuGfuUfcUfuGfcdAdCUfadTdAsAf	AS1403	1513	udTdAUfadGdAGfcAfaGfaGfcAfcAfgUfusUfsu	0.73	0.89	0.77	
D1404	S1404	422	aacAgugUucuUgcuCuauAsa	AS1404	1514	uUaUAgAGCaAGAaCACuGUUsusu	0.12	0.39	0.79	
D1405	S1405	423	AacaGuguUcuuGcucUauasA	AS1405	1515	uUAUaGAGcAAGaACAcUGUusUsu	0.12	0.37	0.77	
D1406	S1406	424	AfaCfaAfuGfuUfcUfudGdCAfcUfadTdAsAf	AS1406	1516	udTdAUfaGfadGdCAfaGfaGfcAfcAfgUfusUfsu	0.59	0.93	0.89	
D1407	S1407	425	aACagUGuuCUugCUcuAUasa	AS1407	1517	UUauAGagCAagAAcaCUguUsUsu	0.09	0.16	0.55	
D1408	S1408	426	AfaCfaAfuGfuUfcUfuGfcAfcdTdAdTdAsAf	AS1408	1518	udTdAdTdAGfaGfcAfaGfaAfcAfcAfgUfusUfsu	0.22	0.64	0.86	
D1409	S1409	427	aaCAguGUucUUgcUCuaUAsa	AS1409	1519	uUaUAgaGCaaGAacACugUUsusu	0.13	0.31	0.76	
D1410	S1410	428	AfaCfaAfuGfuUfcUfuGfcAfdCdTdAdTdAsAf	AS1410	1520	udTdAdTdAdGaGfcAfaGfaGfcAfcAfgUfusUfsu	0.77	0.94	0.93	
D1411	S1411	429	aacAfgugUfucuUfgcuCfuauAfsa	AS1411	1521	uUfaUfAfgAfGfCfaAfGfAfaCfAfCfuGfUfUfsusu	0.23	0.53	1.04	
D1412	S1412	430	aacdAgugdTucudTgcudCuaudAsa	AS1412	1522	udTadTdAgdAdGdCadAdGdAadCdAdCudGdTdTsusu	0.30	0.64	0.90	
D1413	S1413	431	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1413	1523	UfUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.19	0.63	
D1414	S1414	432	AfaCfaGfuGfUfUfcUfuGfcUfcUfaUfasa	AS1414	1524	UfUfaUfaGfaGfcAfaGfaacAfcUfgUfusUfsu	0.11	0.28	0.66	
D1415	S1415	433	AfaCfaGfuGfuUfcUfuGfCfUfcUfaUfasa	AS1415	1525	UfUfaUfaGfagcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.53	
D1416	S1416	434	aacaguguucuugcucuauasa	AS1416	1526	UfUfAfUfAfGfAfGfCfAfAfGfAfAfCfAfCfUfGfUfUfsusu	0.20	0.53	0.99	
D1417	S1417	435	AfaCfaGfuGfuUfcUfuGfcUfcUfAfUfasa	AS1417	1527	UfUfauaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.17	0.53	
D1418	S1418	436	aAfCfagUfGfuuCfUfugCfUfcuAfUfasa	AS1418	1528	UfUfauAfGfagCfAfagAfAfcaCfUfguUfsUfsu	0.08	0.20	0.70	
D1419	S1419	437	AfaCfAfGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1419	1529	uUfaUfaGfaGfcAfaGfaAfcAfcugUfusUfsUf	0.08	0.20	0.70	
D1420	S1420	438	GfaCfuUfcUfcCfUfCfcAfgugGfaCfcUfL96	AS1420	1530	aGfgUfcCfAfCfuGfgagGfaGfaAfgUfcsCfsc				
D1421	S1421	439	GfaCfuUfcUfcCfUfCfcAfgUfGfGfaCfcUfL96	AS1421	1531	aGfgUfccaCfuGfgagGfaGfaAfgUfcsCfsc				
D1422	S1422	440	AfcUfuCfuCfcUfCfCfaGfuggAfcCfuGfL96	AS1422	1532	cAfgGfuCfCfAfcUfggaGfgAfgAfaGfusCfsc				
D1423	S1423	441	AfcUfuCfuCfcUfCfCfaGfuGfGfAfcCfuGfL96	AS1423	1533	cAfgGfuccAfcUfggaGfgAfgAfaGfusCfsc				
D1424	S1424	442	CfuUfcUfcCfuCfCfAfgUfggaCfcUfgAfL96	AS1424	1534	uCfaGfgUfCfCfaCfuggAfgGfaGfaAfgsUfsc				
D1425	S1425	443	CfuUfcUfcCfuCfCfAfgUfgGfAfCfcUfgAfL96	AS1425	1535	uCfaGfgucCfaCfuggAfgGfaGfaAfgsUfsc				

D1426	S1426	444	UfuCfuCfcUfcCfAfGfuGfgacCfuGfaAfL96	AS1426	1536	uUfcAfgGfUfCfcAfcugGfaGfgAfgAfasGfsu					
D1427	S1427	445	UfuCfuCfcUfcCfAfGfuGfgAfCfCfcfuGfaAfL96	AS1427	1537	uUfcAfgguCfcAfcugGfaGfgAfgAfasGfsu					
D1428	S1428	446	UfcUfcCfuCfcAfGfUfgGfaccUfgAfaGfL96	AS1428	1538	cUfuCfaGfGfUfcCfacuGfgAfgGfaGfasAfsg					
D1429	S1429	447	UfcUfcCfuCfcAfGfUfgGfaCfCfUfgAfaGfL96	AS1429	1539	cUfuCfaggUfcCfacuGfgAfgGfaGfasAfsg					
D1430	S1430	448	CfuCfcUfcCfaGfUfGfgAfccuGfaAfgGfL96	AS1430	1540	cCfuUfcAfGfGfuCfcacUfgGfaGfgAfgsAfsa					
D1431	S1431	449	CfuCfcUfcCfaGfUfGfgAfcCfUfGfaAfgGfL96	AS1431	1541	cCfuUfcagGfuCfcacUfgGfaGfgAfgsAfsa					
D1432	S1432	450	UfcCfuCfcAfgUfGfGfaCfcugAfaGfgAfL96	AS1432	1542	uCfcUfuCfAfGfgUfccaCfuGfgAfgGfasGfsa					
D1433	S1433	451	UfcCfuCfcAfgUfGfGfaCfcUfGfAfaGfgAfL96	AS1433	1543	uCfcUfucaGfgUfccaCfuGfgAfgGfasGfsa					
D1434	S1434	452	CfcUfcCfaGfuGfGfAfcCfugaAfgGfaCfL96	AS1434	1544	gUfcCfuUfCfAfgGfuccAfcUfgGfaGfgsAfsg					
D1435	S1435	453	CfcUfcCfaGfuGfGfAfcCfuGfAfAfgGfaCfL96	AS1435	1545	gUfcCfuucAfgGfuccAfcUfgGfaGfgsAfsg					
D1436	S1436	454	CfuCfcAfgUfgGfAfCfcUfgaaGfgAfcGfL96	AS1436	1546	cGfuCfcUfUfCfaGfgucCfaCfuGfgAfgsGfsa					
D1437	S1437	455	CfuCfcAfgUfgGfAfCfcUfgAfAfGfgAfcGfL96	AS1437	1547	cGfuCfcuuCfaGfgucCfaCfuGfgAfgsGfsa					
D1438	S1438	456	UfcCfaGfuGfgAfCfCfuGfaagGfaCfgAfL96	AS1438	1548	uCfgUfcCfUfUfCfAfgguCfcAfcUfgGfasGfsg					
D1439	S1439	457	UfcCfaGfuGfgAfCfCfuGfaAfGfGfaCfgAfL96	AS1439	1549	uCfgUfccuUfcAfgguCfcAfcUfgGfasGfsg					
D1440	S1440	458	CfcAfgUfgGfaCfCfUfgAfaggAfcGfaGfL96	AS1440	1550	cUfcGfuCfCfUfuCfaggUfcCfaCfuGfgsAfsg					
D1441	S1441	459	CfcAfgUfgGfaCfCfUfgAfaGfGfAfcGfaGfL96	AS1441	1551	cUfcGfuccUfuCfaggUfcCfaCfuGfgsAfsg					
D1442	S1442	460	CfaGfuGfgAfcCfUfGfaAfggaCfgAfgGfL96	AS1442	1552	cCfuCfgUfCfCfuUfcagGfuCfcAfcUfgsGfsa					
D1443	S1443	461	CfaGfuGfgAfcCfUfGfaAfgGfAfCfgAfgGfL96	AS1443	1553	cCfuCfgucCfuUfcagGfuCfcAfcUfgsGfsa					
D1444	S1444	462	AfgUfgGfaCfcUfGfAfaGfgacGfaGfgGfL96	AS1444	1554	cCfcUfcGfUfCfcUfucaGfgUfcCfaCfusGfsg					
D1445	S1445	463	AfgUfgGfaCfcUfGfAfaGfgAfCfGfaGfgGfL96	AS1445	1555	cCfcUfcguCfcUfucaGfgUfcCfaCfusGfsg					
D1446	S1446	464	GfuGfgAfcCfuGfAfAfgGfacgAfgGfgAfL96	AS1446	1556	uCfcCfuCfGfUfcCfuucAfgGfuCfcAfcsUfsg					
D1447	S1447	465	GfuGfgAfcCfuGfAfAfgGfaCfGfAfgGfgAfL96	AS1447	1557	uCfcCfucgUfcCfuucAfgGfuCfcAfcsUfsg					
D1448	S1448	466	UfgGfaCfcUfgAfAfGfgAfcgaGfgGfaUfL96	AS1448	1558	aUfcCfcUfCfGfuCfcuuCfaGfgUfcCfasCfsu					
D1449	S1449	467	UfgGfaCfcUfgAfAfGfgAfcGfAfGfgGfaUfL96	AS1449	1559	aUfcCfcucGfuCfcuuCfaGfgUfcCfasCfsu					
D1450	S1450	468	GfgAfcCfuGfaAfGfGfaCfgagGfgAfuGfL96	AS1450	1560	cAfuCfcCfUfCfgUfccuUfcAfgGfuCfcsAfsc					
D1451	S1451	469	GfgAfcCfuGfaAfGfGfaCfgAfGfGfgAfuGfL96	AS1451	1561	cAfuCfccuCfgUfccuUfcAfgGfuCfcsAfsc					
D1452	S1452	470	GfaCfcUfgAfaGfGfAfcGfaggGfaUfgGfL96	AS1452	1562	cCfaUfcCfCfUfCfGfuccUfuCfaGfgUfcsCfsa					

D1453	S1453	471	GfaCfcUfgAfaGfGfAfcGfaGfGfGfaUfgGfL96	AS1453	1563	cCfaUfcccUfcGfuccUfuCfaGfgUfcsCfsa					
D1454	S1454	472	AfcCfuGfaAfgGfAfCfgAfggAfuGfgGfL96	AS1454	1564	cCfcAfuCfCfCfuCfgucCfuUfcAfgGfusCfsc					
D1455	S1455	473	AfcCfuGfaAfgGfAfCfgAfgGfGfAfuGfgGfL96	AS1455	1565	cCfcAfuccCfuCfgucCfuUfcAfgGfusCfsc					
D1456	S1456	474	CfcUfgAfaGfgAfCfGfaGfggaUfgGfgAfL96	AS1456	1566	uCfcCfaUfCfCfCfUfcguCfcUfuCfaGfgsUfsc					
D1457	S1457	475	CfcUfgAfaGfgAfCfGfaGfgGfAfUfgGfgAfL96	AS1457	1567	uCfcCfaucCfcUfcguCfcUfuCfaGfgsUfsc					
D1458	S1458	476	CfuGfaAfgGfaCfGfAfgGfgauGfgGfaUfL96	AS1458	1568	aUfcCfcAfUfCfcCfucgUfcCfuUfcAfgsGfsu					
D1459	S1459	477	CfuGfaAfgGfaCfGfAfgGfgAfUfGfgGfaUfL96	AS1459	1569	aUfcCfcauCfccCfucgUfccCfuUfcAfgsGfsu					
D1460	S1460	478	UfgAfaGfgAfcGfAfGfgGfaugGfgAfuUfL96	AS1460	1570	aAfuCfcCfAfUfcCfcucGfuCfcUfuCfasGfsg					
D1461	S1461	479	UfgAfaGfgAfcGfAfGfgGfaUfGfGfgAfuUfL96	AS1461	1571	aAfuCfccaUfcCfcucGfuCfcUfuCfasGfsg					
D1462	S1462	480	GfaAfgGfaCfgAfGfGfgAfuggGfaUfuUfL96	AS1462	1572	aAfaUfcCfCfAfuCfccuCfgUfcCfuUfcsAfsg					
D1463	S1463	481	GfaAfgGfaCfgAfGfGfgAfuGfGfGfaUfuUfL96	AS1463	1573	aAfaUfcccAfuCfccuCfgUfcCfuUfcsAfsg					
D1464	S1464	482	AfaGfgAfcGfaGfGfGfaUfgggAfuUfuCfL96	AS1464	1574	gAfaAfuCfCfCfaUfcccUfcGfuCfcUfusCfsa					
D1465	S1465	483	AfaGfgAfcGfaGfGfGfaUfgGfGfAfuUfuCfL96	AS1465	1575	gAfaAfuccCfaUfcccUfcGfuCfcUfusCfsa					
D1466	S1466	484	AfgGfaCfgAfgGfGfAfuGfggaUfuUfcAfL96	AS1466	1576	uGfaAfaUfCfCfcAfuccCfuCfgUfcCfusUfsc					
D1467	S1467	485	AfgGfaCfgAfgGfGfAfuGfgGfAfUfuUfcAfL96	AS1467	1577	uGfaAfaucCfcAfuccCfuCfgUfcCfusUfsc					
D1468	S1468	486	GfgAfcGfaGfgGfAfUfgGfgauUfuCfaUfL96	AS1468	1578	aUfgAfaAfUfCfCfcAfaucCfcUfCfGfuCfcsUfsu					
D1469	S1469	487	GfgAfcGfaGfgGfAfUfgGfgAfUfUfuCfaUfL96	AS1469	1579	aUfgAfaauCfcCfaucCfcUfCfGfuCfcsUfsu					
D1470	S1470	488	GfaCfgAfgGfgAfUfGfgGfauuUfcAfuGfL96	AS1470	1580	cAfuGfaAfAfUfcCfcauCfcCfuCfgUfcsCfsu					
D1471	S1471	489	GfaCfgAfgGfgAfUfGfgGfaUfUfUfcAfuGfL96	AS1471	1581	cAfuGfaaaUfcCfcauCfcCfuCfgUfcsCfsu					
D1472	S1472	490	AfcGfaGfgGfaUfGfGfgAfuuuCfaUfgUfL96	AS1472	1582	aCfaUfgAfAfAfUfCfccaUfcCfcUfCfgUfusCfsc					
D1473	S1473	491	AfcGfaGfgGfaUfGfGfgAfuUfUfCfaUfgUfL96	AS1473	1583	aCfaUfgaaAfuCfccaUfcCfcUfCfgUfusCfsc					
D1474	S1474	492	CfgAfgGfgAfuGfGfGfaUfuucAfuGfuAfL96	AS1474	1584	uAfcAfuGfAfAfaUfcccAfuCfcCfuCfgsUfsc					
D1475	S1475	493	CfgAfgGfgAfuGfGfGfaUfuUfCfAfuGfuAfL96	AS1475	1585	uAfcAfugaAfaUfcccAfuCfcCfuCfgsUfsc					
D1476	S1476	494	GfaGfgGfaUfgGfGfAfuUfucaUfgUfaAfL96	AS1476	1586	uUfaCfaUfGfAfaAfucccCfaUfcCfCfUfcsGfsu					
D1477	S1477	495	GfaGfgGfaUfgGfGfAfuUfuCfAfUfgUfaAfL96	AS1477	1587	uUfaCfaugAfaAfuccCfaUfcCfcUfcsGfsu					
D1478	S1478	496	AfgGfgAfuGfgGfAfUfuUfcauGfuAfaCfL96	AS1478	1588	gUfuAfcAfUfGfaAfaucCfcAfuCfcCfusCfsg					
D1479	S1479	497	AfgGfgAfuGfgGfAfUfuUfcAfUfGfuAfaCfL96	AS1479	1589	gUfuAfcauGfaAfaucCfcAfuCfcCfusCfsg					

D1480	S1480	498	GfgGfaUfgGfgAfUfUfuCfaugUfaAfcCfL96	AS1480	1590	gGfuUfaCfAfUfgAfaauCfcCfaUfcCfcsUfsc					
D1481	S1481	499	GfgGfaUfgGfgAfUfUfuCfaUfgUfaAfcCfL96	AS1481	1591	gGfuUfacaUfgAfaauCfcCfaUfcCfcsUfsc					
D1482	S1482	500	GfgAfuGfgGfaUfUfUfcAfuguAfaCfcAfL96	AS1482	1592	uGfgUfuAfCfAfuGfaaaUfcCfcAfuCfcsCfsu					
D1483	S1483	501	GfgAfuGfgGfaUfUfUfcAfuGfUfAfaCfcAfL96	AS1483	1593	uGfgUfuacAfuGfaaaUfcCfcAfuCfcsCfsu					
D1484	S1484	502	GfaUfgGfgAfuUfUfCfaUfguaAfcCfaAfL96	AS1484	1594	uUfgGfuUfAfCfaUfgaaAfuCfcCfaUfcsCfsc					
D1485	S1485	503	GfaUfgGfgAfuUfUfCfaUfgUfAfAfcCfaAfL96	AS1485	1595	uUfgGfuuaCfaUfgaaAfuCfcCfaUfcsCfsc					
D1486	S1486	504	AfuGfgGfaUfuUfCfAfuGfuaaCfcAfaGfL96	AS1486	1596	cUfuGfgUfUfAfcAfugaAfaUfcCfcAfusCfsc					
D1487	S1487	505	AfuGfgGfaUfuUfCfAfuGfuAfAfCfcAfaGfL96	AS1487	1597	cUfuGfguuAfcAfugaAfaUfcCfcAfusCfsc					
D1488	S1488	506	UfgGfgAfuUfuCfAfUfgUfaaccCfaAfgAfL96	AS1488	1598	uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc					
D1489	S1489	507	UfgGfgAfuUfuCfAfUfgUfaAfCfCfcAfgAfL96	AS1489	1599	uCfuUfggUfaCfaugAfaAfuCfcCfasUfsc					
D1490	S1490	508	GfgGfaUfuUfcAfUfGfuAfaccAfaGfaGfL96	AS1490	1600	cUfcUfuGfGfUfuAfcauGfaAfaUfcCfcsAfsu					
D1491	S1491	509	GfgGfaUfuUfcAfUfGfuAfaCfCfAfaGfaGfL96	AS1491	1601	cUfcUfuggUfuAfcauGfaAfaUfcCfcsAfsu					
D1492	S1492	510	GfgAfuUfuCfaUfGfUfaAfccaAfgAfgUfL96	AS1492	1602	aCfuCfuUfGfGfuUfacaUfgAfaAfuCfcsCfsa					
D1493	S1493	511	GfgAfuUfuCfaUfGfUfaAfcCfAfAfgAfgUfL96	AS1493	1603	aCfuCfuugGfuUfacaUfgAfaAfuCfcsCfsa					
D1494	S1494	512	GfaUfuUfcAfuGfUfAfaCfcaaGfaGfuAfL96	AS1494	1604	uAfcUfcUfUfGfgUfuacAfuGfaAfaUfcsCfsc					
D1495	S1495	513	GfaUfuUfcAfuGfUfAfaCfcAfAfGfaGfuAfL96	AS1495	1605	uAfcUfcuuGfgUfuacAfuGfaAfaUfcsCfsc					
D1496	S1496	514	AfuUfuCfaUfgUfAfAfcCfaagAfgUfaUfL96	AS1496	1606	aUfaCfuCfUfUfgGfuuaCfaUfgAfaAfusCfsc					
D1497	S1497	515	AfuUfuCfaUfgUfAfAfcCfaAfGfAfgUfaUfL96	AS1497	1607	aUfaCfucuUfgGfuuaCfaUfgAfaAfusCfsc					
D1498	S1498	516	UfuUfcAfuGfuAfAfCfcAfagaGfuAfuUfL96	AS1498	1608	aAfuAfcUfCfUfuGfguuAfcAfuGfaAfasUfsc					
D1499	S1499	517	UfuUfcAfuGfuAfAfCfcAfaGfAfGfuAfuUfL96	AS1499	1609	aAfuAfcucUfuGfguuAfcAfuGfaAfasUfsc					
D1500	S1500	518	UfuCfaUfgUfaAfCfCfaAfgagUfaUfuCfL96	AS1500	1610	gAfaUfaCfUfCfuUfggUfaCfaUfgAfasAfsu					
D1501	S1501	519	UfuCfaUfgUfaAfCfCfaAfgAfGfUfaUfuCfL96	AS1501	1611	gAfaUfacuCfuUfggUfaCfaUfgAfasAfsu					
D1502	S1502	520	UfcAfuGfuAfaCfCfAfaGfagaUfuUfccCfL96	AS1502	1612	gGfaAfuAfCfUfcUfuggUfuAfcAfuGfasAfsa					
D1503	S1503	521	UfcAfuGfuAfaCfCfAfaGfaGfUfAfuUfccCfL96	AS1503	1613	gGfaAfuacUfcUfuggUfuAfcAfuGfasAfsa					
D1504	S1504	522	CfaUfgUfaAfcCfAfAfgAfguaUfuCfcAfL96	AS1504	1614	uGfgAfaUfAfCfuCfuugGfuUfaCfaUfgsAfsa					
D1505	S1505	523	CfaUfgUfaAfcCfAfAfgAfgUfAfUfuCfcAfL96	AS1505	1615	uGfgAfauaCfuCfuugGfuUfaCfaUfgsAfsa					
D1506	S1506	524	AfuGfuAfaCfcAfAfGfaGfuauUfcCfaUfL96	AS1506	1616	aUfgGfaAfUfAfcUfcuuGfgUfuAfcAfusGfsa					

D1507	S1507	525	AfuGfuAfaCfcAfAfGfaGfuAfUfUfcCfaUfL96	AS1507	1617	aUfgGfaauAfcUfcuuGfgUfuAfcAfusGfsa				
D1508	S1508	526	UfgUfaAfcCfaAfGfAfgUfauuCfcAfuUfL96	AS1508	1618	aAfuGfgAfAfUfaCfucuUfgGfuUfaCfasUfsg				
D1509	S1509	527	UfgUfaAfcCfaAfGfAfgUfaUfUfCfcAfuUfL96	AS1509	1619	aAfuGfgaaUfaCfucuUfgGfuUfaCfasUfsg				
D1510	S1510	528	GfuAfaCfcAfaGfAfGfuAfuucCfaUfuUfL96	AS1510	1620	aAfaUfgGfAfAfuAfcucUfuGfgUfuAfcsAfsu				
D1511	S1511	529	GfuAfaCfcAfaGfAfGfuAfuUfCfCfaUfuUfL96	AS1511	1621	aAfaUfggaAfuAfcucUfuGfgUfuAfcsAfsu				
D1512	S1512	530	UfaAfcCfaAfgAfGfUfaUfuccAfuUfuUfL96	AS1512	1622	aAfaAfuGfGfAfaUfacuCfuUfgGfuUfasCfsa				
D1513	S1513	531	UfaAfcCfaAfgAfGfUfaUfuCfCfAfuUfuUfL96	AS1513	1623	aAfaAfuggAfaUfacuCfuUfgGfuUfasCfsa				
D1514	S1514	532	AfaCfcAfaGfaGfUfAfuUfccaUfuUfuUfL96	AS1514	1624	aAfaAfaUfGfGfaAfuacUfcUfuGfgUfusAfsc				
D1515	S1515	533	AfaCfcAfaGfaGfUfAfuUfcCfAfUfuUfuUfL96	AS1515	1625	aAfaAfaugGfaAfuacUfcUfuGfgUfusAfsc				
D1516	S1516	534	AfcCfaAfgAfgUfAfUfuCfcauUfuUfuAfL96	AS1516	1626	uAfaAfaAfUfGfgAfauaCfuCfuUfgGfusUfsa				
D1517	S1517	535	AfcCfaAfgAfgUfAfUfuCfcAfUfUfuUfuAfL96	AS1517	1627	uAfaAfaauGfgAfauaCfuCfuUfgGfusUfsa				
D1518	S1518	536	CfcAfaGfaGfuAfUfUfcCfauuUfuUfaCfL96	AS1518	1628	gUfaAfaAfAfUfgGfaauAfcUfcUfuGfgsUfsu				
D1519	S1519	537	CfcAfaGfaGfuAfUfUfcCfaUfUfUfuUfaCfL96	AS1519	1629	gUfaAfaaaUfgGfaauAfcUfcUfuGfgsUfsu				
D1520	S1520	538	CfaAfgAfgUfaUfUfCfcAfuuuUfuAfcUfL96	AS1520	1630	aGfuAfaAfAfAfuGfgaaUfaCfuCfuUfgsGfsu				
D1521	S1521	539	CfaAfgAfgUfaUfUfCfcAfuUfUfUfuAfcUfL96	AS1521	1631	aGfuAfaaaAfuGfgaaUfaCfuCfuUfgsGfsu				
D1522	S1522	540	AfaGfaGfuAfuUfCfCfaUfuuuUfaCfuAfL96	AS1522	1632	uAfgUfaAfAfAfaUfggaAfuAfcUfcUfusGfsg				
D1523	S1523	541	AfaGfaGfuAfuUfCfCfaUfuUfUfUfaCfuAfL96	AS1523	1633	uAfgUfaaaAfaUfggaAfuAfcUfcUfusGfsg				
D1524	S1524	542	AfgAfgUfaUfuCfCfAfuUfuuuAfcUfaAfL96	AS1524	1634	uUfaGfuAfAfAfaAfuggAfaUfaCfuCfusUfsg				
D1525	S1525	543	AfgAfgUfaUfuCfCfAfuUfuUfUfAfcUfaAfL96	AS1525	1635	uUfaGfuaaAfaAfuggAfaUfaCfuCfusUfsg				
D1526	S1526	544	GfaGfuAfuUfcCfAfUfuUfuuaCfuAfaAfL96	AS1526	1636	uUfuAfgUfAfAfaAfaugGfaAfuAfcUfcsUfsu				
D1527	S1527	545	GfaGfuAfuUfcCfAfUfuUfuUfAfCfuAfaAfL96	AS1527	1637	uUfuAfguaAfaAfaugGfaAfuAfcUfcsUfsu				
D1528	S1528	546	AfgUfaUfuCfcAfUfUfuUfuacUfaAfaGfL96	AS1528	1638	cUfuUfaGfUfAfaAfaauGfgAfaUfaCfusCfsu				
D1529	S1529	547	AfgUfaUfuCfcAfUfUfuUfuAfCfUfaAfaGfL96	AS1529	1639	cUfuUfaguAfaAfaauGfgAfaUfaCfusCfsu				
D1530	S1530	548	GfuAfuUfcCfaUfUfUfuUfacuAfaAfgCfL96	AS1530	1640	gCfuUfuAfGfUfaAfaaaUfgGfaAfuAfcsUfsc				
D1531	S1531	549	GfuAfuUfcCfaUfUfUfuUfaCfUfAfaAfgCfL96	AS1531	1641	gCfuUfuagUfaAfaaaUfgGfaAfuAfcsUfsc				
D1532	S1532	550	UfaUfuCfcAfuUfUfUfuAfcuaAfaGfcAfL96	AS1532	1642	uGfcUfuUfAfGfuAfaaaAfuGfgAfaUfasCfsu				
D1533	S1533	551	UfaUfuCfcAfuUfUfUfuAfcUfAfAfaGfcAfL96	AS1533	1643	uGfcUfuuaGfuAfaaaAfuGfgAfaUfasCfsu				

D1534	S1534	552	AfuUfcCfaUfuUfUfUfaCfuaaAfgCfaGfL96	AS1534	1644	cUfgCfuUfUfAfgUfaaaAfaUfgGfaAfusAfsc					
D1535	S1535	553	AfuUfcCfaUfuUfUfUfaCfuAfAfAfgCfaGfL96	AS1535	1645	cUfgCfuuuAfgUfaaaAfaUfgGfaAfusAfsc					
D1536	S1536	554	UfuCfcAfuUfuUfUfAfcUfaaaGfcAfgUfL96	AS1536	1646	aCfuGfcUfUfUfaGfuaaAfaAfuGfgAfasUfsa					
D1537	S1537	555	UfuCfcAfuUfuUfUfAfcUfaAfAfGfcAfgUfL96	AS1537	1647	aCfuGfcuuUfaGfuaaAfaAfuGfgAfasUfsa					
D1538	S1538	556	UfccfaUfuUfuUfAfCfuAfaagCfaGfuGfL96	AS1538	1648	cAfcUfgCfUfUfuAfguaAfaAfaUfgGfasAfsu					
D1539	S1539	557	UfccfaUfuUfuUfAfCfuAfaAfaGfCfaGfuGfL96	AS1539	1649	cAfcUfgcuUfuAfguaAfaAfaUfgGfasAfsu					
D1540	S1540	558	CfcAfuUfuUfuAfCfUfaAfagcAfgUfgUfL96	AS1540	1650	aCfaCfuGfCfUfuUfaguAfaAfaAfuGfgsAfsa					
D1541	S1541	559	CfcAfuUfuUfuAfCfUfaAfaGfCfAfgUfgUfL96	AS1541	1651	aCfaCfugcUfuUfaguAfaAfaAfuGfgsAfsa					
D1542	S1542	560	CfaUfuUfuUfaCfUfAfaAfgcaGfuGfuUfL96	AS1542	1652	aAfcAfcUfGfCfuUfuagUfaAfaAfaUfgsGfsa					
D1543	S1543	561	CfaUfuUfuUfaCfUfAfaAfgCfAfGfuGfuUfL96	AS1543	1653	aAfcAfcugCfuUfuagUfaAfaAfaUfgsGfsa					
D1544	S1544	562	AfuUfuUfuAfcUfAfAfaGfcagUfgUfuUfL96	AS1544	1654	aAfaCfaCfUfGfCfUfuuaGfuAfaAfaAfusGfsg					
D1545	S1545	563	AfuUfuUfuAfcUfAfAfaGfcAfGfUfgUfuUfL96	AS1545	1655	aAfaCfacuGfcUfuuaGfuAfaAfaAfusGfsg					
D1546	S1546	564	UfuUfuUfaCfuAfAfAfgCfaguGfuUfuUfL96	AS1546	1656	aAfaAfcAfCfUfgCfuuuAfgUfaAfaAfasUfsg					
D1547	S1547	565	UfuUfuUfaCfuAfAfAfgCfaGfUfGfuUfuUfL96	AS1547	1657	aAfaAfcacUfgCfuuuAfgUfaAfaAfasUfsg					
D1548	S1548	566	UfuUfuAfcUfaAfAfGfcAfgugUfuUfuCfL96	AS1548	1658	gAfaAfaCfAfCfuGfcuuUfaGfuAfaAfasAfsu					
D1549	S1549	567	UfuUfuAfcUfaAfAfGfcAfgUfGfUfuUfuCfL96	AS1549	1659	gAfaAfacaCfuGfcuuUfaGfuAfaAfasAfsu					
D1550	S1550	568	UfuUfaCfuAfaAfGfCfaGfuguUfuUfcAfL96	AS1550	1660	uGfaAfaAfCfAfcUfgcuUfuAfgUfaAfasAfsa					
D1551	S1551	569	UfuUfaCfuAfaAfGfCfaGfuGfUfUfuUfcAfL96	AS1551	1661	uGfaAfaacAfcUfgcuUfuAfgUfaAfasAfsa					
D1552	S1552	570	UfuAfcUfaAfaGfCfAfgUfguuUfuCfaCfL96	AS1552	1662	gUfgAfaAfAfCfaCfugcUfuUfaGfuAfasAfsa					
D1553	S1553	571	UfuAfcUfaAfaGfCfAfgUfgUfUfUfuCfaCfL96	AS1553	1663	gUfgAfaaaCfaCfugcUfuUfaGfuAfasAfsa					
D1554	S1554	572	UfaCfuAfaAfgCfAfGfuGfuuuUfcAfcCfL96	AS1554	1664	gGfuGfaAfAfAfcAfcugCfuUfuAfgUfasAfsa					
D1555	S1555	573	UfaCfuAfaAfgCfAfGfuGfuUfUfUfcAfcCfL96	AS1555	1665	gGfuGfaaaAfcAfcugCfuUfuAfgUfasAfsa					
D1556	S1556	574	AfcUfaAfaGfcAfGfUfgUfuuuCfaCfcUfL96	AS1556	1666	aGfgUfgAfAfAfaCfacuGfcUfuUfaGfusAfsa					
D1557	S1557	575	AfcUfaAfaGfcAfGfUfgUfuUfUfCfaCfcUfL96	AS1557	1667	aGfgUfgaaAfaCfacuGfcUfuUfaGfusAfsa					
D1558	S1558	576	CfuAfaAfgCfaGfUfGfuUfuucAfcCfuCfL96	AS1558	1668	gAfgGfuGfAfAfaAfcacUfgCfuUfuAfgsUfsa					
D1559	S1559	577	CfuAfaAfgCfaGfUfGfuUfuUfCfAfcCfuCfL96	AS1559	1669	gAfgGfugaAfaAfcacUfgCfuUfuAfgsUfsa					
D1560	S1560	578	UfaAfaGfcAfgUfGfUfuUfucaCfcUfcAfL96	AS1560	1670	uGfaGfgUfGfAfaAfacaCfuGfcUfuUfasGfsu					

D1561	S1561	579	UfaAfaGfcAfgUfGfUfuUfuCfAfCfcUfcAfL96	AS1561	1671	uGfaGfgugAfaAfacaCfuGfcUfuUfasGfsu					
D1562	S1562	580	AfaAfgCfaGfuGfUfUfuUfcacCfuCfaUfL96	AS1562	1672	aUfgAfgGfUfGfaAfaacAfcUfgCfuUfusAfsg					
D1563	S1563	581	AfaAfgCfaGfuGfUfUfuUfcAfCfCfuCfaUfL96	AS1563	1673	aUfgAfgguGfaAfaacAfcUfgCfuUfusAfsg					
D1564	S1564	582	AfaGfcAfgUfgUfUfUfuCfaccUfcAfuAfL96	AS1564	1674	uAfuGfaGfGfUfgAfaaaCfaCfuGfcUfusUfsa					
D1565	S1565	583	AfaGfcAfgUfgUfUfUfuCfaCfCfUfcAfuAfL96	AS1565	1675	uAfuGfaggUfgAfaaaCfaCfuGfcUfusUfsa					
D1566	S1566	584	AfgCfaGfuGfuUfUfUfcAfccuCfaUfaUfL96	AS1566	1676	aUfaUfgAfGfGfuGfaaaAfcAfcUfgCfusUfsu					
D1567	S1567	585	AfgCfaGfuGfuUfUfUfcAfcCfUfCfaUfaUfL96	AS1567	1677	aUfaUfgagGfuGfaaaAfcAfcUfgCfusUfsu					
D1568	S1568	586	GfcAfgUfgUfuUfUfCfaCfcucAfuAfuGfL96	AS1568	1678	cAfuAfuGfAfGfgUfgaaAfaCfaCfuGfcsUfsu					
D1569	S1569	587	GfcAfgUfgUfuUfUfCfaCfcUfCfAfuAfuGfL96	AS1569	1679	cAfuAfugaGfgUfgaaAfaCfaCfuGfcsUfsu					
D1570	S1570	588	CfaGfuGfuUfuUfCfAfcCfucaUfaUfgCfL96	AS1570	1680	gCfaUfaUfGfAfgGfugaAfaAfcAfcUfgsCfsu					
D1571	S1571	589	CfaGfuGfuUfuUfCfAfcCfuCfAfUfaUfgCfL96	AS1571	1681	gCfaUfaugAfgGfugaAfaAfcAfcUfgsCfsu					
D1572	S1572	590	AfgUfgUfuUfuCfAfCfCfUfcauAfuGfcUfL96	AS1572	1682	aGfcAfuAfUfGfaGfgugAfaAfaCfaCfusGfsc					
D1573	S1573	591	AfgUfgUfuUfuCfAfCfCfUfcAfUfAfuGfcUfL96	AS1573	1683	aGfcAfuauGfaGfgugAfaAfaCfaCfusGfsc					
D1574	S1574	592	GfuGfuUfuUfcAfCfCfuCfauaUfgCfuAfL96	AS1574	1684	uAfgCfaUfAfUfgAfgguGfaAfaAfcAfcsUfsg					
D1575	S1575	593	GfuGfuUfuUfcAfCfCfuCfaUfAfUfgCfuAfL96	AS1575	1685	uAfgCfauaUfgAfgguGfaAfaAfcAfcsUfsg					
D1576	S1576	594	UfgUfuUfuCfaCfCfUfcAfuauGfcUfaUfL96	AS1576	1686	aUfaGfcAfUfAfuGfaggUfgAfaAfaCfasCfsu					
D1577	S1577	595	UfgUfuUfuCfaCfCfUfcAfuAfUfGfcUfaUfL96	AS1577	1687	aUfaGfcauAfuGfaggUfgAfaAfaCfasCfsu					
D1578	S1578	596	GfuUfuUfcAfcCfUfCfaUfaugCfuAfuGfL96	AS1578	1688	cAfuAfgCfAfUfaUfgagGfuGfaAfaAfcsAfsc					
D1579	S1579	597	GfuUfuUfcAfcCfUfCfaUfaUfGfCfuAfuGfL96	AS1579	1689	cAfuAfgcaUfaUfgagGfuGfaAfaAfcsAfsc					
D1580	S1580	598	UfuUfuCfaCfcUfCfAfuAfugcUfaUfgUfL96	AS1580	1690	aCfaUfaGfCfAfuAfugaGfgUfgAfaAfasCfsa					
D1581	S1581	599	UfuUfuCfaCfcUfCfAfuAfuGfCfUfaUfgUfL96	AS1581	1691	aCfaUfagcAfuAfugaGfgUfgAfaAfasCfsa					
D1582	S1582	600	UfuUfcAfcCfuCfAfUfaUfgcuAfuGfuUfL96	AS1582	1692	aAfcAfuAfGfCfaUfaugAfgGfuGfaAfasAfsc					
D1583	S1583	601	UfuUfcAfcCfuCfAfUfaUfgCfUfAfuGfuUfL96	AS1583	1693	aAfcAfuagCfaUfaugAfgGfuGfaAfasAfsc					
D1584	S1584	602	UfuCfaCfcUfcAfUfAfuGfcuaUfgUfuAfL96	AS1584	1694	uAfaCfaUfAfGfcAfuauGfaGfgUfgAfasAfsa					
D1585	S1585	603	UfuCfaCfcUfcAfUfAfuGfcUfAfUfgUfuAfL96	AS1585	1695	uAfaCfauaGfcAfuauGfaGfgUfgAfasAfsa					
D1586	S1586	604	UfcAfcCfuCfaUfAfUfgCfuauGfuUfaGfL96	AS1586	1696	cUfaAfcAfUfAfgCfauaUfgAfgGfuGfasAfsa					
D1587	S1587	605	UfcAfcCfuCfaUfAfUfgCfuAfUfGfuUfaGfL96	AS1587	1697	cUfaAfcauAfgCfauaUfgAfgGfuGfasAfsa					

D1588	S1588	606	CfaCfcUfcAfuAfUfGfcUfaugUfuAfgAfL96	AS1588	1698	uCfuAfaCfAfUfaGfcauAfuGfaGfgUfgsAfsa				
D1589	S1589	607	CfaCfcUfcAfuAfUfGfcUfaUfGfUfuAfgAfL96	AS1589	1699	uCfuAfacaUfaGfcauAfuGfaGfgUfgsAfsa				
D1590	S1590	608	AfcCfuCfaUfaUfGfCfuAfuguUfaGfaAfL96	AS1590	1700	uUfcUfaAfCfAfuAfgcaUfaUfgAfgGfusGfsa				
D1591	S1591	609	AfcCfuCfaUfaUfGfCfuAfuGfUfUfaGfaAfL96	AS1591	1701	uUfcUfaacAfuAfgcaUfaUfgAfgGfusGfsa				
D1592	S1592	610	CfcUfcAfuAfuGfCfUfaUfguuAfgAfaGfL96	AS1592	1702	cUfuCfuAfAfCfaUfagcAfuAfuGfaGfgsUfsg				
D1593	S1593	611	CfcUfcAfuAfuGfCfUfaUfgUfUfAfgAfaGfL96	AS1593	1703	cUfuCfuaaCfaUfagcAfuAfuGfaGfgsUfsg				
D1594	S1594	612	CfuCfaUfaUfgCfUfAfuGfuuaGfaAfgUfL96	AS1594	1704	aCfuUfcUfAfAfcAfcAfuagCfaUfaUfgAfgsGfsu				
D1595	S1595	613	CfuCfaUfaUfgCfUfAfuGfuUfAfGfaAfgUfL96	AS1595	1705	aCfuUfcuaAfcAfuagCfaUfaUfgAfgsGfsu				
D1596	S1596	614	UfcAfuAfuGfcUfAfUfgUfuagAfaGfuCfL96	AS1596	1706	gAfcUfuCfUfAfaCfauaGfcAfuAfuGfasGfsg				
D1597	S1597	615	UfcAfuAfuGfcUfAfUfgUfuAfGfAfaGfuCfL96	AS1597	1707	gAfcUfucuAfaCfauaGfcAfuAfuGfasGfsg				
D1598	S1598	616	CfaUfaUfgCfuAfUfGfuUfagaAfgUfcCfL96	AS1598	1708	gGfaCfuUfCfUfaAfcauAfgCfaUfaUfgsAfsg				
D1599	S1599	617	CfaUfaUfgCfuAfUfGfuUfaGfAfAfgUfcCfL96	AS1599	1709	gGfaCfuucUfaAfcauAfgCfaUfaUfgsAfsg				
D1600	S1600	618	AfuAfuGfcUfaUfGfUfuAfgaaGfuCfcAfL96	AS1600	1710	uGfgAfcUfUfCfuAfacaUfaGfcAfuAfusGfsa				
D1601	S1601	619	AfuAfuGfcUfaUfGfUfuAfgAfAfGfuCfcAfL96	AS1601	1711	uGfgAfcuuCfuAfacaUfaGfcAfuAfusGfsa				
D1602	S1602	620	UfaUfgCfuAfuGfUfUfaGfaagUfcCfaGfL96	AS1602	1712	cUfgGfaCfUfUfcUfaacAfuAfgCfaUfasUfsg				
D1603	S1603	621	UfaUfgCfuAfuGfUfUfaGfaAfGfUfcCfaGfL96	AS1603	1713	cUfgGfacuUfcUfaacAfuAfgCfaUfasUfsg				
D1604	S1604	622	AfuGfcUfaUfgUfUfAfgAfaguCfcAfgGfL96	AS1604	1714	cCfuGfgAfCfUfuCfuaaCfaUfaGfcAfusAfsu				
D1605	S1605	623	AfuGfcUfaUfgUfUfAfgAfaGfUfCfcAfgGfL96	AS1605	1715	cCfuGfgacUfuCfuaaCfaUfaGfcAfusAfsu				
D1606	S1606	624	UfgCfuAfuGfuUfAfGfaAfgucCfaGfgCfL96	AS1606	1716	gCfcUfgGfAfCfuUfcuaAfcAfuAfgCfasUfsa				
D1607	S1607	625	UfgCfuAfuGfuUfAfGfaAfgUfCfCfaGfgCfL96	AS1607	1717	gCfcUfggaCfuUfcuaAfcAfuAfgCfasUfsa				
D1608	S1608	626	GfcUfaUfgUfuAfGfAfaGfuccAfgGfcAfL96	AS1608	1718	uGfcCfuGfGfAfcUfucuAfaCfaUfaGfcsAfsu				
D1609	S1609	627	GfcUfaUfgUfuAfGfAfaGfuCfCfAfgGfcAfL96	AS1609	1719	uGfcCfuggAfcUfucuAfaCfaUfaGfcsAfsu				
D1610	S1610	628	CfuAfuGfuUfaGfAfAfgUfccaGfgCfaGfL96	AS1610	1720	cUfgCfcUfGfGfaCfuucUfaAfcAfuAfgsCfsa				
D1611	S1611	629	CfuAfuGfuUfaGfAfAfgUfcCfAfGfgCfaGfL96	AS1611	1721	cUfgCfcugGfaCfuucUfaAfcAfuAfgsCfsa				
D1612	S1612	630	UfaUfgUfuAfgAfAfGfuCfcagGfcAfgAfL96	AS1612	1722	uCfuGfcCfUfGfgAfcuuCfuAfaCfaUfasGfsc				
D1613	S1613	631	UfaUfgUfuAfgAfAfGfuCfcAfGfGfcAfgAfL96	AS1613	1723	uCfuGfccuGfgAfcuuCfuAfaCfaUfasGfsc				
D1614	S1614	632	AfuGfuUfaGfaAfGfUfCfCfaggCfaGfaGfL96	AS1614	1724	cUfcUfgCfCfUfgGfacuUfcUfaAfcAfusAfsg				

D1615	S1615	633	AfuGfuUfaGfaAfGfUfcCfaGfGfCfaGfaGfL96	AS1615	1725	cUfcUfgccUfgGfacuUfcUfaAfcAfusAfsg				
D1616	S1616	634	UfgUfuAfgAfaGfUfCfcAfggcAfgAfgAfL96	AS1616	1726	uCfuCfuGfCfCfuGfgacUfuCfuAfaCfasUfsa				
D1617	S1617	635	UfgUfuAfgAfaGfUfCfcAfgGfCfAfgAfgAfL96	AS1617	1727	uCfuCfugcCfuGfgacUfuCfuAfaCfasUfsa				
D1618	S1618	636	GfuUfaGfaAfgUfCfCfaGfgcaGfaGfaCfL96	AS1618	1728	gUfcUfcUfGfCfcUfggaCfuUfcUfaAfcsAfsu				
D1619	S1619	637	GfuUfaGfaAfgUfCfCfaGfgCfAfGfaGfaCfL96	AS1619	1729	gUfcUfcugCfcUfggaCfuUfcUfaAfcsAfsu				
D1620	S1620	638	UfuAfgAfaGfuCfCfAfgGfcagAfgAfcAfL96	AS1620	1730	uGfuCfuCfUfGfcCfuggAfcUfuCfuAfasCfsa				
D1621	S1621	639	UfuAfgAfaGfuCfCfAfgGfcAfGfAfgAfcAfL96	AS1621	1731	uGfuCfucuGfcCfuggAfcUfuCfuAfasCfsa				
D1622	S1622	640	UfaGfaAfgUfcCfAfGfgCfagaGfaCfaAfL96	AS1622	1732	uUfgUfcUfCfUfgCfcugGfaCfuUfcUfasAfsc				
D1623	S1623	641	UfaGfaAfgUfcCfAfGfgCfaGfAfGfaCfaAfL96	AS1623	1733	uUfgUfcucUfgCfcugGfaCfuUfcUfasAfsc				
D1624	S1624	642	AfgAfaGfuCfcAfGfGfcAfgagAfcAfaUfL96	AS1624	1734	aUfuGfuCfUfCfuGfccuGfgAfcUfuCfusAfsa				
D1625	S1625	643	AfgAfaGfuCfcAfGfGfcAfgAfGfAfcAfaUfL96	AS1625	1735	aUfuGfucuCfuGfccuGfgAfcUfuCfusAfsa				
D1626	S1626	644	GfaAfgUfcCfaGfGfCfaGfagaCfaAfuAfL96	AS1626	1736	uAfuUfgUfCfUfcUfgccUfgGfaCfuUfcsUfsa				
D1627	S1627	645	GfaAfgUfcCfaGfGfCfaGfaGfAfCfaAfuAfL96	AS1627	1737	uAfuUfgucUfcUfgccUfgGfaCfuUfcsUfsa				
D1628	S1628	646	AfaGfuCfcAfgGfCfAfgAfgacAfaUfaAfL96	AS1628	1738	uUfaUfuGfUfCfuCfugccUfgGfgAfcUfusCfsu				
D1629	S1629	647	AfaGfuCfcAfgGfCfAfgAfgAfCfAfaUfaAfL96	AS1629	1739	uUfaUfuguCfuCfugccUfgGfgAfcUfusCfsu				
D1630	S1630	648	AfgUfcCfaGfgCfAfGfaGfacaAfuAfaAfL96	AS1630	1740	uUfuAfuUfGfUfcUfcugCfcUfgGfaCfusUfsc				
D1631	S1631	649	AfgUfcCfaGfgCfAfGfaGfaCfAfAfuAfaAfL96	AS1631	1741	uUfuAfuugUfCfUfcugCfcUfgGfaCfusUfsc				
D1632	S1632	650	GfuCfcAfgGfcAfGfAfgAfcaaUfaAfaAfL96	AS1632	1742	uUfuUfaUfUfGfuCfucuGfcCfuGfgAfcsUfsu				
D1633	S1633	651	GfuCfcAfgGfcAfGfAfgAfcAfAfUfaAfaAfL96	AS1633	1743	uUfuUfauuGfuCfucuGfcCfuGfgAfcsUfsu				
D1634	S1634	652	UfcCfaGfgCfaGfAfGfaCfaauAfaAfaCfL96	AS1634	1744	gUfuUfuAfUfUfgUfcucUfgCfcUfgGfasCfsu				
D1635	S1635	653	UfcCfaGfgCfaGfAfGfaCfaAfUfAfaAfaCfL96	AS1635	1745	gUfuUfuauUfgUfcucUfgCfcUfgGfasCfsu				
D1636	S1636	654	CfcAfgGfcAfgAfGfAfcAfauaAfaAfcAfL96	AS1636	1746	uGfuUfuUfAfUfuGfucuCfuGfcCfuGfgsAfsc				
D1637	S1637	655	CfcAfgGfcAfgAfGfAfcAfaUfAfAfaAfcAfL96	AS1637	1747	uGfuUfuuaUfuGfucuCfuGfcCfuGfgsAfsc				
D1638	S1638	656	CfaGfgCfaGfaGfAfCfaAfuaaAfaCfaUfL96	AS1638	1748	aUfgUfuUfUfAfuUfgucUfcUfgCfcUfgsGfsa				
D1639	S1639	657	CfaGfgCfaGfaGfAfCfaAfuAfAfAfaCfaUfL96	AS1639	1749	aUfgUfuuuAfuUfgucUfcUfgCfcUfgsGfsa				
D1640	S1640	658	AfgGfcAfgAfgAfCfAfaUfaaaAfcAfuUfL96	AS1640	1750	aAfuGfuUfUfAfuUfuguCfuCfuGfcCfusGfsg				
D1641	S1641	659	AfgGfcAfgAfgAfCfAfaUfaAfAfAfcAfuUfL96	AS1641	1751	aAfuGfuuuUfaUfuguCfuCfuGfcCfusGfsg				

D1642	S1642	660	GfgCfaGfaGfaGfaCfAfAfuAfaaaCfaUfuCfL96	AS1642	1752	gAfaUfgUfUfUfuAfuugUfcUfcUfgCfcsUfsg					
D1643	S1643	661	GfgCfaGfaGfaCfAfAfuAfaAfAfCfaUfuCfL96	AS1643	1753	gAfaUfguuUfuAfuugUfcUfcUfgCfcsUfsg					
D1644	S1644	662	GfcAfgAfgAfcAfAfUfaAfaacAfuUfcCfL96	AS1644	1754	gGfaAfuGfUfUfuUfauuGfuCfuCfuGfcsCfsu					
D1645	S1645	663	GfcAfgAfgAfcAfAfUfaAfaAfCfAfuUfccCfL96	AS1645	1755	gGfaAfuguUfuUfauuGfuCfuCfuGfcsCfsu					
D1646	S1646	664	CfaGfaGfaCfaAfUfAfaAfacaUfuCfcUfL96	AS1646	1756	aGfgAfaUfGfUfuUfuauUfgUfcUfcUfgsCfsc					
D1647	S1647	665	CfaGfaGfaCfaAfUfAfaAfaCfAfUfuCfcUfL96	AS1647	1757	aGfgAfaugUfuUfuauUfgUfcUfcUfgsCfsc					
D1648	S1648	666	AfgAfgAfcAfaUfAfAfaAfcauUfcCfuGfL96	AS1648	1758	cAfgGfaAfUfGfuUfuuaUfuGfuCfuCfusGfsc					
D1649	S1649	667	AfgAfgAfcAfaUfAfAfaAfcAfUfUfcCfuGfL96	AS1649	1759	cAfgGfaauGfuUfuuaUfuGfuCfuCfusGfsc					
D1650	S1650	668	GfaGfaCfaAfuAfAfAfAfaCfauuCfcUfgUfL96	AS1650	1760	aCfaGfgAfAfUfgUfuuuAfuUfgUfcUfcsUfsg					
D1651	S1651	669	GfaGfaCfaAfuAfAfAfAfaCfaUfUfCfcUfgUfL96	AS1651	1761	aCfaGfgaaUfgUfuuuAfuUfgUfcUfcsUfsg					
D1652	S1652	670	AfgAfcAfaUfaAfAfAfAfcAfuucCfuGfuGfL96	AS1652	1762	cAfcAfgGfAfAfuGfuuuUfaUfuGfuCfusCfsu					
D1653	S1653	671	AfgAfcAfaUfaAfAfAfAfcAfuUfCfCfuGfuGfL96	AS1653	1763	cAfcAfggaAfuGfuuuUfaUfuGfuCfusCfsu					
D1654	S1654	672	GfaCfaAfuAfaAfAfCfaUfuccUfgUfgAfL96	AS1654	1764	uCfaCfaGfGfAfaUfguuUfuAfuUfgUfcsUfsc					
D1655	S1655	673	GfaCfaAfuAfaAfAfCfaUfuCfCfUfgUfgAfL96	AS1655	1765	uCfaCfaggAfaUfguuUfuAfuUfgUfcsUfsc					
D1656	S1656	674	AfcAfaUfaAfaAfCfAfuUfccuGfuGfaAfL96	AS1656	1766	uUfcAfcAfGfGfaAfuguUfuUfaUfuGfusCfsu					
D1657	S1657	675	AfcAfaUfaAfaAfCfAfuUfcCfUfGfuGfaAfL96	AS1657	1767	uUfcAfcagGfaAfuguUfuUfaUfuGfusCfsu					
D1658	S1658	676	CfaAfuAfaAfaCfAfUfuCfcugUfgAfaAfL96	AS1658	1768	uUfuCfaCfAfGfgAfaugUfuUfuAfuUfgsUfsc					
D1659	S1659	677	CfaAfuAfaAfaCfAfUfuCfcUfGfUfgAfaAfL96	AS1659	1769	uUfuCfacaGfgAfaugUfuUfuAfuUfgsUfsc					
D1660	S1660	678	AfaUfaAfaAfcAfUfUfcCfuguGfaAfaGfL96	AS1660	1770	cUfuUfcAfCfAfgGfaauGfuUfuUfaUfusGfsu					
D1661	S1661	679	AfaUfaAfaAfcAfUfUfcCfuGfUfGfaAfaGfL96	AS1661	1771	cUfuUfcacAfgGfaauGfuUfuUfaUfusGfsu					
D1662	S1662	680	AfuAfaAfaCfaUfUfCfCfUfgugAfaAfgGfL96	AS1662	1772	cCfuUfuCfAfCfaGfgaaUfgUfuUfuAfusUfsg					
D1663	S1663	681	AfuAfaAfaCfaUfUfCfCfUfgUfGfAfaAfgGfL96	AS1663	1773	cCfuUfucaCfaGfgaaUfgUfuUfuAfusUfsg					
D1664	S1664	682	UfaAfaAfcAfuUfCfCfCfuGfugAfaAfgGfCfL96	AS1664	1774	gCfcUfuUfCfAfcAfggaAfuGfuUfuUfasUfsu					
D1665	S1665	683	UfaAfaAfcAfuUfCfCfCfuGfuGfAfAfaGfgCfL96	AS1665	1775	gCfcUfuucAfcAfggaAfuGfuUfuUfasUfsu					
D1666	S1666	684	AfaAfaCfaCfaUfuCfCfCfUfgUfgaaAfgGfcAfL96	AS1666	1776	uGfcCfuUfUfCfaCfaggAfaUfgUfuUfusAfsu					
D1667	S1667	685	AfaAfaCfaUfuCfCfCfUfgUfgAfAfAfgGfcAfL96	AS1667	1777	uGfcCfuuuCfaCfaggAfaUfgUfuUfusAfsu					
D1668	S1668	686	AfaAfcAfuUfCfCfUfGfuGfaaaGfgCfaCfL96	AS1668	1778	gUfgCfcUfUfUfcAfcagGfaAfuGfuUfusUfsa					

D1669	S1669	687	AfaAfcAfuUfcCfUfGfuGfaAfAfGfgCfaCfL96	AS1669	1779	gUfgCfcuuUfcAfcagGfaAfuGfuUfusUfsa					
D1670	S1670	688	AfaCfaUfuCfcUfGfUfgAfaagGfcAfcUfL96	AS1670	1780	aGfuGfcCfUfUfuCfacaGfgAfaUfgUfusUfsu					
D1671	S1671	689	AfaCfaUfuCfcUfGfUfgAfaAfGfGfcAfcUfL96	AS1671	1781	aGfuGfccuUfuCfacaGfgAfaUfgUfusUfsu					
D1672	S1672	690	AfcAfuUfcCfuGfUfGfaAfaggCfaCfuUfL96	AS1672	1782	aAfgUfgCfCfUfuUfcacAfgGfaAfuGfusUfsu					
D1673	S1673	691	AfcAfuUfcCfuGfUfGfaAfaGfGfCfaCfuUfL96	AS1673	1783	aAfgUfgccUfuUfcacAfgGfaAfuGfusUfsu					
D1674	S1674	692	CfaUfuCfcUfgUfGfAfaAfggcAfcUfuUfL96	AS1674	1784	aAfaGfuGfCfCfuUfucaCfaGfgAfaUfgsUfsu					
D1675	S1675	693	CfaUfuCfcUfgUfGfAfaAfgGfCfAfcUfuUfL96	AS1675	1785	aAfaGfugcCfuUfucaCfaGfgAfaUfgsUfsu					
D1676	S1676	694	AfuUfcCfuGfuGfAfAfaGfgcaCfuUfuUfL96	AS1676	1786	aAfaAfgUfGfCfCfUfuucAfcAfgGfaAfusGfsu					
D1677	S1677	695	AfuUfcCfuGfuGfAfAfaGfgCfAfCfuUfuUfL96	AS1677	1787	aAfaAfgugCfcUfuucAfcAfgGfaAfusGfsu					
D1678	S1678	696	UfuCfcUfgUfgAfAfAfgGfcacUfuUfuCfL96	AS1678	1788	gAfaAfaGfUfGfcCfuuuCfaCfaGfgAfasUfsg					
D1679	S1679	697	UfuCfcUfgUfgAfAfAfgGfcAfCfUfuUfuCfL96	AS1679	1789	gAfaAfaguGfcCfuuuCfaCfaGfgAfasUfsg					
D1680	S1680	698	UfcCfuGfuGfaAfAfGfgCfacuUfuUfcAfL96	AS1680	1790	uGfaAfaAfGfUfgCfcuuUfcAfcAfgGfasAfsu					
D1681	S1681	699	UfcCfuGfuGfaAfAfGfgCfaCfUfUfuUfcAfL96	AS1681	1791	uGfaAfaagUfgCfcuuUfcAfcAfgGfasAfsu					
D1682	S1682	700	CfcUfgUfgAfaAfGfGfcAfcuuUfuCfaUfL96	AS1682	1792	aUfgAfaAfAfGfuGfccuUfuCfaCfaGfgsAfsa					
D1683	S1683	701	CfcUfgUfgAfaAfGfGfcAfcUfUfUfuCfaUfL96	AS1683	1793	aUfgAfaaaGfuGfccuUfuCfaCfaGfgsAfsa					
D1684	S1684	702	CfuGfuGfaAfaGfGfCfaCfuuuUfcAfuUfL96	AS1684	1794	aAfuGfaAfAfAfAfgUfgccUfuUfcAfcAfgsGfsa					
D1685	S1685	703	CfuGfuGfaAfaGfGfCfaCfuUfUfUfcAfuUfL96	AS1685	1795	aAfuGfaaaAfgUfgccUfuUfcAfcAfgsGfsa					
D1686	S1686	704	UfgUfgAfaAfgGfCfAfcUfuuuCfaUfuCfL96	AS1686	1796	gAfaUfgAfAfAfaGfugccCfuUfuCfaCfasGfsg					
D1687	S1687	705	UfgUfgAfaAfgGfCfAfcUfuUfUfCfaUfuCfL96	AS1687	1797	gAfaUfgaaAfaGfugccCfuUfuCfaCfasGfsg					
D1688	S1688	706	GfuGfaAfaGfgCfAfCfuUfuucAfuUfcCfL96	AS1688	1798	gGfaAfuGfAfAfaAfgugCfcUfuUfcAfcAfsg					
D1689	S1689	707	GfuGfaAfaGfgCfAfCfuUfuUfCfAfuUfcCfL96	AS1689	1799	gGfaAfugaAfaAfgugCfcUfuUfcAfcAfsg					
D1690	S1690	708	UfgAfaAfgGfcAfCfUfuUfucaUfuCfcAfL96	AS1690	1800	uGfgAfaUfGfAfaAfaguGfcCfuUfuCfasCfsa					
D1691	S1691	709	UfgAfaAfgGfcAfCfUfuUfuCfAfUfuCfcAfL96	AS1691	1801	uGfgAfaugAfaAfaguGfcCfuUfuCfasCfsa					
D1692	S1692	710	GfaAfaGfgCfaCfUfUfUfcauUfcCfaCfL96	AS1692	1802	gUfgGfaAfUfGfaAfaagUfgCfcUfuUfcsAfsc					
D1693	S1693	711	GfaAfaGfgCfaCfUfUfUfcAfUfUfcCfaCfL96	AS1693	1803	gUfgGfaauGfaAfaagUfgCfcUfuUfcsAfsc					
D1694	S1694	712	AfaAfgGfcAfcUfUfUfcCfauuCfcAfcUfL96	AS1694	1804	aGfuGfgAfAfUfgAfaaaGfuGfcCfuUfusCfsa					
D1695	S1695	713	AfaAfgGfcAfcUfUfUfcCfaUfUfCfcAfcUfL96	AS1695	1805	aGfuGfgaaUfgAfaaaGfuGfcCfuUfusCfsa					

D1696	S1696	714	AfaGfgCfaCfuUfUfUfcAfuucCfaCfuUfL96	AS1696	1806	aAfgUfgGfAfAfuGfaaaAfgUfgCfcUfusUfsc				
D1697	S1697	715	AfaGfgCfaCfuUfUfUfcAfuUfCfCfaCfuUfL96	AS1697	1807	aAfgUfggaAfuGfaaaAfgUfgCfcUfusUfsc				
D1698	S1698	716	AfgGfcAfcUfuUfUfUfCfaUfuccAfcUfuUfL96	AS1698	1808	aAfaGfuGfGfAfaUfgaaAfaGfuGfcCfusUfsu				
D1699	S1699	717	AfgGfcAfcUfuUfUfUfCfaUfuCfCfAfcUfuUfL96	AS1699	1809	aAfaGfuggAfaUfgaaAfaGfuGfcCfusUfsu				
D1700	S1700	718	GfgCfaCfuUfuUfUfCfAfuUfccaCfuUfuAfL96	AS1700	1810	uAfaAfgUfGfGfaAfugaAfaAfgUfgCfcsUfsu				
D1701	S1701	719	GfgCfaCfuUfuUfUfCfAfuUfcCfAfCfuUfuAfL96	AS1701	1811	uAfaAfgugGfaAfugaAfaAfgUfgCfcsUfsu				
D1702	S1702	720	GfcAfcUfuUfuCfAfUfuCfcacUfuUfaAfL96	AS1702	1812	uUfaAfaGfUfGfgAfaugAfaAfaGfuGfcsCfsu				
D1703	S1703	721	GfcAfcUfuUfuCfAfUfuCfcAfCfUfuUfaAfL96	AS1703	1813	uUfaAfaguGfgAfaugAfaAfaGfuGfcsCfsu				
D1704	S1704	722	CfaCfuUfuUfcAfUfUfcCfacuUfuAfaCfL96	AS1704	1814	gUfuAfaAfGfUfgGfaauGfaAfaAfgUfgsCfsc				
D1705	S1705	723	CfaCfuUfuUfcAfUfUfcCfaCfUfUfuAfaCfL96	AS1705	1815	gUfuAfaagUfgGfaauGfaAfaAfgUfgsCfsc				
D1706	S1706	724	AfcUfuUfuCfaUfUfCfcAfcuuUfaAfcUfL96	AS1706	1816	aGfuUfaAfAfGfuGfgaaUfgAfaAfaGfusGfsc				
D1707	S1707	725	AfcUfuUfuCfaUfUfCfcAfcUfUfUfaAfcUfL96	AS1707	1817	aGfuUfaaaGfuGfgaaUfgAfaAfaGfusGfsc				
D1708	S1708	726	CfuUfuUfcAfuUfCfCfaCfuuuAfaCfuUfL96	AS1708	1818	aAfgUfuAfAfAfgUfggaAfuGfaAfaAfgsUfsg				
D1709	S1709	727	CfuUfuUfcAfuUfCfCfaCfuUfUfAfaCfuUfL96	AS1709	1819	aAfgUfuaaAfgUfggaAfuGfaAfaAfgsUfsg				
D1710	S1710	728	UfuUfuCfaUfuCfCfAfcUfuuaAfcUfuGfL96	AS1710	1820	cAfaGfuUfAfAfaGfuggAfaUfgAfaAfasGfsu				
D1711	S1711	729	UfuUfuCfaUfuCfCfAfcUfuUfAfAfcUfuGfL96	AS1711	1821	cAfaGfuuaAfaGfuggAfaUfgAfaAfasGfsu				
D1712	S1712	730	UfuUfcAfuUfcCfAfCfuUfuaaCfuUfgAfL96	AS1712	1822	uCfaAfgUfUfAfaAfgugGfaAfuGfaAfasAfsg				
D1713	S1713	731	UfuUfcAfuUfcCfAfCfuUfuAfAfCfuUfgAfL96	AS1713	1823	uCfaAfguuAfaAfgugGfaAfuGfaAfasAfsg				
D1714	S1714	732	UfuCfaUfuCfcAfCfUfuUfaacUfuGfaUfL96	AS1714	1824	aUfcAfaGfUfUfaAfaguGfgAfaUfgAfasAfsa				
D1715	S1715	733	UfuCfaUfuCfcAfCfUfuUfaAfCfUfuGfaUfL96	AS1715	1825	aUfcAfaguUfaAfaguGfgAfaUfgAfasAfsa				
D1716	S1716	734	UfcAfuUfcCfaCfUfUfuAfacuUfgAfuUfL96	AS1716	1826	aAfuCfaAfGfUfuAfaagUfgGfaAfuGfasAfsa				
D1717	S1717	735	UfcAfuUfcCfaCfUfUfuAfaCfUfUfgAfuUfL96	AS1717	1827	aAfuCfaagUfuAfaagUfgGfaAfuGfasAfsa				
D1718	S1718	736	CfaUfuCfcAfcUfUfUfaAfcuuGfaUfuUfL96	AS1718	1828	aAfaUfcAfAfGfuUfaaaGfuGfgAfaUfgsAfsa				
D1719	S1719	737	CfaUfuCfcAfcUfUfUfaAfcUfUfGfaUfuUfL96	AS1719	1829	aAfaUfcaaGfuUfaaaGfuGfgAfaUfgsAfsa				
D1720	S1720	738	AfuUfcCfaCfuUfUfAfaCfuugAfuUfuUfL96	AS1720	1830	aAfaAfuCfAfAfgUfuaaAfgUfgGfaAfusGfsa				
D1721	S1721	739	AfuUfcCfaCfuUfUfAfaCfuUfGfAfuUfuUfL96	AS1721	1831	aAfaAfucaAfgUfuaaAfgUfgGfaAfusGfsa				
D1722	S1722	740	UfuCfcAfcUfuUfAfAfcUfugaUfuUfuUfL96	AS1722	1832	aAfaAfaUfCfAfaGfuuaAfaGfuGfgAfasUfsg				

D1723	S1723	741	UfuCfcAfcUfuUfAfAfcUfuGfAfUfuUfuUfL96	AS1723	1833	aAfaAfaucAfaGfuuaAfaGfuGfgAfasUfsg					
D1724	S1724	742	UfcCfaCfuUfuAfAfAfCfuUfgauUfuUfuUfL96	AS1724	1834	aAfaAfaAfUfCfaAfguuAfaAfgUfgGfasAfsu					
D1725	S1725	743	UfcCfaCfuUfuAfAfAfCfuUfgAfUfUfuUfuUfL96	AS1725	1835	aAfaAfaauCfaAfguuAfaAfgUfgGfasAfsu					
D1726	S1726	744	CfcAfcUfuUfaAfCfUfuGfauuUfuUfuAfL96	AS1726	1836	uAfaAfaAfAfUfcAfaguUfaAfaGfuGfgsAfsa					
D1727	S1727	745	CfcAfcUfuUfaAfCfUfuGfaUfUfUfuUfuAfL96	AS1727	1837	uAfaAfaaaUfcAfaguUfaAfaGfuGfgsAfsa					
D1728	S1728	746	CfaCfuUfuAfaCfUfUfgAfuuuUfuUfaAfL96	AS1728	1838	uUfaAfaAfAfAfUfuCfaagUfuAfaAfgUfgsGfsa					
D1729	S1729	747	CfaCfuUfuAfaCfUfUfgAfuUfUfUfuUfaAfL96	AS1729	1839	uUfaAfaaaAfuCfaagUfuAfaAfgUfgsGfsa					
D1730	S1730	748	AfcUfuUfaAfcUfUfGfaUfuuuUfuAfaAfL96	AS1730	1840	uUfuAfaAfAfAfaUfcaaGfuUfaAfaGfusGfsg					
D1731	S1731	749	AfcUfuUfaAfcUfUfGfaUfuUfUfUfuAfaAfL96	AS1731	1841	uUfuAfaaaAfaUfcaaGfuUfaAfaGfusGfsg					
D1732	S1732	750	CfuUfuAfaCfuUfGfAfuUfuuuUfaAfaUfL96	AS1732	1842	aUfuUfaAfAfAfaAfucaAfgUfuAfaAfgsUfsg					
D1733	S1733	751	CfuUfuAfaCfuUfGfAfuUfuUfUfUfaAfaUfL96	AS1733	1843	aUfuUfaaaAfaAfucaAfgUfuAfaAfgsUfsg					
D1734	S1734	752	UfuUfaAfcUfuGfAfUfuUfuuuAfaAfuUfL96	AS1734	1844	aAfuUfuAfAfAfaAfaucAfaGfuUfaAfasGfsu					
D1735	S1735	753	UfuUfaAfcUfuGfAfUfuUfUfAfaAfuUfL96	AS1735	1845	aAfuUfuaaAfaAfaucAfaGfuUfaAfasGfsu					
D1736	S1736	754	UfuAfaCfuUfgAfUfUfuUfuuaAfaUfuCfL96	AS1736	1846	gAfaUfuUfAfAfaAfaauCfaAfgUfuAfasAfsg					
D1737	S1737	755	UfuAfaCfuUfgAfUfUfuUfUfAfAfaUfuCfL96	AS1737	1847	gAfaUfuuaAfaAfaauCfaAfgUfuAfasAfsg					
D1738	S1738	756	UfaAfcUfuGfaUfUfUfuUfuaaAfuUfcCfL96	AS1738	1848	gGfaAfuUfUfAfaAfaaaUfcAfaGfuUfasAfsa					
D1739	S1739	757	UfaAfcUfuGfaUfUfUfuUfuAfAfAfuUfcCfL96	AS1739	1849	gGfaAfuuuAfaAfaaaUfcAfaGfuUfasAfsa					
D1740	S1740	758	AfaCfuUfgAfuUfUfUfuUfaaaUfuCfcCfL96	AS1740	1850	gGfgAfaUfUfUfaAfaaaAfuCfaAfgUfusAfsa					
D1741	S1741	759	AfaCfuUfgAfuUfUfUfuUfaAfAfUfuCfcCfL96	AS1741	1851	gGfgAfauuUfaAfaaaAfuCfaAfgUfusAfsa					
D1742	S1742	760	AfcUfuGfaUfuUfUfUfuAfaauUfcCfcUfL96	AS1742	1852	aGfgGfaAfUfUfuAfaaaAfaUfcAfaGfusUfsa					
D1743	S1743	761	AfcUfuGfaUfuUfUfUfuAfaAfUfUfcCfcUfL96	AS1743	1853	aGfgGfaauUfuAfaaaAfaUfcAfaGfusUfsa					
D1744	S1744	762	CfuUfgAfuUfuUfUfUfaAfauuCfcCfuUfL96	AS1744	1854	aAfgGfgAfAfUfuUfaaaAfaAfuCfaAfgsUfsu					
D1745	S1745	763	CfuUfgAfuUfuUfUfUfaAfaUfUfCfcCfuUfL96	AS1745	1855	aAfgGfgaaUfuUfaaaAfaAfuCfaAfgsUfsu					
D1746	S1746	764	UfuGfaUfuUfuUfUfAfaAfuuuCfcUfuAfL96	AS1746	1856	uAfaGfgGfAfAfuUfuaaAfaAfaUfcAfasGfsu					
D1747	S1747	765	UfuGfaUfuUfuUfUfAfaAfuUfCfCfcUfuAfL96	AS1747	1857	uAfaGfggaAfuUfuaaAfaAfaUfcAfasGfsu					
D1748	S1748	766	UfgAfuUfuUfuUfAfAfaUfuccCfuUfaUfL96	AS1748	1858	aUfaAfgGfGfAfaUfuuaAfaAfaAfuCfasAfsg					
D1749	S1749	767	UfgAfuUfuUfuUfAfAfaUfuCfCfCfCfuUfaUfL96	AS1749	1859	aUfaAfgggAfaUfuuaAfaAfaAfuCfasAfsg					

D1750	S1750	768	GfaUfuUfuUfuAfAfAfuUfcccUfuAfuUfL96	AS1750	1860	aAfuAfaGfGfGfaAfuuuAfaAfaAfaUfcsAfsa					
D1751	S1751	769	GfaUfuUfuUfuAfAfAfuUfcCfCfUfuAfuUfL96	AS1751	1861	aAfuAfaggGfaAfuuuAfaAfaAfaUfcsAfsa					
D1752	S1752	770	AfuUfuUfuUfaAfAfUfuCfccuUfaUfuGfL96	AS1752	1862	cAfaUfaAfGfGfgAfauuUfaAfaAfaAfusCfsa					
D1753	S1753	771	AfuUfuUfuUfaAfAfUfuCfcCfUfUfaUfuGfL96	AS1753	1863	cAfaUfaagGfgAfauuUfaAfaAfaAfusCfsa					
D1754	S1754	772	UfuUfuUfuAfaAfUfUfCfCfcuuAfuUfgUfL96	AS1754	1864	aCfaAfuAfAfGfgGfaauUfuAfaAfaAfasUfsc					
D1755	S1755	773	UfuUfuUfuAfaAfUfUfCfcCfcUfUfAfuUfgUfL96	AS1755	1865	aCfaAfuaaGfgGfaauUfuAfaAfaAfasUfsc					
D1756	S1756	774	UfuUfuUfaAfaUfUfCfcCfuuaUfuGfuCfL96	AS1756	1866	gAfcAfaUfAfAfgGfgaaUfuUfaAfaAfasAfsu					
D1757	S1757	775	UfuUfuUfaAfaUfUfCfcCfuUfAfUfuGfuCfL96	AS1757	1867	gAfcAfauaAfgGfgaaUfuUfaAfaAfasAfsu					
D1758	S1758	776	UfuUfuAfaAfuUfCfCfcUfuauUfgUfcCfL96	AS1758	1868	gGfaCfaAfUfAfaGfggaAfuUfuAfaAfasAfsa					
D1759	S1759	777	UfuUfuAfaAfuUfCfCfcUfuAfUfUfgUfcCfL96	AS1759	1869	gGfaCfaauAfaGfggaAfuUfuAfaAfasAfsa					
D1760	S1760	778	UfuUfaAfaUfuCfCfCfuUfauuGfuCfcCfL96	AS1760	1870	gGfgAfcAfAfUfuAfgggAfaUfuUfaAfasAfsa					
D1761	S1761	779	UfuUfaAfaUfuCfCfCfuUfaUfUfGfuCfcCfL96	AS1761	1871	gGfgAfcaaUfaAfgggAfaUfuUfaAfasAfsa					
D1762	S1762	780	UfuAfaAfuUfcCfCfUfuAfuugUfcCfcUfL96	AS1762	1872	aGfgGfaCfAfAfuAfaggGfaAfuUfuAfasAfsa					
D1763	S1763	781	UfuAfaAfuUfcCfCfUfuAfuUfGfUfcCfcUfL96	AS1763	1873	aGfgGfacaAfuAfaggGfaAfuUfuAfasAfsa					
D1764	S1764	782	UfaAfaUfuCfcCfUfUfaUfuguCfcCfuUfL96	AS1764	1874	aAfgGfgAfCfAfaUfaagGfgAfaUfuUfasAfsa					
D1765	S1765	783	UfaAfaUfuCfcCfUfUfaUfuGfUfCfcCfuUfL96	AS1765	1875	aAfgGfgacAfaUfaagGfgAfaUfuUfasAfsa					
D1766	S1766	784	AfaAfuUfcCfcUfUfAfuUfgucCfcUfuCfL96	AS1766	1876	gAfaGfgGfAfCfaAfuaaGfgGfaAfuUfusAfsa					
D1767	S1767	785	AfaAfuUfcCfcUfUfAfuUfgUfCfCfcUfuCfL96	AS1767	1877	gAfaGfggaCfaAfuaaGfgGfaAfuUfusAfsa					
D1768	S1768	786	AfaUfuCfcCfuUfAfUfuGfucccCfuUfcCfL96	AS1768	1878	gGfaAfgGfGfAfcAfauaAfgGfgAfaUfusUfsa					
D1769	S1769	787	AfaUfuCfcCfuUfAfUfuGfuCfCfCfuUfcCfL96	AS1769	1879	gGfaAfgggAfcAfauaAfgGfgAfaUfusUfsa					
D1770	S1770	788	AfuUfcCfcUfuAfUfUfgUfcccUfuCfcAfL96	AS1770	1880	uGfgAfaGfGfGfaCfaauAfaGfgGfaAfusUfsu					
D1771	S1771	789	AfuUfcCfcUfuAfUfUfgUfCfCfCfUfuCfcAfL96	AS1771	1881	uGfgAfaggGfaCfaauAfaGfgGfaAfusUfsu					
D1772	S1772	790	UfuCfcCfuUfaUfUfGfuCfccuUfcCfaAfL96	AS1772	1882	uUfgGfaAfGfGfgAfcaaUfaAfgGfgAfasUfsu					
D1773	S1773	791	UfuCfcCfuUfaUfUfGfuCfcCfUfUfcCfaAfL96	AS1773	1883	uUfgGfaagGfgAfcaaUfaAfgGfgAfasUfsu					
D1774	S1774	792	UfcCfcUfuAfuUfGfUfcCfcuuCfcAfaAfL96	AS1774	1884	uUfuGfgAfAfGfgGfacaAfuAfaGfgGfasAfsu					
D1775	S1775	793	UfcCfcUfuAfuUfGfUfcCfcUfUfCfcAfaAfL96	AS1775	1885	uUfuGfgaaGfgGfacaAfuAfaGfgGfasAfsu					
D1776	S1776	794	CfcCfuUfaUfuGfUfCfcCfuucCfaAfaAfL96	AS1776	1886	uUfuUfgGfAfAfgGfgacAfaUfaAfgGfgsAfsa					

D1777	S1777	795	CfcCfuUfaUfuGfUfCfcCfuUfCfCfaAfaAfL96	AS1777	1887	uUfuUfggaAfgGfgacAfaUfaAfgGfgsAfsa					
D1778	S1778	796	CfcUfuAfuUfgUfCfCfcUfuccAfaAfaAfL96	AS1778	1888	uUfuUfuGfGfAfaGfggaCfaAfuAfaGfgsGfsa					
D1779	S1779	797	CfcUfuAfuUfgUfCfCfcUfuCfCfAfaAfaAfL96	AS1779	1889	uUfuUfuggAfaGfggaCfaAfuAfaGfgsGfsa					
D1780	S1780	798	CfuUfaUfuGfuCfCfCfuUfccaAfaAfaAfL96	AS1780	1890	uUfuUfuUfGfGfaAfgggAfcAfaUfaAfgsGfsg					
D1781	S1781	799	CfuUfaUfuGfuCfCfCfuUfCfCfAfaAfaAfL96	AS1781	1891	uUfuUfuugGfaAfgggAfcAfaUfaAfgsGfsg					
D1782	S1782	800	UfuAfuUfgUfcCfCfUfuCfcaaAfaAfaAfL96	AS1782	1892	uUfuUfuUfGfgAfaggGfaCfaAfuAfasGfsg					
D1783	S1783	801	UfuAfuUfgUfcCfCfUfuCfcAfAfAfaAfaAfL96	AS1783	1893	uUfuUfuuuGfgAfaggGfaCfaAfuAfasGfsg					
D1784	S1784	802	UfaUfuGfuCfcCfUfUfCfCfaaaAfaAfaAfL96	AS1784	1894	uUfuUfuUfUfUfgGfaagGfgAfcAfaUfasAfsg					
D1785	S1785	803	UfaUfuGfuCfcCfUfUfCfCfaAfAfAfaAfaAfL96	AS1785	1895	uUfuUfuuuUfgGfaagGfgAfcAfaUfasAfsg					
D1786	S1786	804	AfuUfgUfcCfcUfUfUfCfcAfaaaAfaAfaGfL96	AS1786	1896	cUfuUfuUfUfUfuGfgaaGfgGfaCfaAfusAfsa					
D1787	S1787	805	AfuUfgUfcCfcUfUfUfCfcAfaAfAfAfaAfaGfL96	AS1787	1897	cUfuUfuuuUfuGfgaaGfgGfaCfaAfusAfsa					
D1788	S1788	806	UfuGfuCfcCfuUfCfCfaAfaaaAfaAfgAfL96	AS1788	1898	uCfuUfuUfUfUfUfggaAfgGfgAfcAfasUfsa					
D1789	S1789	807	UfuGfuCfcCfuUfCfCfaAfaAfAfAfaAfgAfL96	AS1789	1899	uCfuUfuuuUfuUfggaAfgGfgAfcAfasUfsa					
D1790	S1790	808	UfgUfcCfcUfuCfCfAfaAfaaaAfaGfaGfL96	AS1790	1900	cUfcUfuUfUfUfUfuggAfaGfgGfaCfasAfsu					
D1791	S1791	809	UfgUfcCfcUfuCfCfAfaAfaAfAfAfaGfaGfL96	AS1791	1901	cUfcUfuuuUfuUfuggAfaGfgGfaCfasAfsu					
D1792	S1792	810	GfuCfcCfuUfcCfAfAfaAfaaaAfgAfgAfL96	AS1792	1902	uCfuCfuUfUfUfUfuugGfaAfgGfgAfcsAfsa					
D1793	S1793	811	GfuCfcCfuUfcCfAfAfaAfaAfAfAfgAfgAfL96	AS1793	1903	uCfuCfuuuUfuUfuugGfaAfgGfgAfcsAfsa					
D1794	S1794	812	UfcCfcUfuCfcAfAfAfaAfaaaGfaGfaAfL96	AS1794	1904	uUfcUfcUfUfUfUfuuuGfgAfaGfgGfasCfsa					
D1795	S1795	813	UfcCfcUfuCfcAfAfAfaAfaAfAfGfaGfaAfL96	AS1795	1905	uUfcUfcuuUfuUfuuuGfgAfaGfgGfasCfsa					
D1796	S1796	814	CfcCfuUfcCfaAfAfAfaAfaagAfgAfaUfL96	AS1796	1906	aUfuCfuCfUfUfUfuuuUfgGfaAfgGfgsAfsc					
D1797	S1797	815	CfcCfuUfcCfaAfAfAfaAfaAfGfAfgAfaUfL96	AS1797	1907	aUfuCfucuUfUfUfuuuUfgGfaAfgGfgsAfsc					
D1798	S1798	816	CfcUfuCfcAfaAfAfAfaAfagaGfaAfuCfL96	AS1798	1908	gAfuUfcUfCfUfUfUfuuuUfuGfgAfaGfgsGfsa					
D1799	S1799	817	CfcUfuCfcAfaAfAfAfaAfaGfAfGfaAfuCfL96	AS1799	1909	gAfuUfcucUfuUfUfuuuUfuGfgAfaGfgsGfsa					
D1800	S1800	818	CfuUfcCfaAfaAfAfAfaAfgagAfaUfcAfL96	AS1800	1910	uGfaUfuCfUfCfuUfUfuuuUfuUfgGfaAfgsGfsg					
D1801	S1801	819	CfuUfcCfaAfaAfAfAfaAfgAfGfAfaUfcAfL96	AS1801	1911	uGfaUfucuCfuUfUfuuuUfuUfgGfaAfgsGfsg					
D1802	S1802	820	UfuCfcAfaAfaAfAfAfaGfagaAfuCfaAfL96	AS1802	1912	uUfgAfuUfCfUfcUfuuuUfuUfuGfgAfasGfsg					
D1803	S1803	821	UfuCfcAfaAfaAfAfAfaGfaGfAfAfuCfaAfL96	AS1803	1913	uUfgAfuucUfcUfUfuuuUfuUfuGfgAfasGfsg					

D1804	S1804	822	UfcCfaAfaAfaAfAfAfgAfgaaUfcAfaAfL96	AS1804	1914	uUfuGfaUfUfCfuCfuuuUfuUfuUfgGfasAfsg					
D1805	S1805	823	UfcCfaAfaAfaAfAfAfgAfgAfAfUfcAfaAfL96	AS1805	1915	uUfuGfauuCfuCfuuuUfuUfuUfgGfasAfsg					
D1806	S1806	824	CfcAfaAfaAfaAfAfGfaGfaauCfaAfaAfL96	AS1806	1916	uUfuUfgAfUfUfcUfcuuUfuUfuUfuGfgsAfsa					
D1807	S1807	825	CfcAfaAfaAfaAfAfGfaGfaAfUfCfaAfaAfL96	AS1807	1917	uUfuUfgauUfcUfcuuUfuUfuUfuGfgsAfsa					
D1808	S1808	826	CfaAfaAfaAfaAfGfAfgAfaucAfaAfaUfL96	AS1808	1918	aUfuUfuGfAfUfuCfucuUfuUfuUfuUfgsGfsa					
D1809	S1809	827	CfaAfaAfaAfaAfGfAfgAfaUfCfAfaAfaUfL96	AS1809	1919	aUfuUfugaUfuCfucuUfuUfuUfuUfgsGfsa					
D1810	S1810	828	AfaAfaAfaAfaGfAfGfaAfucaAfaAfuUfL96	AS1810	1920	aAfuUfuUfGfAfuUfucuCfuUfuUfuUfusGfsg					
D1811	S1811	829	AfaAfaAfaAfaGfAfGfaAfuCfAfAfaAfuUfL96	AS1811	1921	aAfuUfuugAfuUfucuCfuUfuUfuUfusGfsg					
D1812	S1812	830	AfaAfaAfaAfgAfGfAfaUfcaaAfaUfuUfL96	AS1812	1922	aAfaUfuUfUfGfaUfucuCfuUfuUfuUfusUfsg					
D1813	S1813	831	AfaAfaAfaAfgAfGfAfaUfcAfAfAfaUfuUfL96	AS1813	1923	aAfaUfuuuGfaUfucuCfuUfuUfuUfusUfsg					
D1814	S1814	832	AfaAfaAfaGfaGfAfAfuCfaaaAfuUfuUfL96	AS1814	1924	aAfaAfuUfUfUfgAfuucUfcUfuUfuUfusUfsu					
D1815	S1815	833	AfaAfaAfaGfaGfAfAfuCfaAfAfAfuUfuUfL96	AS1815	1925	aAfaAfuuuUfgAfuucUfcUfuUfuUfusUfsu					
D1816	S1816	834	AfaAfaAfgAfgAfAfUfcAfaaaUfuUfuAfL96	AS1816	1926	uAfaAfaUfUfUfuGfauuCfuCfuUfuUfusUfsu					
D1817	S1817	835	AfaAfaAfgAfgAfAfUfcAfaAfAfUfuUfuAfL96	AS1817	1927	uAfaAfauuUfuGfauuCfuCfuUfuUfusUfsu					
D1818	S1818	836	AfaAfaGfaGfaAfUfCfaAfaauUfuUfaCfL96	AS1818	1928	gUfaAfaAfUfUfuUfgauUfcUfcUfuUfusUfsu					
D1819	S1819	837	AfaAfaGfaGfaAfUfCfaAfaAfUfUfuUfaCfL96	AS1819	1929	gUfaAfaauUfuUfgauUfcUfcUfuUfusUfsu					
D1820	S1820	838	AfaAfgAfgAfaUfCfAfaAfauuUfuAfcAfL96	AS1820	1930	uGfuAfaAfAfUfuUfugaUfuCfuCfuUfusUfsu					
D1821	S1821	839	AfaAfgAfgAfaUfCfAfaAfaUfUfUfuAfcAfL96	AS1821	1931	uGfuAfaaaUfuUfugaUfuCfuCfuUfusUfsu					
D1822	S1822	840	AfaGfaGfaAfuCfAfAfaAfauuuUfaCfaAfL96	AS1822	1932	uUfgUfaAfAfAfuUfuugAfuUfcUfcUfusUfsu					
D1823	S1823	841	AfaGfaGfaAfuCfAfAfaAfuUfUfUfuCfaAfL96	AS1823	1933	uUfgUfaaaAfuUfuugAfuUfcUfcUfusUfsu					
D1824	S1824	842	AfgAfgAfaUfcAfAfAfaUfuuuAfcAfaAfL96	AS1824	1934	uUfuGfuAfAfAfaUfuuuGfaUfuCfuCfusUfsu					
D1825	S1825	843	AfgAfgAfaUfcAfAfAfaUfuUfUfAfcAfaAfL96	AS1825	1935	uUfuGfuaaAfaUfuuuGfaUfuCfuCfusUfsu					
D1826	S1826	844	GfaGfaAfuCfaAfAfAfuUfuuaCfaAfaGfL96	AS1826	1936	cUfuUfgUfAfAfaAfuuuUfgAfuUfcUfcsUfsu					
D1827	S1827	845	GfaGfaAfuCfaAfAfAfuUfuUfAfCfaAfaGfL96	AS1827	1937	cUfuUfguaAfaAfuuuUfgAfuUfcUfcsUfsu					
D1828	S1828	846	AfgAfaUfcAfaAfAfUfuUfuacAfaAfgAfL96	AS1828	1938	uCfuUfuGfUfAfaAfauuUfuGfaUfuCfusCfsu					
D1829	S1829	847	AfgAfaUfcAfaAfAfUfuUfuAfCfAfaAfgAfL96	AS1829	1939	uCfuUfuguAfaAfauuUfuGfaUfuCfusCfsu					
D1830	S1830	848	GfaAfuCfaAfaAfUfUfuUfacaAfaGfaAfL96	AS1830	1940	uUfcUfuUfGfUfaAfaauUfuUfgAfuUfcsUfsc					

D1831	S1831	849	GfaAfuCfaAfaAfUfUfuUfaCfAfAfaGfaAfL96	AS1831	1941	uUfcUfuugUfaAfaauUfuUfgAfuUfcsUfsc					
D1832	S1832	850	AfaUfcAfaAfaUfUfUfuAfcaaAfgAfaUfL96	AS1832	1942	aUfuCfuUfUfGfuAfaaaUfuUfuGfaUfusCfsu					
D1833	S1833	851	AfaUfcAfaAfaUfUfUfuAfcAfAfAfAfgAfaUfL96	AS1833	1943	aUfuCfuuuGfuAfaaaUfuUfuGfaUfusCfsu					
D1834	S1834	852	AfuCfaAfaAfuUfUfUfaCfaaaGfaAfuCfL96	AS1834	1944	gAfuUfcUfUfUfgUfaaaAfuUfuUfgAfusUfsc					
D1835	S1835	853	AfuCfaAfaAfuUfUfUfaCfaAfAfGfaAfuCfL96	AS1835	1945	gAfuUfcuuUfgUfaaaAfuUfuUfgAfusUfsc					
D1836	S1836	854	UfcAfaAfaUfuUfUfAfcAfaagAfaUfcAfL96	AS1836	1946	uGfaUfuCfUfUfuGfuaaAfaUfuUfuGfasUfsu					
D1837	S1837	855	UfcAfaAfaUfuUfUfAfcAfaAfGfAfaUfcAfL96	AS1837	1947	uGfaUfucuUfuGfuaaAfaUfuUfuGfasUfsu					
D1838	S1838	856	CfaAfaAfuUfuUfAfCfaAfagaAfuCfaAfL96	AS1838	1948	uUfgAfuUfCfUfuUfguaAfaAfuUfuUfgsAfsu					
D1839	S1839	857	CfaAfaAfuUfuUfAfCfaAfaGfAfAfuCfaAfL96	AS1839	1949	uUfgAfuucUfuUfguaAfaAfuUfuUfgsAfsu					
D1840	S1840	858	AfaAfaUfuUfuAfCfAfaAfgaaUfcAfaAfL96	AS1840	1950	uUfuGfaUfUfCfuUfuguAfaAfaUfuUfusGfsa					
D1841	S1841	859	AfaAfaUfuUfuAfCfAfaAfgAfAfUfcAfaAfL96	AS1841	1951	uUfuGfauuCfuUfuguAfaAfaUfuUfusGfsa					
D1842	S1842	860	AfaAfuUfuUfaCfAfAfaGfaauCfaAfaGfL96	AS1842	1952	cUfuUfgAfUfUfcUfuugUfaAfaAfuUfusUfsg					
D1843	S1843	861	AfaAfuUfuUfaCfAfAfaGfaAfUfCfaAfaGfL96	AS1843	1953	cUfuUfgauUfcUfuugUfaAfaAfuUfusUfsg					
D1844	S1844	862	AfaUfuUfuAfcAfAfAfgAfaucAfaAfgGfL96	AS1844	1954	cCfuUfuGfAfUfuCfuuuGfuAfaAfaUfusUfsu					
D1845	S1845	863	AfaUfuUfuAfcAfAfAfgAfaUfCfAfaAfgGfL96	AS1845	1955	cCfuUfugaUfuCfuuuGfuAfaAfaUfusUfsu					
D1846	S1846	864	AfuUfuUfaCfaAfAfGfaAfucaAfaGfgAfL96	AS1846	1956	uCfcUfuUfGfAfuUfcuuUfgUfaAfaAfusUfsu					
D1847	S1847	865	AfuUfuUfaCfaAfAfGfaAfuCfAfAfaGfgAfL96	AS1847	1957	uCfcUfuugAfuUfcuuUfgUfaAfaAfusUfsu					
D1848	S1848	866	UfuUfuAfcAfaAfGfAfaUfcaaAfgGfaAfL96	AS1848	1958	uUfcCfuUfUfGfaUfucuUfuGfuAfaAfasUfsu					
D1849	S1849	867	UfuUfuAfcAfaAfGfAfaUfcAfAfAfgGfaAfL96	AS1849	1959	uUfcCfuuuGfaUfucuUfuGfuAfaAfasUfsu					
D1850	S1850	868	UfuUfaCfaAfaGfAfAfuCfaaaGfgAfaUfL96	AS1850	1960	aUfuCfcUfUfUfgAfuucUfuUfgUfaAfasAfsu					
D1851	S1851	869	UfuUfaCfaAfaGfAfAfuCfaAfAfGfgAfaUfL96	AS1851	1961	aUfuCfcuuUfgAfuucUfuUfgUfaAfasAfsu					
D1852	S1852	870	UfuAfcAfaAfgAfAfUfcAfaagGfaAfuUfL96	AS1852	1962	aAfuUfcCfUfUfuGfauuCfuUfuGfuAfasAfsa					
D1853	S1853	871	UfuAfcAfaAfgAfAfUfcAfaAfGfGfaAfuUfL96	AS1853	1963	aAfuUfccuUfuGfauuCfuUfuGfuAfasAfsa					
D1854	S1854	872	UfaCfaAfaGfaAfUfCfaAfaggAfaUfuCfL96	AS1854	1964	gAfaUfuCfCfUfuUfgauUfcUfuUfgUfasAfsa					
D1855	S1855	873	UfaCfaAfaGfaAfUfCfaAfaGfGfAfaUfuCfL96	AS1855	1965	gAfaUfuccUfuUfgauUfcUfuUfgUfasAfsa					
D1856	S1856	874	AfcAfaAfgAfaUfCfAfaAfggaAfuUfcUfL96	AS1856	1966	aGfaAfuUfCfCfuUfugaUfuCfuUfuGfusAfsa					
D1857	S1857	875	AfcAfaAfgAfaUfCfAfaAfgGfAfAfuUfcUfL96	AS1857	1967	aGfaAfuucCfuUfugaUfuCfuUfuGfusAfsa					

D1858	S1858	876	CfaAfaGfaAfuCfAfAfaGfgaaUfuCfuAfL96	AS1858	1968	uAfgAfaUfUfCfcUfuugAfuUfcUfuUfgsUfsa					
D1859	S1859	877	CfaAfaGfaAfuCfAfAfaGfgAfAfUfuCfuAfL96	AS1859	1969	uAfgAfauuCfcUfuugAfuUfcUfuUfgsUfsa					
D1860	S1860	878	AfaAfgAfaUfcAfAfAfgGfaauUfcUfaGfL96	AS1860	1970	cUfaGfaAfUfUfcCfuuuGfaUfuCfuUfusGfsu					
D1861	S1861	879	AfaAfgAfaUfcAfAfAfgGfaAfUfUfcUfaGfL96	AS1861	1971	cUfaGfaauUfcCfuuuGfaUfuCfuUfusGfsu					
D1862	S1862	880	AfaGfaAfuCfaAfAfGfgAfauuCfuAfgAfL96	AS1862	1972	uCfuAfgAfAfUfuCfcuuUfgAfuUfcUfusUfsg					
D1863	S1863	881	AfaGfaAfuCfaAfAfGfgAfaUfUfCfuAfgAfL96	AS1863	1973	uCfuAfgaaUfuCfcuuUfgAfuUfcUfusUfsg					
D1864	S1864	882	AfgAfaUfcAfaAfGfGfaAfuucUfaGfaAfL96	AS1864	1974	uUfcUfaGfAfAfuUfccuUfuGfaUfuCfusUfsu					
D1865	S1865	883	AfgAfaUfcAfaAfGfGfaAfuUfCfUfaGfaAfL96	AS1865	1975	uUfcUfagaAfuUfccuUfuGfaUfuCfusUfsu					
D1866	S1866	884	GfaAfuCfaAfaGfGfAfaUfucuAfgAfaAfL96	AS1866	1976	uUfuCfuAfGfAfaUfuccUfuUfgAfuUfcsUfsu					
D1867	S1867	885	GfaAfuCfaAfaGfGfAfaUfuCfUfAfgAfaAfL96	AS1867	1977	uUfuCfuagAfaUfuccUfuUfgAfuUfcsUfsu					
D1868	S1868	886	AfaUfcAfaAfgGfAfAfuUfcuaGfaAfaGfL96	AS1868	1978	cUfuUfcUfAfGfaAfuucCfuUfuGfaUfusCfsu					
D1869	S1869	887	AfaUfcAfaAfgGfAfAfuUfcUfAfGfaAfaGfL96	AS1869	1979	cUfuUfcuaGfaAfuucCfuUfuGfaUfusCfsu					
D1870	S1870	888	AfuCfaAfaGfgAfAfUfuCfuagAfaAfgUfL96	AS1870	1980	aCfuUfuCfUfAfgAfauuCfcUfuUfgAfusUfsc					
D1871	S1871	889	AfuCfaAfaGfgAfAfUfuCfuAfGfAfaAfgUfL96	AS1871	1981	aCfuUfucuAfgAfauuCfcUfuUfgAfusUfsc					
D1872	S1872	890	UfcAfaAfgGfaAfUfUfcUfagaAfaGfuAfL96	AS1872	1982	uAfcUfuUfCfUfaGfaauUfcCfuUfuGfasUfsu					
D1873	S1873	891	UfcAfaAfgGfaAfUfUfcUfaGfAfAfaGfuAfL96	AS1873	1983	uAfcUfuucUfaGfaauUfcCfuUfuGfasUfsu					
D1874	S1874	892	CfaAfaGfgAfaUfUfCfuAfgaaAfgUfaUfL96	AS1874	1984	aUfaCfuUfUfCfuAfgaaUfuCfcUfuUfgsAfsu					
D1875	S1875	893	CfaAfaGfgAfaUfUfCfuAfgAfAfAfgUfaUfL96	AS1875	1985	aUfaCfuuuCfuAfgaaUfuCfcUfuUfgsAfsu					
D1876	S1876	894	AfaAfgGfaAfuUfCfUfaGfaaaGfuAfuCfL96	AS1876	1986	gAfuAfcUfUfUfcUfagaAfuUfcCfuUfusGfsa					
D1877	S1877	895	AfaAfgGfaAfuUfCfUfaGfaAfAfGfuAfuCfL96	AS1877	1987	gAfuAfcuuUfcUfagaAfuUfcCfuUfusGfsa					
D1878	S1878	896	AfaGfgAfaUfuCfUfAfgAfaagUfaUfcUfL96	AS1878	1988	aGfaUfacUfUfUfcUfuagAfaUfuCfcUfusUfsg					
D1879	S1879	897	AfaGfgAfaUfuCfUfAfgAfaAfGfUfaUfcUfL96	AS1879	1989	aGfaUfacuUfuCfuagAfaUfuCfcUfusUfsg					
D1880	S1880	898	AfgGfaAfuUfcUfAfGfaAfaguAfuCfuGfL96	AS1880	1990	cAfgAfuAfCfUfuUfcuaGfaAfuUfcCfusUfsu					
D1881	S1881	899	AfgGfaAfuUfcUfAfGfaAfaGfUfAfuCfuGfL96	AS1881	1991	cAfgAfuacUfuUfcuaGfaAfuUfcCfusUfsu					
D1882	S1882	900	GfgAfaUfuCfuAfGfAfaAfguaUfcUfgGfL96	AS1882	1992	cCfaGfaUfAfCfuUfucuAfgAfaUfuCfcsUfsu					
D1883	S1883	901	GfgAfaUfuCfuAfGfAfaAfgUfAfUfcUfgGfL96	AS1883	1993	cCfaGfauaCfuUfucuAfgAfaUfuCfcsUfsu					
D1884	S1884	902	GfaAfuUfcUfaGfAfAfaGfuauCfuGfgGfL96	AS1884	1994	cCfcAfgAfUfAfcUfuucUfaGfaAfuUfcsCfsu					

D1885	S1885	903	GfaAfuUfcUfaGfAfAfaGfuAfUfCfuGfgGfL96	AS1885	1995	cCfcAfgauAfcUfuucUfaGfaAfuUfcsCfsu					
D1886	S1886	904	AfaUfuCfuAfgAfAfAfgUfaucUfgGfgCfL96	AS1886	1996	gCfcCfaGfAfUfaCfuuuCfuAfgAfaUfusCfsc					
D1887	S1887	905	AfaUfuCfuAfgAfAfAfgUfaUfCfUfgGfgCfL96	AS1887	1997	gCfcCfagaUfaCfuuuCfuAfgAfaUfusCfsc					
D1888	S1888	906	AfuUfcUfaGfaAfAfGfuAfucuGfgGfcAfL96	AS1888	1998	uGfcCfcAfGfAfuAfcuuUfcUfaGfaAfusUfsc					
D1889	S1889	907	AfuUfcUfaGfaAfAfGfuAfuCfUfGfgGfcAfL96	AS1889	1999	uGfcCfcagAfuAfcuuUfcUfaGfaAfusUfsc					
D1890	S1890	908	UfuCfuAfgAfaAfGfUfaUfcugGfgCfaGfL96	AS1890	2000	cUfgCfcCfAfGfaUfacuUfuCfuAfgAfasUfsu					
D1891	S1891	909	UfuCfuAfgAfaAfGfUfaUfcUfGfGfgCfaGfL96	AS1891	2001	cUfgCfccaGfaUfacuUfuCfuAfgAfasUfsu					
D1892	S1892	910	UfcUfaGfaAfaGfUfAfuCfuggGfcAfgAfL96	AS1892	2002	uCfuGfcCfCfAfgAfuacUfuUfcUfaGfasAfsu					
D1893	S1893	911	UfcUfaGfaAfaGfUfAfuCfuGfGfGfcAfgAfL96	AS1893	2003	uCfuGfcccAfgAfuacUfuUfcUfaGfasAfsu					
D1894	S1894	912	CfuAfgAfaAfgUfAfUfcUfgggCfaGfaAfL96	AS1894	2004	uUfcUfgCfCfCfaGfauaCfuUfuCfuAfgsAfsa					
D1895	S1895	913	CfuAfgAfaAfgUfAfUfcUfgGfGfCfaGfaAfL96	AS1895	2005	uUfcUfgccCfaGfauaCfuUfuCfuAfgsAfsa					
D1896	S1896	914	UfaGfaAfaGfuAfUfCfuGfggcAfgAfaCfL96	AS1896	2006	gUfuCfuGfCfCfcAfgauAfcUfuUfcUfasGfsa					
D1897	S1897	915	UfaGfaAfaGfuAfUfCfuGfgGfCfAfgAfaCfL96	AS1897	2007	gUfuCfugcCfcAfgauAfcUfuUfcUfasGfsa					
D1898	S1898	916	AfgAfaAfgUfaUfCfUfgGfgcaGfaAfcGfL96	AS1898	2008	cGfuUfcUfGfCfcCfagaUfaCfuUfuCfusAfsg					
D1899	S1899	917	AfgAfaAfgUfaUfCfUfgGfgCfAfGfaAfcGfL96	AS1899	2009	cGfuUfcugCfcCfagaUfaCfuUfuCfusAfsg					
D1900	S1900	918	GfaAfaGfuAfuCfUfGfgGfcagAfaCfgCfL96	AS1900	2010	gCfgUfuCfUfGfcCfcagAfuAfcUfuUfcsUfsa					
D1901	S1901	919	GfaAfaGfuAfuCfUfGfgGfcAfGfAfaCfgCfL96	AS1901	2011	gCfgUfucuGfcCfcagAfuAfcUfuUfcsUfsa					
D1902	S1902	920	AfaAfgUfaUfcUfGfGfgCfagaAfcGfcUfL96	AS1902	2012	aGfcGfuUfCfUfgCfccaGfaUfaCfuUfusCfsu					
D1903	S1903	921	AfaAfgUfaUfcUfGfGfgCfaGfAfAfcGfcUfL96	AS1903	2013	aGfcGfuucUfgCfccaGfaUfaCfuUfusCfsu					
D1904	S1904	922	AfaGfuAfuCfuGfGfGfcAfgaaCfgCfuAfL96	AS1904	2014	uAfgCfgUfUfCfuGfcccAfgAfuAfcUfusUfsc					
D1905	S1905	923	AfaGfuAfuCfuGfGfGfcAfgAfAfCfgCfuAfL96	AS1905	2015	uAfgCfguuCfuGfcccAfgAfuAfcUfusUfsc					
D1906	S1906	924	AfgUfaUfcUfgGfGfCfaGfaacGfcUfaGfL96	AS1906	2016	cUfaGfcGfUfUfcUfgccCfaGfaUfaCfusUfsu					
D1907	S1907	925	AfgUfaUfcUfgGfGfCfaGfaAfCfGfcUfaGfL96	AS1907	2017	cUfaGfcguUfcUfgcccCfaGfaUfaCfusUfsu					
D1908	S1908	926	GfuAfuCfuGfgGfCfAfgAfacgCfuAfgGfL96	AS1908	2018	cCfuAfgCfGfUfuCfugccCfcAfgAfuAfcsUfsu					
D1909	S1909	927	GfuAfuCfuGfgGfCfAfgAfaCfGfCfuAfgGfL96	AS1909	2019	cCfuAfgcgUfuCfugcCfcAfgAfuAfcsUfsu					
D1910	S1910	928	UfaUfcUfgGfgCfAfGfaAfcgcUfaGfgAfL96	AS1910	2020	uCfcUfaGfCfGfuUfcugCfcCfaGfaUfasCfsu					
D1911	S1911	929	UfaUfcUfgGfgCfAfGfaAfcGfCfUfaGfgAfL96	AS1911	2021	uCfcUfagcGfuUfcugCfcCfaGfaUfasCfsu					

D1912	S1912	930	AfuCfuGfgGfcAfGfAfaCfgcuAfgGfaGfL96	AS1912	2022	cUfcCfuAfGfCfgUfucuGfcCfcAfgAfusAfsc					
D1913	S1913	931	AfuCfuGfgGfcAfGfAfaCfgCfUfAfgGfaGfL96	AS1913	2023	cUfcCfuagCfgUfucuGfcCfcAfgAfusAfsc					
D1914	S1914	932	UfcUfgGfgCfaGfAfAfcGfcuaGfgAfgAfL96	AS1914	2024	uCfuCfcUfAfGfcGfuucUfgCfcCfaGfasUfsa					
D1915	S1915	933	UfcUfgGfgCfaGfAfAfcGfcUfAfGfgAfgAfL96	AS1915	2025	uCfuCfcuaGfcGfuucUfgCfcCfaGfasUfsa					
D1916	S1916	934	CfuGfgGfcAfgAfAfCfgCfuagGfaGfaGfL96	AS1916	2026	cUfcUfccCfUfAfgCfguuCfuGfcCfcAfgsAfsu					
D1917	S1917	935	CfuGfgGfcAfgAfAfCfgCfuAfGfGfaGfaGfL96	AS1917	2027	cUfcUfccuAfgCfguuCfuGfcCfcAfgsAfsu					
D1918	S1918	936	UfgGfgCfaGfaAfCfGfcUfaggAfgAfgAfL96	AS1918	2028	uCfuCfuCfCfUfaGfcguUfcUfgCfcCfasGfsa					
D1919	S1919	937	UfgGfgCfaGfaAfCfGfcUfaGfGfAfgAfgAfL96	AS1919	2029	uCfuCfuccUfaGfcguUfcUfgCfcCfasGfsa					
D1920	S1920	938	GfgGfcAfgAfaCfGfCfcUfaAfggaGfaGfaUfL96	AS1920	2030	aUfcUfcUfCfCfcUfaAfgcgUfuCfuGfcCfcsAfsg					
D1921	S1921	939	GfgGfcAfgAfaCfGfCfcUfaAfgGfAfGfaGfaUfL96	AS1921	2031	aUfcUfcucCfuAfgcgUfuCfuGfcCfcsAfsg					
D1922	S1922	940	GfgCfaGfaAfcGfCfUfaGfgagAfgAfuCfL96	AS1922	2032	gAfuCfuCfUfCfCfUfagcGfuUfcUfgCfcsCfsa					
D1923	S1923	941	GfgCfaGfaAfcGfCfUfaGfgAfGfAfgAfuCfL96	AS1923	2033	gAfuCfucuCfcUfagcGfuUfcUfgCfcsCfsa					
D1924	S1924	942	GfcAfgAfaCfgCfUfAfgGfagaGfaUfCcfL96	AS1924	2034	gGfaUfcUfCfUfCfCfuagCfgUfuCfuGfcsCfsc					
D1925	S1925	943	GfcAfgAfaCfgCfUfAfgGfaGfAfGfaUfCcCfL96	AS1925	2035	gGfaUfcucUfcCfuagCfgUfuCfuGfcsCfsc					
D1926	S1926	944	CfaGfaAfcGfcUfAfGfgAfgagAfuCfcAfL96	AS1926	2036	uGfgAfuCfUfCfuCfcuaGfcGfuUfcUfgsCfsc					
D1927	S1927	945	CfaGfaAfcGfcUfAfGfgAfgAfGfAfuCfcAfL96	AS1927	2037	uGfgAfucuCfuCfcuaGfcGfuUfcUfgsCfsc					
D1928	S1928	946	AfgAfaCfgCfuAfGfGfaGfagaUfcCfaAfL96	AS1928	2038	uUfgGfaUfCfUfCfUfccuAfgCfgUfuCfusGfsc					
D1929	S1929	947	AfgAfaCfgCfuAfGfGfaGfaGfAfUfcCfaAfL96	AS1929	2039	uUfgGfaucUfcUfCfccuAfgCfgUfuCfusGfsc					
D1930	S1930	948	GfaAfcGfcUfaGfGfAfgAfgauCfcAfaAfL96	AS1930	2040	uUfuGfgAfUfCfuCfuccUfaGfcGfuUfcsUfsg					
D1931	S1931	949	GfaAfcGfcUfaGfGfAfgAfgAfUfCfcAfaAfL96	AS1931	2041	uUfuGfgauCfuCfuccUfaGfcGfuUfcsUfsg					
D1932	S1932	950	AfaCfgCfuAfgGfAfGfaGfaucCfaAfaUfL96	AS1932	2042	aUfuUfgGfAfUfcUfcucCfuAfgCfgUfusCfsu					
D1933	S1933	951	AfaCfgCfuAfgGfAfGfaGfaUfCfCfaAfaUfL96	AS1933	2043	aUfuUfggaUfcUfcucCfuAfgCfgUfusCfsu					
D1934	S1934	952	AfcGfcUfaGfgAfGfAfgAfuccAfaAfuUfL96	AS1934	2044	aAfuUfuGfGfAfuCfucuCfcUfaGfcGfusUfsc					
D1935	S1935	953	AfcGfcUfaGfgAfGfAfgAfuCfCfAfaAfuUfL96	AS1935	2045	aAfuUfuggAfuCfucuCfcUfaGfcGfusUfsc					
D1936	S1936	954	CfgCfuAfgGfaGfAfGfaUfccaAfaUfuUfL96	AS1936	2046	aAfaUfuUfGfGfaUfcucUfcCfuAfgCfgsUfsu					
D1937	S1937	955	CfgCfuAfgGfaGfAfGfaUfcCfAfAfaUfuUfL96	AS1937	2047	aAfaUfuugGfaUfcucUfcCfuAfgCfgsUfsu					
D1938	S1938	956	GfcUfaGfgAfgAfGfAfuCfcaaAfuUfuCfL96	AS1938	2048	gAfaAfuUfUfGfgAfucuCfuCfcUfaGfcsGfsu					

D1939	S1939	957	GfcUfaGfgAfgAfGfAfuCfcAfAfAfuUfuCfL96	AS1939	2049	gAfaAfuuuGfgAfucuCfuCfcUfaGfcsGfsu					
D1940	S1940	958	CfuAfgGfaGfaGfAfUfcCfaaaUfuUfcCfL96	AS1940	2050	gGfaAfaUfUfUfgGfaucUfcUfcCfuAfgsCfsg					
D1941	S1941	959	CfuAfgGfaGfaGfAfUfcCfaAfAfUfuUfcCfL96	AS1941	2051	gGfaAfauuUfgGfaucUfcUfcCfuAfgsCfsg					
D1942	S1942	960	UfaGfgAfgAfgAfUfCfcAfaauUfuCfcAfL96	AS1942	2052	uGfgAfaAfUfUfuGfgauCfuCfuCfcUfasGfsc					
D1943	S1943	961	UfaGfgAfgAfgAfUfUfCfcAfaAfUfUfuCfcAfL96	AS1943	2053	uGfgAfaauUfuGfgauCfuCfuCfcUfasGfsc					
D1944	S1944	962	AfgGfaGfaGfaUfCfCfaAfauuUfcCfaUfL96	AS1944	2054	aUfgGfaAfAfUfuUfggaUfcUfcUfcCfusAfsg					
D1945	S1945	963	AfgGfaGfaGfaUfCfCfaAfaUfUfUfcCfaUfL96	AS1945	2055	aUfgGfaaaUfuUfggaUfcUfcUfcCfusAfsg					
D1946	S1946	964	GfgAfgAfgAfaUfCfCfAfaAfuuuCfcAfuUfL96	AS1946	2056	aAfuGfgAfAfAfuUfuggAfuCfuCfuCfcsUfsa					
D1947	S1947	965	GfgAfgAfgAfaUfCfCfAfaAfuUfUfCfcAfuUfL96	AS1947	2057	aAfuGfgaaAfuUfuggAfuCfuCfuCfcsUfsa					
D1948	S1948	966	GfaGfaGfaUfcCfAfAfaUfuuccCfaUfuGfL96	AS1948	2058	cAfaUfgGfAfAfaUfuugGfaUfcUfcUfccsCfsu					
D1949	S1949	967	GfaGfaGfaUfcCfAfAfaUfuUfCfCfCfaUfuGfL96	AS1949	2059	cAfaUfggaAfaUfuugGfaUfcUfcUfccsCfsu					
D1950	S1950	968	AfgAfgAfuCfcAfAfAfuUfuccAfuUfgUfL96	AS1950	2060	aCfaUfgGfGfAfaAfuuuGfgAfuCfuCfusCfsc					
D1951	S1951	969	AfgAfgAfuCfcAfAfAfuUfuCfCfCfAfuUfgUfL96	AS1951	2061	aCfaAfuggAfaAfuuuGfgAfuCfuCfusCfsc					
D1952	S1952	970	GfaGfaUfcCfaAfAfUfuUfccaUfuGfuCfL96	AS1952	2062	gAfcAfaUfGfGfaAfauuUfgGfaUfcUfcsUfsc					
D1953	S1953	971	GfaGfaUfcCfaAfAfUfuUfcCfAfUfuGfuCfL96	AS1953	2063	gAfcAfaugGfaAfauuUfgGfaUfcUfcsUfsc					
D1954	S1954	972	AfgAfuCfcAfaAfUfUfuCfcauUfgUfcUfL96	AS1954	2064	aGfaCfaAfUfGfgAfaauUfuGfgAfuCfusCfsu					
D1955	S1955	973	AfgAfuCfcAfaAfUfUfuCfcAfUfUfgUfcUfL96	AS1955	2065	aGfaCfaauGfgAfaauUfuGfgAfuCfusCfsu					
D1956	S1956	974	GfaUfcCfaAfaUfUfUfcCfauuGfuCfuUfL96	AS1956	2066	aAfgAfcAfAfUfgGfaaaUfuUfgGfaUfcsUfsc					
D1957	S1957	975	GfaUfcCfaAfaUfUfUfcCfaUfUfGfuCfuUfL96	AS1957	2067	aAfgAfcaaUfgGfaaaUfuUfgGfaUfcsUfsc					
D1958	S1958	976	AfuCfcAfaAfuUfUfCfcAfuugUfcUfuGfL96	AS1958	2068	cAfaGfaCfAfAfuGfgaaAfuUfuGfgAfusCfsu					
D1959	S1959	977	AfuCfcAfaAfuUfUfCfcAfuUfGfUfcUfuGfL96	AS1959	2069	cAfaGfacaAfuGfgaaAfuUfuGfgAfusCfsu					
D1960	S1960	978	UfcCfaAfaUfuUfCfCfaUfuguCfuUfgCfL96	AS1960	2070	gCfaAfgAfCfAfaUfggaAfaUfuUfgGfasUfsc					
D1961	S1961	979	UfcCfaAfaUfuUfCfCfaUfuGfUfCfuUfgCfL96	AS1961	2071	gCfaAfgacAfaUfggaAfaUfuUfgGfasUfsc					
D1962	S1962	980	CfcAfaAfuUfuCfCfAfuUfgucUfuGfcAfL96	AS1962	2072	uGfcAfaGfAfCfaAfuggAfaAfuUfuGfgsAfsu					
D1963	S1963	981	CfcAfaAfuUfuCfCfAfuUfgUfCfUfuGfcAfL96	AS1963	2073	uGfcAfagaCfaAfuggAfaAfuUfuGfgsAfsu					
D1964	S1964	982	CfaAfaUfuUfcCfAfUfuGfucuUfgCfaAfL96	AS1964	2074	uUfgCfaAfGfAfcAfaugGfaAfaUfuUfgsGfsa					
D1965	S1965	983	CfaAfaUfuUfcCfAfUfuGfuCfUfUfgCfaAfL96	AS1965	2075	uUfgCfaagAfcAfaugGfaAfaUfuUfgsGfsa					

D1966	S1966	984	AfaAfuUfuCfcAfUfUfgUfcuuGfcAfaGfL96	AS1966	2076	cUfuGfcAfAfGfaCfaauGfgAfaAfuUfusGfsg				
D1967	S1967	985	AfaAfuUfuCfcAfUfUfgUfcUfUfGfcAfaGfL96	AS1967	2077	cUfuGfcaaGfaCfaauGfgAfaAfuUfusGfsg				
D1968	S1968	986	AfaUfuUfcCfaUfUfGfuCfuugCfaAfgCfL96	AS1968	2078	gCfuUfgCfAfAfgAfcaaUfgGfaAfaUfusUfsg				
D1969	S1969	987	AfaUfuUfcCfaUfUfGfuCfuUfGfCfaAfgCfL96	AS1969	2079	gCfuUfgcaAfgAfcaaUfgGfaAfaUfusUfsg				
D1970	S1970	988	AfuUfuCfcAfuUfGfUfcUfugcAfaGfcAfL96	AS1970	2080	uGfcUfuGfCfAfaGfacaAfuGfgAfaAfusUfsu				
D1971	S1971	989	AfuUfuCfcAfuUfGfUfcUfuGfCfAfaGfcAfL96	AS1971	2081	uGfcUfugcAfaGfacaAfuGfgAfaAfusUfsu				
D1972	S1972	990	UfuUfcCfaUfuGfUfCfuUfgcaAfgCfaAfL96	AS1972	2082	uUfgCfuUfGfCfaAfgacAfaUfgGfaAfasUfsu				
D1973	S1973	991	UfuUfcCfaUfuGfUfCfuUfgCfAfAfgCfaAfL96	AS1973	2083	uUfgCfuugCfaAfgacAfaUfgGfaAfasUfsu				
D1974	S1974	992	UfuCfcAfuUfgUfCfUfuGfcaaGfcAfaAfL96	AS1974	2084	uUfuGfcUfUfGfcAfagaCfaAfuGfgAfasAfsu				
D1975	S1975	993	UfuCfcAfuUfgUfCfUfuGfcAfAfGfcAfaAfL96	AS1975	2085	uUfuGfcuuGfcAfagaCfaAfuGfgAfasAfsu				
D1976	S1976	994	UfcCfaUfuGfuCfUfUfgCfaagCfaAfaGfL96	AS1976	2086	cUfuUfgCfUfUfgCfaagAfcAfaUfgGfasAfsa				
D1977	S1977	995	UfcCfaUfuGfuCfUfUfgCfaAfGfCfaAfaGfL96	AS1977	2087	cUfuUfgcuUfgCfaagAfcAfaUfgGfasAfsa				
D1978	S1978	996	CfcAfuUfgUfcUfUfGfcAfagcAfaAfgCfL96	AS1978	2088	gCfuUfuGfCfUfuGfcaaGfaCfaAfuGfgsAfsa				
D1979	S1979	997	CfcAfuUfgUfcUfUfGfcAfaGfCfAfaAfgCfL96	AS1979	2089	gCfuUfugcUfuGfcaaGfaCfaAfuGfgsAfsa				
D1980	S1980	998	CfaUfuGfuCfuUfGfCfaAfgcaAfaGfcAfL96	AS1980	2090	uGfcUfuUfGfCfuUfgcaAfgAfcAfaUfgsGfsa				
D1981	S1981	999	CfaUfuGfuCfuUfGfCfaAfgCfAfAfaGfcAfL96	AS1981	2091	uGfcUfuugCfuUfgcaAfgAfcAfaUfgsGfsa				
D1982	S1982	1000	AfuUfgUfcUfuGfCfAfaGfcaaAfgCfaCfL96	AS1982	2092	gUfgCfuUfUfGfcUfugcAfaGfaCfaAfusGfsg				
D1983	S1983	1001	AfuUfgUfcUfuGfCfAfaGfcAfAfAfgCfaCfL96	AS1983	2093	gUfgCfuuuGfcUfugcAfaGfaCfaAfusGfsg				
D1984	S1984	1002	UfuGfuCfuUfgCfAfAfgCfaaaGfcAfcGfL96	AS1984	2094	cGfuGfcUfUfUfgCfuugCfaAfgAfcAfasUfsg				
D1985	S1985	1003	UfuGfuCfuUfgCfAfAfgCfaAfAfGfcAfcGfL96	AS1985	2095	cGfuGfcuuUfgCfuugCfaAfgAfcAfasUfsg				
D1986	S1986	1004	UfgUfcUfuGfcAfAfGfcAfaagCfaCfgUfL96	AS1986	2096	aCfgUfgCfUfUfuGfcuuGfcAfaGfaCfasAfsu				
D1987	S1987	1005	UfgUfcUfuGfcAfAfGfcAfaAfGfCfaCfgUfL96	AS1987	2097	aCfgUfgcuUfuGfcuuGfcAfaGfaCfasAfsu				
D1988	S1988	1006	GfuCfuUfgCfaAfGfCfaAfagcAfcGfuAfL96	AS1988	2098	uAfcGfuGfCfUfuUfgcuUfgCfaAfgAfcsAfsa				
D1989	S1989	1007	GfuCfuUfgCfaAfGfCfaAfaGfCfAfcGfuAfL96	AS1989	2099	uAfcGfugcUfuUfgcuUfgCfaAfgAfcsAfsa				
D1990	S1990	1008	UfcUfuGfcAfaGfCfAfaAfgcaCfgUfaUfL96	AS1990	2100	aUfaCfgUfGfCfuUfugcUfuGfcAfaGfasCfsa				
D1991	S1991	1009	UfcUfuGfcAfaGfCfAfaAfgCfAfCfgUfaUfL96	AS1991	2101	aUfaCfgugCfuUfugcUfuGfcAfaGfasCfsa				
D1992	S1992	1010	CfuUfgCfaAfgCfAfAfaGfcacGfuAfuUfL96	AS1992	2102	aAfuAfcGfUfGfcUfuugCfuUfgCfaAfgsAfsc				

D1993	S1993	1011	CfuUfgCfaAfgCfAfAfaGfcAfCfGfuAfuUfL96	AS1993	2103	aAfuAfcguGfcUfuugCfuUfgCfaAfgsAfsc					
D1994	S1994	1012	UfuGfcAfaGfcAfAfAfgCfacgUfaUfuAfL96	AS1994	2104	uAfaUfaCfGfUfgCfuuuGfcUfuGfcAfasGfsa					
D1995	S1995	1013	UfuGfcAfaGfcAfAfAfgCfaCfGfUfaUfuAfL96	AS1995	2105	uAfaUfacgUfgCfuuuGfcUfuGfcAfasGfsa					
D1996	S1996	1014	UfgCfaAfgCfaAfAfGfcAfcguAfuUfaAfL96	AS1996	2106	uUfaAfuAfCfGfuGfcuuUfgCfuUfgCfasAfsg					
D1997	S1997	1015	UfgCfaAfgCfaAfAfGfcAfcGfUfAfuUfaAfL96	AS1997	2107	uUfaAfuacGfuGfcuuUfgCfuUfgCfasAfsg					
D1998	S1998	1016	GfcAfaGfcAfaAfGfCfaCfguaUfuAfaAfL96	AS1998	2108	uUfuAfaUfAfCfgUfgcuUfuGfcUfuGfcsAfsa					
D1999	S1999	1017	GfcAfaGfcAfaAfGfCfaCfgUfAfUfuAfaAfL96	AS1999	2109	uUfuAfauaCfgUfgcuUfuGfcUfuGfcsAfsa					
D2000	S2000	1018	CfaAfgCfaAfaGfCfAfcGfuauUfaAfaUfL96	AS2000	2110	aUfuUfaAfUfAfcGfugcUfuUfgCfuUfgsCfsa					
D2001	S2001	1019	CfaAfgCfaAfaGfCfAfcGfuAfUfUfaAfaUfL96	AS2001	2111	aUfuUfaauAfcGfugcUfuUfgCfuUfgsCfsa					
D2002	S2002	1020	AfaGfcAfaAfgCfAfCfgUfauuAfaAfuAfL96	AS2002	2112	uAfuUfuAfAfUfaCfgugCfuUfuGfcUfusGfsc					
D2003	S2003	1021	AfaGfcAfaAfgCfAfCfgUfaUfUfAfaAfuAfL96	AS2003	2113	uAfuUfuaaUfaCfgugCfuUfuGfcUfusGfsc					
D2004	S2004	1022	AfgCfaAfaGfcAfCfGfuAfuuaAfaUfaUfL96	AS2004	2114	aUfaUfuUfAfAfuAfcguGfcUfuUfgCfusUfsg					
D2005	S2005	1023	AfgCfaAfaGfcAfCfGfuAfuUfAfAfaUfaUfL96	AS2005	2115	aUfaUfuuaAfuAfcguGfcUfuUfgCfusUfsg					
D2006	S2006	1024	GfcAfaAfgCfaCfGfUfaUfuaaAfuAfuAfGfL96	AS2006	2116	cAfuAfuUfUfAfaUfacgUfgCfuUfuGfcsUfsu					
D2007	S2007	1025	GfcAfaAfgCfaCfGfUfaUfuAfAfAfuAfuAfGfL96	AS2007	2117	cAfuAfuuuAfaUfacgUfgCfuUfuGfcsUfsu					
D2008	S2008	1026	CfaAfaGfcAfcGfUfAfuUfaaaUfaUfgAfL96	AS2008	2118	uCfaUfaUfUfUfaAfauacGfuGfcUfuUfgsCfsu					
D2009	S2009	1027	CfaAfaGfcAfcGfUfAfuUfaAfAfUfaUfgAfL96	AS2009	2119	uCfaUfauuUfaAfuacGfuGfcUfuUfgsCfsu					
D2010	S2010	1028	AfaAfgCfaCfgUfAfUfuAfaauAfuGfaUfL96	AS2010	2120	aUfcAfuAfUfUfuAfauaCfgUfgCfuUfusGfsc					
D2011	S2011	1029	AfaAfgCfaCfgUfAfUfuAfaAfUfAfuGfaUfL96	AS2011	2121	aUfcAfauaUfuAfauaCfgUfgCfuUfusGfsc					
D2012	S2012	1030	AfaGfcAfcGfuAfUfUfaAfauaUfgAfuCfL96	AS2012	2122	gAfuCfaUfAfUfuUfaauAfcGfuGfcUfusUfsg					
D2013	S2013	1031	AfaGfcAfcGfuAfUfUfaAfaUfAfUfgAfuCfL96	AS2013	2123	gAfuCfauaUfuUfaauAfcGfuGfcUfusUfsg					
D2014	S2014	1032	AfgCfaCfgUfaUfUfAfaAfuauGfaUfcUfL96	AS2014	2124	aGfaUfcAfUfAfuUfuaaUfaCfgUfgCfusUfsu					
D2015	S2015	1033	AfgCfaCfgUfaUfUfAfaAfuAfUfGfaUfcUfL96	AS2015	2125	aGfaUfcauAfuUfuaaUfaCfgUfgCfusUfsu					
D2016	S2016	1034	GfcAfcGfuAfuUfAfAfaUfaugAfuCfuGfL96	AS2016	2126	cAfgAfuCfAfUfaUfuuaAfuAfcGfuGfcsUfsu					
D2017	S2017	1035	GfcAfcGfuAfuUfAfAfaUfaUfGfAfuCfuGfL96	AS2017	2127	cAfgAfucaUfaUfuuaAfuAfcGfuGfcsUfsu					
D2018	S2018	1036	CfaCfgUfaUfuAfAfAfuAfugaUfcUfgCfL96	AS2018	2128	gCfaGfaUfCfAfuAfuuuAfaUfaCfgUfgsCfsu					
D2019	S2019	1037	CfaCfgUfaUfuAfAfAfuAfuGfAfUfcUfgCfL96	AS2019	2129	gCfaGfaucAfuAfuuuAfaUfaCfgUfgsCfsu					

D2020	S2020	1038	AfcGfuAfuUfaAfAfUfaUfgauCfuGfcAfL96	AS2020	2130	uGfcAfgAfUfCfaUfauuUfaAfuAfcGfusGfsc				
D2021	S2021	1039	AfcGfuAfuUfaAfAfUfaUfgAfUfCfuGfcAfL96	AS2021	2131	uGfcAfgauCfaUfauuUfaAfuAfcGfusGfsc				
D2022	S2022	1040	CfgUfaUfuAfaAfUfAfuGfaucUfgCfaGfL96	AS2022	2132	cUfgCfaGfAfUfcAfuauUfuAfaAfUfaCfgsUfsg				
D2023	S2023	1041	CfgUfaUfuAfaAfUfAfuGfaUfCfUfgCfaGfL96	AS2023	2133	cUfgCfagaUfcAfuauUfuAfaUfaCfgsUfsg				
D2024	S2024	1042	GfuAfuUfaAfaUfAfUfgAfucuGfcAfgCfL96	AS2024	2134	gCfuGfcAfGfAfuCfauaUfuUfaAfuAfcsGfsu				
D2025	S2025	1043	GfuAfuUfaAfaUfAfUfgAfuCfUfGfcAfgCfL96	AS2025	2135	gCfuGfcagAfuCfauaUfuUfaAfuAfcsGfsu				
D2026	S2026	1044	UfaUfuAfaAfuAfUfGfaUfcugCfaGfcCfL96	AS2026	2136	gGfcUfgCfAfGfaUfcauAfuUfuAfaUfasCfsg				
D2027	S2027	1045	UfaUfuAfaAfuAfUfGfaUfcUfGfCfaGfcCfL96	AS2027	2137	gGfcUfgcaGfaUfcauAfuUfuAfaUfasCfsg				
D2028	S2028	1046	AfuUfaAfaUfaUfGfAfuCfugcAfgCfcAfL96	AS2028	2138	uGfgCfuGfCfAfgAfucaUfaUfuUfaAfusAfsc				
D2029	S2029	1047	AfuUfaAfaUfaUfGfAfuCfuGfCfAfgCfcAfL96	AS2029	2139	uGfgCfugcAfgAfucaUfaUfuUfaAfusAfsc				
D2030	S2030	1048	UfuAfaAfuAfuGfAfUfcUfgcaGfcCfaUfL96	AS2030	2140	aUfgGfcUfGfCfaGfaucAfuAfuUfuAfasUfsa				
D2031	S2031	1049	UfuAfaAfuAfuGfAfUfcUfgCfAfGfcCfaUfL96	AS2031	2141	aUfgGfcugCfaGfaucAfuAfuUfuAfasUfsa				
D2032	S2032	1050	UfaAfaUfaUfgAfUfCfuGfcagCfcAfuUfL96	AS2032	2142	aAfuGfgCfUfGfcAfgauCfaUfaUfuUfasAfsu				
D2033	S2033	1051	UfaAfaUfaUfgAfUfCfuGfcAfGfCfcAfuUfL96	AS2033	2143	aAfuGfgcuGfcAfgauCfaUfaUfuUfasAfsu				
D2034	S2034	1052	AfaAfuAfuGfaUfCfUfgCfagccCfaUfuAfL96	AS2034	2144	uAfaUfgGfCfUfgCfagaUfcAfuAfuUfusAfsa				
D2035	S2035	1053	AfaAfuAfuGfaUfCfUfgCfaGfCfCfaUfuAfL96	AS2035	2145	uAfaUfggcUfgCfagaUfcAfuAfuUfusAfsa				
D2036	S2036	1054	AfaUfaUfgAfuCfUfGfcAfgccAfuUfaAfL96	AS2036	2146	uUfaAfuGfGfCfuGfcagAfuCfaUfaUfusUfsa				
D2037	S2037	1055	AfaUfaUfgAfuCfUfGfcAfgCfCfAfuUfaAfL96	AS2037	2147	uUfaAfuggCfuGfcagAfuCfaUfaUfusUfsa				
D2038	S2038	1056	AfuAfuGfaUfcUfGfCfaGfccaUfuAfaAfL96	AS2038	2148	uUfuAfaUfGfGfcUfgcaGfaUfcAfuAfusUfsu				
D2039	S2039	1057	AfuAfuGfaUfcUfGfCfaGfcCfAfUfuAfaAfL96	AS2039	2149	uUfuAfaugGfcUfgcaGfaUfcAfuAfusUfsu				
D2040	S2040	1058	UfaUfgAfuCfuGfCfAfgCfcauUfaAfaAfL96	AS2040	2150	uUfuUfaAfUfGfgCfugcAfgAfuCfaUfasUfsu				
D2041	S2041	1059	UfaUfgAfuCfuGfCfAfgCfcAfUfUfaAfaAfL96	AS2041	2151	uUfuUfaauGfgCfugcAfgAfuCfaUfasUfsu				
D2042	S2042	1060	AfuGfaUfcUfgCfAfGfcCfauuAfaAfaAfL96	AS2042	2152	uUfuUfuAfAfUfgGfcugCfaGfaUfcAfusAfsu				
D2043	S2043	1061	AfuGfaUfcUfgCfAfGfcCfaUfUfAfaAfaAfL96	AS2043	2153	uUfuUfuaaUfgGfcugCfaGfaUfcAfusAfsu				
D2044	S2044	1062	UfgAfuCfuGfcAfGfCfcAfuuaAfaAfaGfL96	AS2044	2154	cUfuUfuUfAfAfuGfgcuGfcAfgAfuCfasUfsa				
D2045	S2045	1063	UfgAfuCfuGfcAfGfCfcAfuUfAfAfaAfaGfL96	AS2045	2155	cUfuUfuuaAfuGfgcuGfcAfgAfuCfasUfsa				
D2046	S2046	1064	GfaUfcUfgCfaGfCfCfaUfuaaAfaAfgAfL96	AS2046	2156	uCfuUfuUfUfAfaUfggcUfgCfaGfaUfcsAfsu				

D2047	S2047	1065	GfaUfcUfgCfaGfCfCfaUfuAfAfAfaAfgAfL96	AS2047	2157	uCfuUfuuuAfaUfggcUfgCfaGfaUfcsAfsu					
D2048	S2048	1066	AfuCfuGfcAfgCfCfAfuUfaaaAfaGfaCfL96	AS2048	2158	gUfcUfuUfUfUfaAfuggCfuGfcAfgAfusCfsa					
D2049	S2049	1067	AfuCfuGfcAfgCfCfAfuUfaAfAfAfaGfaCfL96	AS2049	2159	gUfcUfuuuUfaAfuggCfuGfcAfgAfusCfsa					
D2050	S2050	1068	UfcUfgCfaGfcCfAfUfuAfaaaAfgAfcAfL96	AS2050	2160	uGfuCfuUfUfUfUfaAfaugGfcUfgCfaGfasUfsc					
D2051	S2051	1069	UfcUfgCfaGfcCfAfUfuAfaAfAfAfgAfcAfL96	AS2051	2161	uGfuCfuuuUfuAfaugGfcUfgCfaGfasUfsc					
D2052	S2052	1070	CfuGfcAfgCfcAfUfUfaAfaaaGfaCfaCfL96	AS2052	2162	gUfgUfcUfUfUfUfaauGfgCfuGfcAfgsAfsu					
D2053	S2053	1071	CfuGfcAfgCfcAfUfUfaAfaAfAfGfaCfaCfL96	AS2053	2163	gUfgUfcuuUfuUfaauGfgCfuGfcAfgsAfsu					
D2054	S2054	1072	UfgCfaGfcCfaUfUfAfaAfaagAfcAfcAfL96	AS2054	2164	uGfuGfuCfUfUfUfuaaUfgGfcUfgCfasGfsa					
D2055	S2055	1073	UfgCfaGfcCfaUfUfAfaAfaAfGfAfcAfcAfL96	AS2055	2165	uGfuGfucuUfuUfuaaUfgGfcUfgCfasGfsa					
D2056	S2056	1074	GfcAfgCfcAfuUfAfAfaAfagaCfaCfaUfL96	AS2056	2166	aUfgUfgUfCfUfUfUfuuaAfuGfgCfuGfcsAfsg					
D2057	S2057	1075	GfcAfgCfcAfuUfAfAfaAfaGfAfCfaCfaUfL96	AS2057	2167	aUfgUfgucUfuUfUfuuaAfuGfgCfuGfcsAfsg					
D2058	S2058	1076	CfaGfcCfaUfuAfAfAfaAfgacAfcAfuUfL96	AS2058	2168	aAfuGfuGfUfCfuUfuuuAfaUfgGfcUfgsCfsa					
D2059	S2059	1077	CfaGfcCfaUfuAfAfAfaAfgAfCfAfcAfuUfL96	AS2059	2169	aAfuGfuguCfuUfuuuAfaUfgGfcUfgsCfsa					
D2060	S2060	1078	AfgCfcAfuUfaAfAfAfaGfacaCfaUfuCfL96	AS2060	2170	gAfaUfgUfGfUfCfuUfuuuUfaAfuGfgCfusGfsc					
D2061	S2061	1079	AfgCfcAfuUfaAfAfAfaGfaCfAfCfaUfuCfL96	AS2061	2171	gAfaUfgugUfcUfuuuUfaAfuGfgCfusGfsc					
D2062	S2062	1080	GfcCfaUfuAfaAfAfAfgAfcacAfuUfcUfL96	AS2062	2172	aGfaAfuGfUfGfuCfuuuUfuAfaUfgGfcsUfsg					
D2063	S2063	1081	GfcCfaUfuAfaAfAfAfgAfcAfCfAfuUfcUfL96	AS2063	2173	aGfaAfuguGfuCfuuuUfuAfaUfgGfcsUfsg					
D2064	S2064	1082	CfcAfuUfaAfaAfAfGfaCfacaUfuCfuGfL96	AS2064	2174	cAfgAfaUfGfUfgUfcuuUfuUfaAfuGfgsCfsu					
D2065	S2065	1083	CfcAfuUfaAfaAfAfGfaCfaCfAfUfuCfuGfL96	AS2065	2175	cAfgAfaugUfgUfcuuUfuUfaAfuGfgsCfsu					
D2066	S2066	1084	CfaUfuAfaAfaAfGfAfcAfcauUfcUfgUfL96	AS2066	2176	aCfaGfaAfUfGfuGfucuUfuUfuAfaUfgsGfsc					
D2067	S2067	1085	CfaUfuAfaAfaAfGfAfcAfcAfUfUfcUfgUfL96	AS2067	2177	aCfaGfaauGfuGfucuUfuUfuAfaUfgsGfsc					
D2068	S2068	1086	AfuUfaAfaAfaGfAfCfaCfauuCfuGfuAfL96	AS2068	2178	uAfcAfgAfAfUfgUfgucUfuUfuUfaAfusGfsg					
D2069	S2069	1087	AfuUfaAfaAfaGfAfCfaCfaUfUfCfuGfuAfL96	AS2069	2179	uAfcAfgaaUfgUfgucUfuUfuUfaAfusGfsg					
D2070	S2070	1088	UfuAfaAfaAfgAfCfAfcAfuucUfgUfaAfL96	AS2070	2180	uUfaCfaGfAfAfuGfuguCfuUfuUfuAfasUfsg					
D2071	S2071	1089	UfuAfaAfaAfgAfCfAfcAfuUfCfUfgUfaAfL96	AS2071	2181	uUfaCfagaAfuGfuguCfuUfuUfuAfasUfsg					
D2072	S2072	1090	UfaAfaAfaGfaCfAfCfaUfucuGfuAfaAfL96	AS2072	2182	uUfuAfcAfGfAfaUfgugUfcUfuUfuUfasAfsu					
D2073	S2073	1091	UfaAfaAfaGfaCfAfCfaUfuCfUfGfuAfaAfL96	AS2073	2183	uUfuAfcagAfaUfgugUfcUfuUfuUfasAfsu					

D2074	S2074	1092	AfaAfaAfgAfcAfCfAfuUfcugUfaAfaAfL96	AS2074	2184	uUfuUfaCfAfGfaAfuguGfuCfuUfuUfusAfsa				
D2075	S2075	1093	AfaAfaAfgAfcAfCfAfuUfcUfGfUfaAfaAfL96	AS2075	2185	uUfuUfacaGfaAfuguGfuCfuUfuUfusAfsa				
D2076	S2076	1094	AfaAfaGfaCfaCfAfUfuCfuguAfaAfaAfL96	AS2076	2186	uUfuUfuAfCfAfgAfaugUfgUfcUfuUfusUfsa				
D2077	S2077	1095	AfaAfaGfaCfaCfAfUfuCfuGfUfAfaAfaAfL96	AS2077	2187	uUfuUfuacAfgAfaugUfgUfcUfuUfusUfsa				
D2078	S2078	1096	AfaAfgAfcAfcAfUfUfcUfguaAfaAfaAfL96	AS2078	2188	uUfuUfuUfAfCfaGfaauGfuGfuCfuUfusUfsu				
D2079	S2079	1097	AfaAfgAfcAfcAfUfUfcUfgUfAfAfaAfaAfL96	AS2079	2189	uUfuUfuuaCfaGfaauGfuGfuCfuUfusUfsu				
D2080	S2080	1098	AfaGfaCfaCfaUfUfCfuGfuaaAfaAfaAfL96	AS2080	2190	uUfuUfuUfUfAfcAfgaaUfgUfgUfcUfusUfsu				
D2081	S2081	1099	AfaGfaCfaCfaUfUfCfuGfuAfAfAfaAfaAfL96	AS2081	2191	uUfuUfuuuAfcAfgaaUfgUfgUfcUfusUfsu				
D2082	S2082	1100	AfgAfcAfcAfuUfCfUfgUfaaaAfaAfaAfL96	AS2082	2192	uUfuUfuUfUfUfaCfagaAfuGfuGfuCfusUfsu				
D2083	S2083	1101	AfgAfcAfcAfuUfCfUfgUfaAfAfAfaAfaAfL96	AS2083	2193	uUfuUfuuuUfaCfagaAfuGfuGfuCfusUfsu				
D2084	S2084	1102	GfaCfaCfaUfuCfUfGfuAfaaaAfaAfaAfL96	AS2084	2194	uUfuUfuUfUfUfuAfcagAfaUfgUfgUfcsUfsu				
D2085	S2085	1103	GfaCfaCfaUfuCfUfGfuAfaAfAfAfaAfaAfL96	AS2085	2195	uUfuUfuuuUfuAfcagAfaUfgUfgUfcsUfsu				
D2086	S2086	1104	AfcAfcAfuUfcUfGfUfaAfaaaAfaAfaAfL96	AS2086	2196	uUfuUfuUfUfUfuUfacaGfaAfuGfuGfusCfsu				
D2087	S2087	1105	AfcAfcAfuUfcUfGfUfaAfaAfAfAfaAfaAfL96	AS2087	2197	uUfuUfuuuUfuUfacaGfaAfuGfuGfusCfsu				
D2088	S2088	1106	CfaCfaUfuCfuGfUfAfaAfaaaAfaAfaAfL96	AS2088	2198	uUfuUfuUfUfUfuUfuacAfgAfaUfgUfgsUfsc				
D2089	S2089	1107	CfaCfaUfuCfuGfUfAfaAfaAfAfAfaAfaAfL96	AS2089	2199	uUfuUfuuuUfuUfuacAfgAfaUfgUfgsUfsc				
D2090	S2090	1108	AfcAfuUfcUfgUfAfAfaAfaaaAfaAfaAfL96	AS2090	2200	uUfuUfuUfUfUfuUfuuaCfaGfaAfuGfusGfsu				
D2091	S2091	1109	AfcAfuUfcUfgUfAfAfaAfaAfAfAfaAfaAfL96	AS2091	2201	uUfuUfuuuUfuUfuuaCfaGfaAfuGfusGfsu				

Lowercase nucleotides (a, u, g, c) are 2'-O-methyl nucleotides; Nf (e.g., Af) is a 2'-fluoro nucleotide; s is a phosphothiorate linkage; L96 indicates a $GalNAc_3$ ligand.

Example 4: *In vitro* screening of RNAi Agents

Cell culture and transfections

Human Hep3B cells or rat H.II.4.E cells (ATCC, Manassas, VA) were grown to near confluence at 37 °C in an atmosphere of 5% CO2 in RPMI (ATCC) supplemented with 10% FBS, streptomycin, and glutamine (ATCC) before being released from the plate by trypsinization. Transfection was carried out by adding 14.8 µl of Opti-MEM plus 0.2 µl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) to 5 µl of siRNA duplexes per well into a 96-well plate and incubated at room temperature for 15 minutes. 80 µl of complete growth media without antibiotic containing ~2 x104 Hep3B cells were then added to the siRNA mixture. Cells were incubated for either 24 or 120 hours prior to RNA purification. Single dose experiments were performed at 10nM and 0.1nM final duplex concentration and dose response experiments were done using 8, 4 fold serial dilutions with a maximum dose of 10nM final duplex concentration.

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

Cells were harvested and lysed in 150 µl of Lysis/Binding Buffer then mixed for 5 minutes at 850rpm using an Eppendorf Thermomixer (the mixing speed was the same throughout the process). Ten microliters of magnetic beads and 80 µl Lysis/Binding Buffer mixture were added to a round bottom plate and mixed for 1 minute. Magnetic beads were captured using magnetic stand and the supernatant was removed without disturbing the beads. After removing the supernatant, the lysed cells were added to the remaining beads and mixed for 5 minutes. After removing the supernatant, magnetic beads were washed 2 times with 150 µl Wash Buffer A and mixed for 1 minute. Beads were capture again and supernatant removed. Beads were then washed with 150 µl Wash Buffer B, captured and supernatant was removed. Beads were next washed with 150 µl Elution Buffer, captured and supernatant removed. Beads were allowed to dry for 2 minutes. After drying, 50 µl of Elution Buffer was added and mixed for 5 minutes at 70°C. Beads were captured on magnet for 5 minutes. 40 µl of supernatant was removed and added to another 96 well plate.

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

A master mix of 1 µl 10X Buffer, 0.4µl 25X dNTPs, 1µl Random primers, 0.5 µl Reverse Transcriptase, 0.5 µl RNase inhibitor and 1.6µl of H_2O per reaction were added into 5 µl total RNA. cDNA was generated using a Bio-Rad C-1000 or S-1000 thermal cycler (Hercules, CA) through the following steps: 25 °C 10 min, 37 °C 120 min, 85 °C 5 sec, 4 °C hold.

Real time PCR

2µl of cDNA were added to a master mix containing 0.5µl GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E (human) Cat # 4308313 (rodent)), 0.5µl TTR TaqMan probe (Applied Biosystems cat # HS00174914 _m1 (human) cat # Rn00562124_m1 (rat)) and 5µl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well plate (Roche cat # 04887301001). Real time PCR was done in a Roche LC 480 Real Time PCR machine (Roche). Each duplex was tested in at least two independent transfections and each transfection was assayed in duplicate, unless otherwise noted.

To calculate relative fold change, real time data were analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC_{50}s were calculated using a 4 parameter fit model using XLFit and normalized to cells transfected with AD-1955 (sense sequence: cuuAcGcuGAGuAcuucGAdTsdT (SEQ ID NO: 2202); antisense sequence: UCGAAGuCUcAGCGuAAGdTsdT (SEQ ID NO: 2203)) or naïve cells over the same dose range, or to its own lowest dose. IC_{50}s were calculated for each individual transfection as well as in combination, where a single IC_{50} was fit to the data from both transfections.

The results of gene silencing of the exemplary siRNA duplex with various motif modifications of the invention are shown in Table 1 above.

Example 5: *In vitro* Silencing Activity of Chemically Modified RNAi Agents that Target TTR

The following experiments demonstrated the beneficial effects of chemical modifications, including the introduction of triplet repeat motifs, together with a $GalNAc_3$ ligand, on the silencing activity of RNAi agents that target TTR. The sequences of the agents investigated are provided in Table 2 below. The regions of complementarity to the TTR mRNA are as follows: the region of complementarity of RNAi agents AD-45165, AD-51546 and AD-51547 is GGATGGGATTTCATGTAACCAAGA (SEQ ID NO: 2204) and the region or complemetarity of RNAi agents AD-45163, AD-51544, and AD-51545 is TTCATGTAACCAAGAGTATTCCAT (SEQ ID NO: 2205).

Protocol for assessment of IC_{50} in Hep3B cells

The IC_{50} for each modified siRNA was determined in Hep3B cells (a human hepatoma cell line) by standard reverse transfection using Lipofectamine RNAiMAX. In brief, reverse transfection was carried out by adding 5 µL of Opti-MEM to 5 µL of siRNA duplex per well into a 96-well plate along with 10 µL of Opti-MEM plus 0.5 µL of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad CA. cat # 13778-150) and incubating at room temperature for 15-20 minutes. Following incubation, 100 µL of complete growth media without antibiotic containing 12,000-15,000 Hep3B cells was then added to each well. Cells were incubated for 24 hours at 37°C in an atmosphere of 5% CO_2 prior to lysis and analysis of TTR and GAPDH mRNA by bDNA (Quantigene). Seven different siRNA concentrations ranging from 10nM to 0.6pM were assessed for IC_{50} determination and TTR/GAPDH for siRNA transfected cells was normalized to cells transfected with 10nM Luc siRNA. The results are shown in Table 2.

Protocol for assessment of free-uptake IC_{50}

Free uptake silencing in primary cynomolgus hepatocytes was assessed following incubation with TTR siRNA for either 4 hours or 24 hours. Silencing was measured at 24 hours from the initial exposure. In brief, 96-well culture plates were coated with 0.05%-0.1% collagen (Sigma C3867-1VL) at room temperature, 24 hours

prior to the start of the experiment. On the day of assay, siRNAs were diluted in pre-warmed Plating Media consisting of DMEM supplemented with GIBCO's Maintenance Media Kit (Serum-Free, Life Technologies CM4000), and added to the collagen-coated 96-well culture plates. Cryopreserved primary cynomolgus hepatocytes were rapidly thawed in a 37°C water bath, and immediately diluted in Plating Media to a concentration of 360,000 cells/mL. A volume of cell suspension was gently pipetted on top of the pre-plated siRNAs such that the final cell count was 18,000 cells/well. The plate was lightly swirled to mix and spread cells evenly across the wells and placed in a 37°C, 5% CO_2 incubator for 24 hours prior to lysis and analysis of TTR and GAPDH mRNA by bDNA (Quantigene, Affymetrix). In the case of the 4h incubation with siRNA, the media was decanted after 4 hours of exposure to the cells, and replaced with fresh Plating Media for the remaining 20 hours of incubation. Downstream analysis for TTR and GAPDH mRNA was the same as described above. For a typical dose reponse curve, siRNAs were titrated from 1uM to 0.24nM by 4 fold serial dilution.

Table 2: *In vitro* Activity Summary for Alternating TTR-GalNAc and Variants with Triplet Motifs

Duplex ID	S (5'-3')	AS (5'-3")	Free-Uptake IC50 (μM)		Hep3B IC50 (nM)
			4h	24h	
AD-45163	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfaUfL96 (SEQ ID NO: 2206)	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfsa (SEQ ID NO: 2212)	0.04101	0.00820	0.0115
AD-51544	AfuGfuAfaCfcAf**Af**GfaGfuAfu**u**cCfaUfL96 (SEQ ID NO: 2207)	aUfgGf**Af**AfuAfcUfc**uu**GfgUfuAfcAfusGfsa (SEQ ID NO: 2213)	0.00346	0.00374	0.0014
AD-51545	AfuGfuAf**Af**CfcAf**Af**GfaGfuAfuUfcCfaUfL96 (SEQ ID NO: 2208)	aUfgGfaAfuAfcUfc**uu**Gfg**u**uAfcAfusGfsa (SEQ ID NO: 2214)	0.00395	0.00389	0.0018
AD-45165	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfgAfL96 (SEQ ID NO: 2209)	uCfuUfgGfuUfaCfaUfgAfaAfuCfcCfasUfsc (SEQ ID NO: 2215)	0.02407	0.00869	0.0112
AD-51546	UfgGf**Gf**AfuUfuCf**Af**UfgUfaAfcCf**Af**AfgAfL96 (SEQ ID NO: 2210)	uCfu**u**gGfuUfaCfa**u**gAfaAfu**cc**CfasUfsc (SEQ ID NO: 2216)	0.00317	0.00263	0.0017
AD-51547	UfgGfgAfuUfuCf**Af**UfgUfa**ac**CfaAfgAfL96 (SEQ ID NO: 2211)	uCfuUfgGf**Uf**UfaCfa**u**gAfaAfuCfcCfasUfsc (SEQ ID NO: 2217)	0.00460	0.00374	0.0028

Lowercase nucleotides (a, u, g, c) indicate 2'-O-methyl nucleotides; Nf (e.g., Af) indicates a 2'-fluoro nucleotide; s indicates a phosphothiorate linkage; L96 indicates a $GalNAc_3$ ligand; bold nucleotides indicate changes relative to the corresponding parent agent. Each bold nucleotide is at the center of a triplet motif.

The results are provided in Table 2 and demonstrate that modified RNAi agents that target TTR provide enhanced silencing activity.

Results: Improved Activity of Modified RNAi Agents

Parent RNAi agents with alternating chemical modifications and a $GalNAc_3$ ligand provided an IC_{50} in Hep3B cells of about 0.01 nM. As shown in Figures 4-5 and in Table 2, agents modified relative to the parent agents, for example, by the addition of one or more repeating triplets of 2'-fluoro and 2'-O-methyl modifications, showed unexpectedly enhanced silencing activity, achieving IC_{50} values in Hep3B cells that were 5-8 fold better than the corresponding parent agent.

Results: Free Uptake IC_{50}s in Hep3B cells

As shown in Table 2 and Figures 6-7, RNAi agents modified relative to the parent AD-45163 also showed enhanced free uptake silencing. The modified agents showed more than double the silencing activity of the parent after a 24 hour incubation period and nearly 10 times the silencing activity of the parent after a 4 hour incubation period.

As shown in Table 2 and Figures 8-9, RNAi agents modified relative to the parent AD-45165 also showed enhanced free uptake silencing. The modified agents showed 2-3 times the silencing activity of the parent after a 24 hour incubation period and 5-8 times the silencing activity of the parent after a 4 hour incubation period.

Taken collectively, these results demonstrate that the modified RNAi agents presented herein, e.g., AD-51544, AD-51545, AD-51546, and AD-51547, all showed unexpectedly good inhibition of TTR mRNA in *in vitro* silencing experiments.

Example 6: TTR mRNA silencing and TTR Protein Suppression in Transgenic Mice

To assess the efficacy of the RNAi agents AD-45163, AD-51544, AD-51545, AD45165, AD-51546, and AD-51547, these agents were administered to transgenic mice that express human transthyretin with the V30M mutation (see Santos, SD., Fernaandes, R., and Saraiva, MJ. (2010) *Neurobiology of Aging*, 31, 280-289). The V30M mutation is known to cause familial amyloid polyneuropathy type I in humans. See, *e.g.*, Lobato, L. (2003) *J Nephrol.*, 16(3):438-42.

The RNAi agents (in PBS buffer) or PBS control were administered to mice (2 male and 2 female) of 18-24 months of age in a single subcutaneous dose of 5 mg/kg or 1 mg/kg. After approximately 48 hours, mice were anesthetized with 200 µl of ketamine, and then exsanguinated by severing the right caudal artery. Whole blood was isolated and plasma was isolated and stored at -80°C until assaying. Liver tissue was collected, flash-frozen and stored at -80°C until processing.

Efficacy of treatment was evaluated by (i) measurement of TTR mRNA in liver at 48 hours post-dose, and (ii) measurement of TTR protein in plasma at pre-bleed and at 48 hours post-dose. TTR liver mRNA levels were assayed utilizing the Branched DNA assays- QuantiGene 2.0 (Panomics cat #: QS0011). Briefly, mouse liver samples were ground and tissue lysates were prepared. Liver lysis mixture (a mixture of 1 volume of lysis mixture, 2 volume of nuclease-free water and 10ul of Proteinase-K/ml for a final concentration of 20mg/ml) was incubated at 65 °C for 35 minutes. 20µl of Working Probe Set (TTR probe for gene target and GAPDH for endogenous control) and 80ul of tissue-lysate were then added into the Capture Plate. Capture Plates were incubated at 55 °C ±1 °C (aprx. 16-20hrs). The next day, the Capture Plates were washed 3 times with 1X Wash Buffer (nuclease-free water, Buffer Component 1 and Wash Buffer Component 2), then dried by centrifuging for 1 minute at 240g. 100µl of pre-Amplifier Working Reagent was added into the Capture Plate, which was sealed with aluminum foil and incubated for 1 hour at 55°C ±1°C. Following 1 hour incubation, the wash step was repeated, then 100µl of Amplifier Working Reagent was added. After 1 hour, the wash and dry steps were repeated, and 100µl of Label Probe was added. Capture plates

were incubated 50 °C ±1 °C for 1 hour. The plate was then washed with 1X Wash Buffer, dried and 100µl Substrate was added into the Capture Plate. Capture Plates were read using the SpectraMax Luminometer following a 5 to 15 minute incubation. bDNA data were analyzed by subtracting the average background from each triplicate sample, averaging the resultant triplicate GAPDH (control probe) and TTR (experimental probe) values, and then computing the ratio: (experimental probe-background)/(control probe-background).

Plasma TTR levels were assayed utilizing the commercially available kit "AssayMax Human Prealbumin ELISA Kit" (AssayPro, St. Charles, MO, Catalog # EP3010-1) according to manufacturer's guidelines. Briefly, mouse plasma was diluted 1:10,000 in 1X mix diluents and added to pre-coated plates along with kit standards, and incubated for 2 hours at room temperature followed by 5X washes with kit wash buffer. Fifty microliters of biotinylated prealbumin antibody was added to each well and incubated for 1 hr at room temperature, followed by 5X washes with wash buffer. Fifty microliters of streptavidin-peroxidase conjugate was added to each well and plates were incubated for 30 minutes at room temperature followed by washing as previously described. The reaction was developed by the addition of 50 µl/well of chromogen substrate and incubation for 10 minutes at room temperature with stopping of reaction by the addition of 50 µl/well of stop solution. Absorbance at 450 nm was read on a Versamax microplate reader (Molecular Devices, Sunnyvale, CA) and data were analyzed utilizing the Softmax 4.6 software package (Molecular Devices).

The results are shown in Figures 10-12. Figure 10 shows that the RNAi agents modified relative to the parent agents AD-45163 and AD-45165 showed RNA silencing activity that was similar or more potent compared with that of the parent agents. Figure 11 shows that the agents AD-51544 and AD-51545 showed dose dependent silencing activity and that the silencing activity of these agents at a dose of 5mg/kg was similar to that of the corresponding parent AD-45163. Figure 12 shows that the agents AD-51546 and AD-51547 also showed dose-dependent silencing activity. Furthermore, the silencing activity of AD-51546 and AD-51547 at a dose of 5mg/kg was superior to that of the corresponding parent AD-45165.

Example 7: Serum and Liver Pharmacokinetic Profiles of RNAi Agents that Target TTR in Mice

To assess the pharmacokinetic profiles of the RNAi agents AD-45163, AD-51544, AD-51545, AD-51546, and AD-51547, these agents, in PBS buffer, were administered to C57BL/6 mice using a single IV bolus or subcutaneous (SC) administration. The plasma concentrations and liver concentrations of the agents were assessed at various timepoints after the administration.

The plasma pharmacokinetic parameters are presented in Tables 3 and 4 below. The mean resident time (MRT) in plasma was about 0.2 hours after IV dosing and about 1 hour after SC dosing. At a dose of 25 mg/kg, the agents AD-51544, AD-51545, AD-51546, and AD-51547 showed similar plasma pharmacokinetic properties. Each of these agents had more than 75% bioavailability from the subcutaneous space. Their bioavailability was superior to that of the parent agent AD-45163 that was administered at a higher dose of 30 mg/kg. The subcutaneous bioavailability of AD-51544 and AD-51547 was about 100%, whereas that of AD-51545 was 90% and that of and AD-51546 was 76%.

Table 3: Summary of Plasma PK Parameter Estimates After SC Administration of TTR-GalNAc siRNAs in Mice

Parameter	30 mpk AD-45163 (h/c TTR-GalNAc)	25 mpk AD-51544 (h/c TTR-GalNAc)	25 mpk AD-51545 (h/c TTR-GalNAc)	25 mpk AD-51546 (h/c TTR-GalNAc)	25 mpk AD-51547 (h/c TTR-GalNAc)
Plasma Tmax (h)	0.25	1	0.5	1	0.5
Plasma Cmax (μg/mL)	9.6	11.7	10.9	11.7	12.1
Plasma AUC (h*μg/mL)	12.4	21.9	19.9	20.9	25.3
F_{sc}(%)	79	100	90.1	76.0	99.2

Table 4: Plasma siRNA PK Parameters in Mice after an IV Bolus or SC Dose of AD-51544, 51545, 51546 or 51547 at 25 mg/kg

Test Article	AD-51544		AD-51545		AD-51546		AD-51547	
siRNA Dose (mg/kg)	25		25		25		25	
Route of Administration	IV	SC	IV	SC	IV	SC	IV	SC
t_{max} (h)	0.083	1	0.083	0.5	0.083	1	0.083	0.5
C_{max} (µg/mL)	96.5[a]	11.7	108[a]	10.9	128[a]	10.9	123[a]	12.1
$AUC_{0\text{-last}}$ (h·µg/mL)	21.6	21.9	22.1	19.9	27.5	20.9	25.5	25.3
$MRT_{0\text{-last}}$ (h)	0.17	1.2	0.16	1.1	0.22	1.4	0.19	1.3
Apparent $t_{1/2\beta}$ (h)[b]	ND	ND	ND	0.49	ND	1.2	ND	0.56
F_{SC} (%)[c]	-	102	-	90.1	-	76.0	-	99.2

a: Concentration at the 1st sampling time (5 min) after IV dosing
b: Apparent elimination half-life ($t_{1/2\beta}$) could not be determined (ND) for all 4 test articles after IV dosing as the terminal phase of the concentration-time profiles was not well defined, as a result, the $t_{1/2\beta}$-associated PK parameters (eg, $AUC_{0\text{-}\infty}$, CL and Vss) were not reported.
c: SC bioavailability, calculated as percentage ratio of $AUC_{0\text{-last}}$ after SC and IV dosing at 25 mg/kg

The results also indicated that the RNAi agents AD-45163, AD-51544, AD-51545, AD-51546, and AD-51547 achieved similar or higher concentrations in the liver when administered subcutaneously than when administered by IV bolus. The liver pharmacokinetic parameters are presented in Tables 5 and 6 below. The peak concentration (C_{max}) and area under the curve ($AUC_{0\text{-}last}$) in the liver were two to three times higher after subcutaneous administration as compared with IV administration of the same agent at the same dose. Liver exposures were highest for AD-51547 and lowest for AD-51545. The mean resident time (MRT) and elimination half-life were longer for AD-51546 and AD-51547 compared with AD-51544 and AD-51545. Following subcutaneous administration, the approximate MRTs were 40 hours for AD-51546 and 25 hours for AD-51547, whereas the MRTs for AD-51544 and AD-51545 were lower (about 6-9 hours). The elimination half life of AD-51546 and AD-51547 was also higher (41-53 hours) than was the elimination half life of AD-51544 and AD-51545 (6-10 hours).

Table 5: Summary of Liver PK Parameter Estimates After SC Administration of TTR-GalNAc siRNAs in Mice

Parameter	30 mpk AD-45163 (h/c TTR-GalNAc)	25 mpk AD-51544 (h/c TTR-GalNAc)	25 mpk AD-51545 (h/c TTR-GalNAc)	25 mpk AD-51546 (h/c TTR-GalNAc)	25 mpk AD-51547 (h/c TTR-GalNAc)
Liver Tmax (h)	8	4	4	2	8
Liver Cmax (μg/g)	313	126	80	117	174
Liver AUC (h*μg/g)	4519	1092	763	2131	4583

Table 6: Liver siRNA PK Parameters in Mice after an IV Bolus or SC Dose of AD-51544, 51545, 51546 or 51547 at 25 mg/kg

Test Article	AD-51544		AD-51545		AD-51546		AD-51547	
siRNA Dose (mg/kg)	25		25		25		25	
Route of Administration	IV	SC	IV	SC	IV	SC	IV	SC
t_{max} (h)	1	4	1	4	4	2	2	8
C_{max} (µg/g)	67.9	126	37.0	80.5	35.3	117	73.8	174
$AUC_{0\text{-last}}$ (h·µg/g)	632	1092	324	763	984	2131	1429	4583
$MRT_{0\text{-last}}$ (h)	8.7	6.5	5.9	8.5	45.7	40.2	29.4	25.3
Apparent $t_{1/2\beta}$ (h)	8.1	8.2	5.7	10.0	51.1	45.3	41.1	52.7

Example 8: *In vitro* Stability of RNAi Agents in Monkey Serum

The serum stability of RNAi agents AD-51544, AD-51545, AD-51546, and AD-51547 was also assessed in monkeys. The results demonstrated that the antisense and sense strands of AD-51544, AD-51545, and AD-51547 showed serum stability over a period of about 24 hours (data not shown).

Example 9: RNAi Agents Produce Lasting Suppression of TTR Protein in Non-Human Primates

The RNA silencing activity of RNAi agents AD-45163, AD-51544, AD-51545, AD-51546, and AD-51547 was assessed by measuring suppression of TTR protein in serum of cynomologous monkeys following subcutaneous administration of five 5 mg/kg doses (one dose each day for 5 days) or a single 25mg/kg dose. Pre-dose TTR protein levels in serum were assessed by averaging the levels at 11 days prior to the first dose, 7 days prior to the first dose, and 1 day prior to the first dose. Post-dose serum levels of TTR protein were assessed by determining the level in serum beginning at 1 day after the final dose (*i.e.*, study day 5 in the 5x5 mg/kg group and study day 1 in the 1x25 mg/kg group) until 49 days after the last dose (*i.e.*, study day 53 in the 5x5 mg/kg group and study day 49 in the 1x25 mg/kg group). See Figure 13.

TTR protein levels were assessed as described in Example 6. The results are shown in Figure 14 and in Tables 7 and 8.

A maximal suppression of TTR protein of up to about 50% was achieved in the groups that received 25 mg/kg of AD-45163, AD-51544, AD-51546, and AD-51547 (see Table 8). A greater maximal suppression of TTR protein of about 70% was achieved in the groups that received 5x5 mg/kg of AD-45163, AD-51544, AD-51546, and AD-51547 (see Table 7). The agent AD-51545 produced a lesser degree of suppression in both administration protocols. Significant suppression of about 20% or more persisted for up to 49 days after the last dose of AD-51546 and AD-51547 in both the 1x25 mg/kg and 5x5 mg/kg protocols. Generally, better suppression was achieved in the 5x5 mg/kg protocol than in the 1x25 mg/kg protocol.

Table 7 Fraction Serum Transthyretin Relative to Pre-dose in Cynomolgus Monkeys (5 mg/kg daily for 5 days)

	D-11	D-7	D-1	D5	D7	D9	D11	D14	D18	D22	D26	D32	D39	D46	D53
AD-45163	0.98	0.99	1.03	0.71	0.52	0.40	0.34	0.27	0.31	0.39	0.48	0.64	0.68	0.81	0.88
AD-51544	1.02	0.99	0.99	0.60	0.47	0.37	0.35	0.39	0.48	0.58	0.66	0.74	0.83	0.91	0.92
AD-51545	1.03	0.97	1.00	0.73	0.65	0.63	0.69	0.68	0.78	0.87	0.97	1.00	1.03	1.06	1.09
AD-51546	1.01	0.97	1.02	0.59	0.42	0.35	0.30	0.32	0.43	0.58	0.66	0.77	0.92	0.93	0.97
AD-51547	0.99	0.99	1.02	0.74	0.54	0.41	0.34	0.34	0.39	0.49	0.51	0.53	0.65	0.70	0.77

Table 8 Fraction Serum Transthyretin Relative to Pre-dose in Cynomolgus Monkeys (25 mg/kg)

	D-11	D-7	D-1	D1	D3	D5	D7	D10	D14	D18	D22	D28	D35	D42	D49
AD-45163	1.04	1.01	0.95	0.99	0.84	0.67	0.57	0.44	0.45	0.51	0.58	0.66	0.72	0.78	0.85
AD-51544	1.01	1.04	0.95	0.92	0.69	0.57	0.49	0.48	0.56	0.65	0.69	0.77	0.83	0.87	0.94
AD-51545	0.98	1.02	0.99	0.87	0.77	0.69	0.71	0.72	0.84	0.90	0.92	0.99	1.00	1.00	1.00
AD-51546	1.04	1.03	0.93	0.89	0.71	0.62	0.53	0.50	0.55	0.70	0.70	0.69	0.72	0.79	0.84
AD-51547	0.96	1.03	1.01	1.19	0.90	0.70	0.54	0.48	0.50	0.50	0.52	0.58	0.62	0.70	0.72

Example 10: Tolerability of RNAi Agents that Target TTR

In Cytokine Evaluation in Whole Blood Assay

To assess the tolerability of RNAi agents that target TTR (including AD-45163, AD-51544, AD-51545, AD-51546, and AD-51547), each agent was tested in a whole blood assay using blood from three human donors. The agents were either 300 nM DOTAP transfected or 1 μM without transfection reagent (free siRNA). There was less than a two fold change for the following cytokines/chemokines: G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα. (Results not shown).

In Vivo Evaluation

To assess *in vivo* tolerability, RNAi agents were injected subcutaneously in CD1 mice at a dose of 125 mg/kg. No cytokine induction was observed at 2, 4, 6, 24, or 48 hours after subcutaneous injection of AD-45163. No significant cytokine induction was observed at 6 or 24 hours after subcucutaneous injection of AD-51544, AD-51545, AD-51546, or AD-51547.

To further assess *in vivo* tolerability, multiple RNAi agents (including AD-45163, AD-51544, AD-51545, AD-51546, and AD-51547) were tested by subcutaneous injection of 5 and 25 mg in non-human primates (cynomologous monkeys) with dose volumes between 1-2 ml per site. No erythema or edema was observed at injection sites.

Single SC Dose Rat Tolerability Study

To assess toxicity, rats were injected with a single subcutaneous dose of 100, 250, 500, or 750 mg/kg of AD-45163 (see Table 9). The following assessments were made: clinical signs of toxicity, body weight, hematology, clinical chemistry and coagulation, organ weights (liver & spleen); gross and microscopic evaluation (kidney, liver, lung, lymph node, spleen, testes, thymus, aorta, heart, intestine (small and large).

Table 9: Single SC Dose Rat Tolerability Study: 100, 250, 500 & 750 mg/kg of AD-45163 in Sprague Dawley Rats

Group	Dose Level (mg/kg)	Dose Volume (ml/kg)	Route & Regimen	No. Male Sprague Dawley Rats	Day of Necropsy
PBS	0	10	SC Injection Day 1 (2 sites)	7/group (5 Tox animals, 2 TK animals)	Day 4
AD-45163 Parent	100				
	250				
	500				
	750				

The results showed no test article-related clinical signs of toxicity, effects on body weight, organ weights, or clinical chemistry. No histopathology was observed in heart, kidneys, testes, spleen, liver, and thymus. There was a non-adverse, slight test article-related increase in WBC (↑68%, primarily attributed to increase in NEUT and MONO) at 750 mg/kg. These results indicate that a single-dose of up to 750 mg/kg is well tolerated in rats.

Tolerability of Repeated Subcutaneous Administrations in Rats

To assess the tolerability of repeated subcutaneous administrations of AD-45163, daily subcutaneous injections of 300 mg/kg were given for 5 days, and a necropsy was performed on day 6. The study design is shown in Table 10.

Table 10: Five Day Repeat Dose Tolerability Study in Rat

Group	Dose Level (kmg/kg	Conc (mg/mL)	No of Tox Animals	Nx Day 6
PBS	0	0	2M, 2F	2M, 2F
AD-45163	300	150	2M, 2F	2M, 2F

The following outcome variables were assessed: clinical signs, body weights, hematology, clinical chemistry and coagulation, organ weights, gross and microscopic evaluation (liver, spleen, kidney, heart, GI tract and first and last injection site). The results showed no test article-related clinical signs, body weight or organ weight effects, and also no test article-related findings in clinical hematology or chemistry. There was a possible slight prolongation of activated partial thromboplastin time (APTT) on day 6 (20.4 vs. 17.4 sec). Histopathology revealed no test article-related findings in the liver, spleen, heart, and GI tract. In the kidney, minimal to slight hypertrophy of the tubular epithelium (not adverse) was observed. At the last injection site, there was minimal multifocal mononuclear infiltration, not adverse. These results indicate that five daily 300 mg/kg doses of the parent RNAi agent AD-45163 are well tolerated in rats.

Example 11: RNAi Agents Produce Lasting Suppression of TTR Protein in Non-Human Primates

The RNA silencing activity of RNAi agent AD-51547 was assessed by measuring suppression of TTR protein in the serum of cynomologous monkeys following subcutaneous administration of a "loading phase" of the RNAi agent: five daily doses of either 2.5 mg/kg, 5 mg/kg or 10 mg/kg (one dose each day for 5 days) followed by a "maintenance phase" of the RNAi agent: weekly dosing of either 2.5 mg/kg, 5 mg/kg or 10 mg/kg for 4 weeks. Pre-dose TTR protein levels in serum were assessed by averaging the levels at 11 days prior to the first dose, 7 days prior to the first dose, and 1 day prior to the first dose. Post-dose serum levels of TTR protein were assessed by determining the level in serum relative to pre-dose beginning at 1 day after

the loading phase was completed until 40 days after the last dose of the maintenance phase (*i.e.*, study day 70).

TTR protein levels were assessed as described in Example 6. The results are shown in Figure 15.

A maximal suppression of TTR protein of up to about 80% was achieved in all of the groups that received either 2.5 mg/kg, 5 mg/kg or 10 mg/kg of AD-51547. Nadir knockdown was achieved in all of the groups by about day 14, the suppression sustained at nadir knockdown levels with a weekly maintenance dose of either 2.5 mg/kg, 5 mg/kg or 10 mg/kg of AD-51547. The levels of TTR had not returned to baseline more than 40 days after the day of administration of the last maintenance dose for the 5 and 2.5 mg/kg dose levels.

Equivalents:

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

We claim:

1. A double stranded RNAi agent comprising a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to part of an mRNA encoding transthyretin (TTR), wherein each strand has about 14 to about 30 nucleotides, wherein said double stranded RNAi agent is represented by formula (III):

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

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

(III)

wherein:

i, j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;

each n_p, n_p', n_q, and n_q' independently represents an overhang nucleotide;

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;

modifications on N_b differ from the modification on Y and modifications on N_b' differ from the modification on Y'; and

wherein the sense strand is conjugated to at least one ligand.

2. The RNAi agent of claim 1, wherein i is 1; j is 1; or both i and j are 1.

3. The RNAi agent of claim 1, wherein k is 1; l is 1; or both k and l are 1.

4. The RNAi agent of claim 1, wherein XXX is complementary to X′X′X′, YYY is complementary to Y′Y′Y′, and ZZZ is complementary to Z′Z′Z′.

5. The RNAi agent of claim 1, wherein the YYY motif occurs at or near the cleavage site of the sense strand.

6. The RNAi agent of claim 1, wherein the Y′Y′Y′ motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

7. The RNAi agent of claim 6, wherein the Y′ is 2′-O-methyl.

8. The RNAi agent of claim 1, wherein formula (III) is represented as formula (IIIa):

sense: 5' n_p -N_a -Y Y Y -N_b -Z Z Z -N_a-n_q 3'

antisense: 3' n_p′-N_a′-Y′Y′Y′-N_b′-Z′Z′Z′-N_a′n_q′ 5'

(IIIa)

wherein each N_b and N_b′ independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

9. The RNAi agent of claim 1, wherein formula (III) is represented as formula (IIIb):

sense: 5' n_p-N_a-X X X -N_b-Y Y Y -N_a-n_q 3'

antisense: 3' n_p′-N_a′-X′X′X′-N_b′-Y′Y′Y′-N_a′-n_q′ 5'

(IIIb)

wherein each N_b and N_b′ independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides.

10. The RNAi agent claim 1, wherein formula (III) is represented as formula (IIIc):

sense: 5' n_p-N_a-X X X -N_b-Y Y Y -N_b-Z Z Z -N_a-n_q 3'

antisense: 3' n_p'-N_a'-X′X′X′-N_b'-Y′Y′Y′-N_b'-Z′Z′Z′-N_a'-n_q' 5'

(IIIc)

wherein each N_b and N_b' independently represents an oligonucleotide sequence comprising 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence comprising 2-10 modified nucleotides.

11. The RNAi agent of claim 1, wherein the duplex region is 15-30 nucleotide pairs in length.

12. The RNAi agent of claim 11, wherein the duplex region is 17-23 nucleotide pairs in length.

13. The RNAi agent of claim 11, wherein the duplex region is 17-25 nucleotide pairs in length.

14. The RNAi agent of claim 11, wherein the duplex region is 23-27 nucleotide pairs in length.

15. The RNAi agent of claim 11, wherein the duplex region is 19-21 nucleotide pairs in length.

16. The RNAi agent of claim 13, wherein the duplex region is 21-23 nucleotide pairs in length.

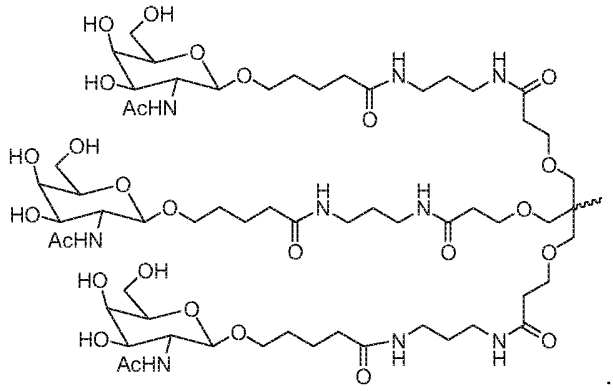
17. The RNAi agent of claim 1, wherein each strand has 15-30 nucleotides.

18. The RNAi agent of claim 1, wherein the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2′-methoxyethyl, 2′-O-alkyl, 2′-O-allyl, 2′-C- allyl, 2′-fluoro, 2′-deoxy, 2'-hydroxyl, and combinations thereof.

19. The RNAi agent of claim 18, wherein the modifications on the nucleotides are 2′-O-methyl,2′-fluoro or both.

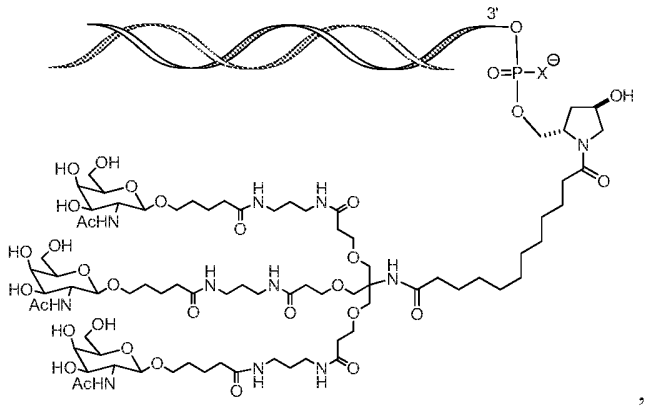
20. The RNAi agent of claim 1, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

21. The RNAi agent of claim 1, wherein the ligand is



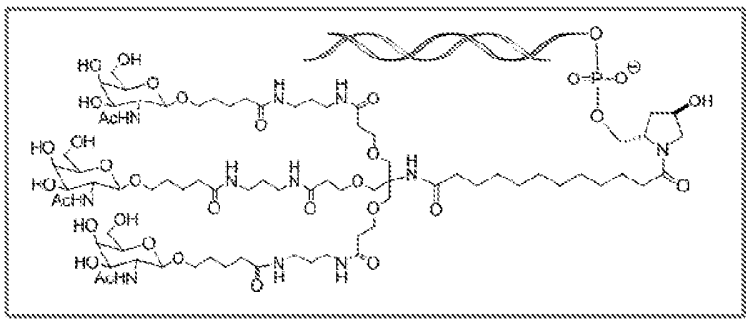
22. The RNAi agent of claim 1, wherein the ligand is attached to the 3' end of the sense strand.

23. The RNAi agent of claim 22, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

24. The RNAi agent of claim 23, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



25. The RNAi agent of claim 1 further comprising at least one phosphorothioate or methylphosphonate internucleotide linkage.

26. The RNAi agent of claim 25, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminal of one strand.

27. The RNAi agent of claim 26, wherein said strand is the antisense strand.

28. The RNAi agent of claim 26, wherein said strand is the sense strand.

29. The RNAi agent of claim 1, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.

30. The RNAi agent of claim 1, wherein the Y nucleotides contain a 2'-fluoro modification.

31. The RNAi agent of claim 1, wherein the Y' nucleotides contain a 2'-O-methyl modification.

32. The RNAi agent of claim 1, wherein p'>0.

33. The RNAi agent of claim 1, wherein p'=2.

34. The RNAi agent of claim 33, wherein q'=0, p=0, q=0, and p' overhang nucleotides are complementary to the target mRNA.

35. The RNAi agent of claim 33, wherein q'=0, p=0, q=0, and p' overhang nucleotides are non-complementary to the target mRNA.

36. The RNAi agent of claim 33, wherein the sense strand has a total of 21 nucleotides and the antisense strand has a total of 23 nucleotides.

37. The RNAi agent of any one of claims 32-36, wherein at least one np′ is linked to a neighboring nucleotide via a phosphorothioate linkage.

38. The RNAi agent of claim 37, wherein all np′ are linked to neighboring nucleotides via phosphorothioate linkages.

39. The RNAi agent of claim 1 selected from the group of RNAi agents listed in Table 1.

40. The RNAi agent of claim 1 selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547.

41. The RNAi agent of claim 40, wherein the RNAi agent is AD-51547.

42. A cell containing the double stranded RNAi agent of any one of claims 1 to 41.

43. A pharmaceutical composition comprising an RNAi agent of any one of claims 1 to 41.

44. The pharmaceutical composition of claim 43, wherein RNAi agent is administered in an unbuffered solution.

45. The pharmaceutical composition of claim 44, wherein said unbuffered solution is saline or water.

46. The pharmaceutical composition of claim 43, wherein said siRNA is administered with a buffer solution.

47. The pharmaceutical composition of claim 46, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

48. The pharmaceutical composition of claim 47, wherein said buffer solution is phosphate buffered saline (PBS).

49. The pharmaceutical composition of claim 43, wherein said pharmaceutical composition is a liposome.

50. The pharmaceutical composition of claim 43, wherein said pharmaceutical composition is a lipid formulation.

51. A method of inhibiting expression of a transthyretin (TTR) in a cell comprising contacting said cell with an RNAi agent of any one of claims 1 to 41 or with a pharmaceutical composition of any one of claims 43 to 50 in an amount effective to inhibit expression of said TTR in said cell, thereby inhibiting expression of said transthyretin (TTR) in said cell.

52. The method of claim 51, wherein expression of said TTR is inhibited by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

53. The method of claim 51, wherein said cell is contacted *in vitro* with said RNAi agent.

54. The method of claim 51, wherein said cell is present within a subject.

55. The method of claim 54, wherein said subject is a human.

56. The method of claim 54, wherein said subject is suffering from a TTR-associated disease and said effective amount is a therapeutically effective amount.

57. The method of claim 54, wherein said subject is a subject at risk for developing a TTR-associated disease and said effective amount is a prophylactically effective amount.

58. The method of claim 57, wherein said subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease.

59. The method of claim 56 or 57, wherein said TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

60. The method of claim 54, wherein said subject has a TTR-associated amyloidosis and said method reduces an amyloid TTR deposit in said subject.

61. The method of claim 54, wherein said RNAi agent is administered to said subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof.

62. The method of claim 54, wherein said RNAi agent is administered to said subject via subcutaneous, intramuscular or intravenous administration.

63. The method of claim 62, wherein said subcutaneous administration comprises administration via a subcutaneous pump or subcutaneous depot.

64. The method of claim 54, wherein said RNAi agent is administered to said subject, such that said RNAi agent is delivered to a specific site within said subject.

65. The method of claim 64, wherein said site is selected from the group consisting of liver, choroid plexus, retina, and pancreas.

66. The method of claim 64, wherein said site is the liver.

67. The method of claim 64, wherein delivery of said RNAi agent is mediated by an asialoglycoprotein receptor (ASGP-R) present in hepatocytes.

68. The method of claim 54, wherein said RNAi agent is administered at a dose of 0.05-50 mg/kg.

69. The method of claim 54, wherein said RNAi agent is administered in two or more doses.

70. The method of claim 69, wherein said RNAi agent is administered at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

71. The method of claim 54, further comprising assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

72. The method of claim 54, wherein administering said RNAi agent does not result in an inflammatory response in said subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from said subject.

73. The method of claim 54, wherein said RNAi agent is administered using a pharmaceutical composition.

74. The method of claim 73, wherein said siRNA is administered in an unbuffered solution.

75. The method of claim 74, wherein said unbuffered solution is saline or water.

76. The method of claim 73, wherein said siRNA is administered with a buffer solution.

77. The method of claim 76, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

78. The method of claim 77, wherein said buffer solution is phosphate buffered saline (PBS).

79. The method of claim 73, wherein said pharmaceutical composition is a liposome.

80. A method of treating or preventing a TTR-associated disease in a subject, comprising administering to said subject a therapeutically effective amount or a prophylactically effective amount of an RNAi agent of any one of claims 1 to 41 or a pharmaceutical composition of any one of claims 43 to 50 thereby treating or preventing said TTR-associated disease in said subject.

81. The method of claim 80, wherein TTR expression in a sample derived from said subject is inhibited by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

82. The method of claim 80, wherein said subject is a human.

83. The method of claim 80, wherein said subject is a subject suffering from a TTR-associated disease.

84. The method of claim 80, wherein said subject is a subject at risk for developing a TTR-associated disease.

85. The method of claim 80, wherein said subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease.

86. The method of claim 80, wherein said TTR-associated disease is selected from the group consisting of senile systemic amyloidosis (SSA), systemic familial amyloidosis, familial amyloidotic polyneuropathy (FAP), familial amyloidotic cardiomyopathy (FAC), leptomeningeal/Central Nervous System (CNS) amyloidosis, and hyperthyroxinemia.

87. The method of claim 80, wherein said subject has a TTR-associated amyloidosis and said method reduces an amyloid TTR deposit in said subject.

88. The method of claim 80, wherein said RNAi agent is administered to said subject by an administration means selected from the group consisting of subcutaneous, intravenous, intramuscular, intrabronchial, intrapleural, intraperitoneal, intraarterial, lymphatic, cerebrospinal, and any combinations thereof.

89. The method of claim 80, wherein said RNAi agent is administered to said subject via subcutaneous, intramuscular or intravenous administration.

90. The method of claim 89, wherein said subcutaneous administration comprises administration via a subcutaneous pump or subcutaneous depot.

91. The method of claim 80, wherein said RNAi agent is administered to said subject, such that said RNAi agent is delivered to a specific site within said subject.

92. The method of claim 91, wherein said site is selected from the group consisting of liver, choroid plexus, retina, and pancreas.

93. The method of claim 91 wherein said site is the liver.

94. The method of claim 91, wherein delivery of said RNAi agent is mediated by an asialoglycoprotein receptor (ASGP-R) present in hepatocytes.

95. The method of claim 80, wherein said RNAi agent is administered at a dose of 0.05-50 mg/kg.

96. The method of claim 80, wherein said RNAi agent is administered in two or more doses.

97. The method of claim 96, wherein said RNAi agent is administered at intervals selected from the group consisting of once every about 12 hours, once every about 24 hours, once every about 48 hours, once every about 72 hours, and once every about 96 hours.

98. The method of claim 80, further comprising assessing the level of TTR mRNA expression or TTR protein expression in a sample derived from the subject.

99. The method of claim 80, wherein administering said RNAi agent does not result in an inflammatory response in said subject as assessed based on the level of a cytokine or chemokine selected from the group consisting of G-CSF, IFN-γ, IL-10, IL-12 (p70), IL1β, IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, TNFα, and any combinations thereof, in a sample from said subject.

100. The method of claim 80, wherein said RNAi agent is administered using a pharmaceutical composition.

101. The method of claim 100, wherein said siRNA is administered in an unbuffered solution.

102. The method of claim 101, wherein said unbuffered solution is saline or water.

103. The method of claim 100, wherein said siRNA is administered with a buffer solution.

104. The method of claim 103, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, or phosphate or any combination thereof.

105. The method of claim 104, wherein said buffer solution is phosphate buffered saline (PBS).

106. The method of claim 100, wherein said pharmaceutical composition is a liposome.

107. A method of inhibiting expression of transthyretin (TTR) in a cell, comprising contacting said cell with a double stranded RNAi agent in an amount effective to inhibit expression of TTR in said cell, wherein said double stranded RNAi agent is selected from the group of RNAi agents listed in Table 1, thereby inhibiting expression of transthyretin (TTR) in said cell.

108. A method of inhibiting expression of transthyretin (TTR) in a cell, comprising contacting said cell with a double stranded RNAi agent in an amount effective to inhibit expression of TTR in said cell, wherein said double stranded RNAi agent is selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547, thereby inhibiting expression of transthyretin (TTR) in said cell.

109. A method of inhibiting expression of transthyretin (TTR) in a cell, comprising contacting said cell with a double stranded RNAi agent in an amount effective to inhibit expression of TTR in said cell, wherein said double stranded RNAi agent is AD-51547, thereby inhibiting expression of transthyretin (TTR) in said cell.

110. A method of treating or preventing a TTR-associated disease in a subject, comprising administering to said subject a therapeutically effective amount or a prophylactically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent is selected from the group of agents listed in Table 1, thereby treating or preventing a TTR-associated disease in said subject.

111. A method of treating or preventing a TTR-associated disease in a subject, comprising administering to said subject a therapeutically effective amount or a prophylactically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent is selected from the group consisting of AD-51544, AD-51545, AD-51546, and AD-51547, thereby treating or preventing a TTR-associated disease in said subject.

112. A method of treating or preventing a TTR-associated disease in a subject, comprising administering to said subject a therapeutically effective amount or a prophylactically effective amount of a double stranded RNAi agent, wherein said double stranded RNAi agent is AD-51547, thereby treating or preventing a TTR-associated disease in said subject.

113. A kit for performing the method of claim 46, comprising

a) said RNAi agent, and

b) instructions for use.

114. A kit for performing the method of claim 74, comprising

a) said RNAi agent,

b) instructions for use, and

c) optionally, means for administering said RNAi agent to said subject.

FIG. 1

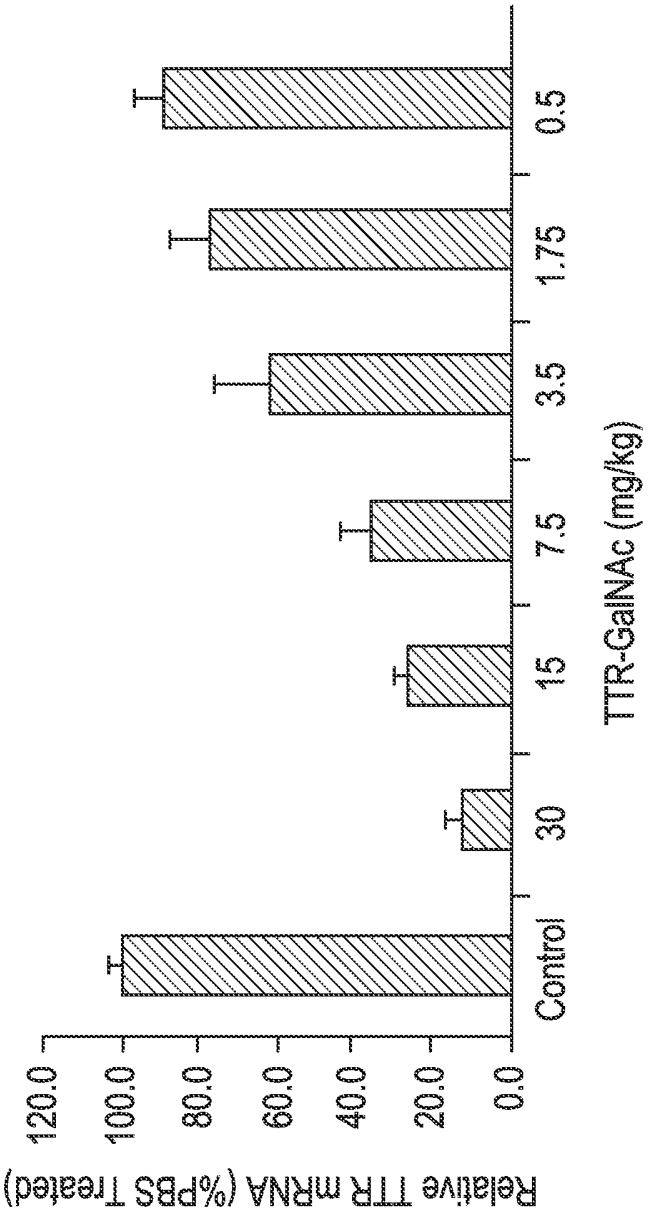

Relative TTR mRNA (%PBS Treated)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Control
30
15
7.5
3.5
1.75
0.5
TTR-GalNAc (mg/kg)

FIG. 2

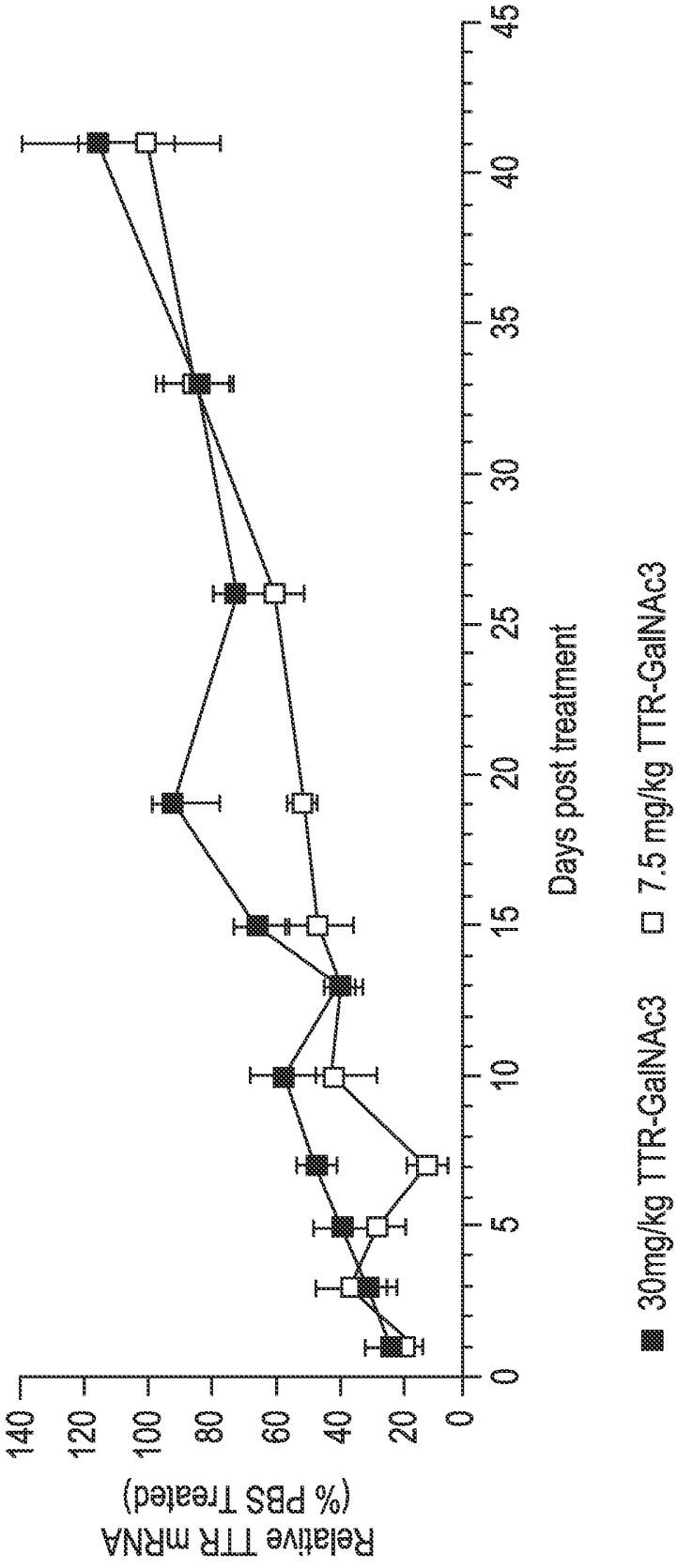

Relative TTR mRNA
(% PBS Treated)
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30
35
40
45
Days post treatment
30mg/kg TTR-GalNAc3
7.5 mg/kg TTR-GalNAc3

FIG. 3

Human TTR mRNA Sequence (SEQ ID NO: 1), Gen Bank Accession No.: M10605,
GI: 189583

```
   1 cagaagtcca ctcattcttg gcaggatggc ttctcatcgt ctgctcctcc tctgccttgc
  61 tggactggta ttgtgtgtct aggctggccc tacgggcacc ggtgaatcca agtgtcctct
 121 gatggtcaaa gttctagatg ctgtccgagg cagtcctgcc atcaatgtgg ccgtgcatgt
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 301 aatagacacc aaatcttact ggaagggcac tggcatctcc ccattccatg agcatgcaga
 361 ggtggtattc acagccaacg actccggccc ccgccgctac accattgccg ccctgctgag
 421 ccgctactcc tattccacca cggctgtcgt caccgtggtc aaggaatgag ggacttctcc
 481 tccagtgggac ctgaagggacg agggatgggga ttttcatgtaa ccaagagtat tccatttttta
 541 ctaaagcagt gttttcacct catatgctat gttagaagtc cagggcagaga caataaaaca
 601 ttcctgtgaa aggc
```

FIG. 4

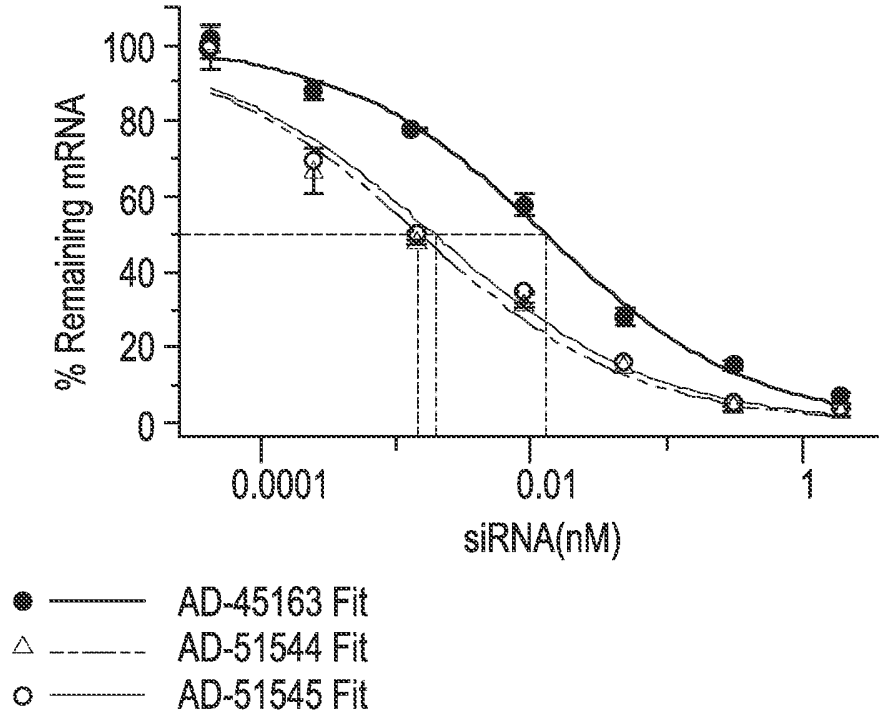

% Remaining mRNA
100
80
60
40
20
0
0.0001
0.01
1
siRNA(nM)
AD-45163 Fit
AD-51544 Fit
AD-51545 Fit

FIG. 5

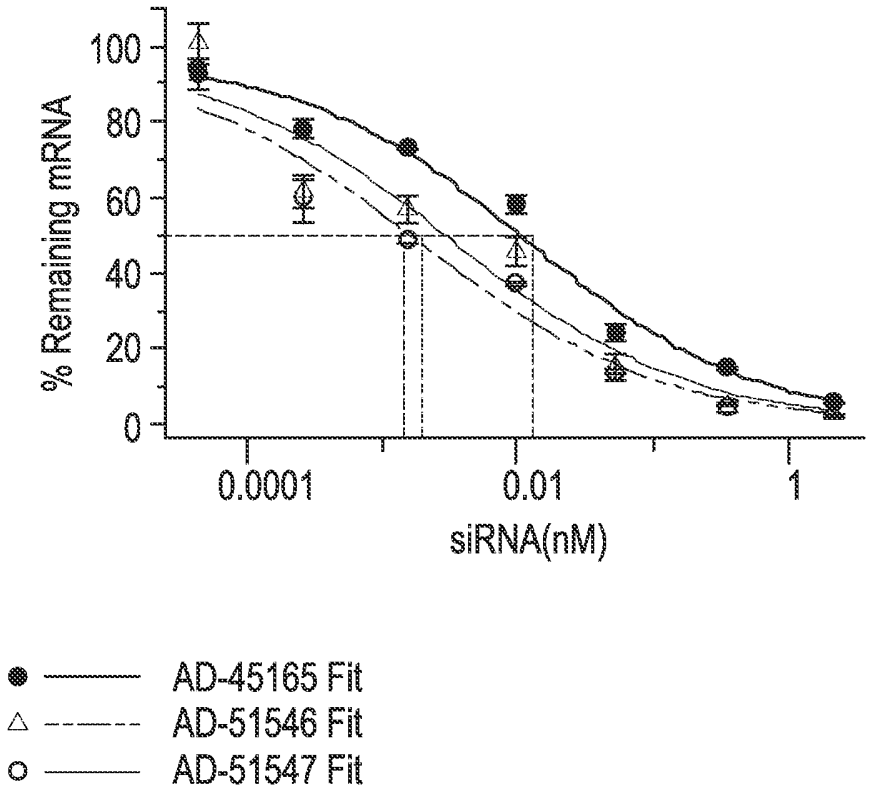

% Remaining mRNA
100
80
60
40
20
0
0.0001
0.01
1
siRNA(nM)
AD-45165 Fit
AD-51546 Fit
AD-51547 Fit

FIG. 6

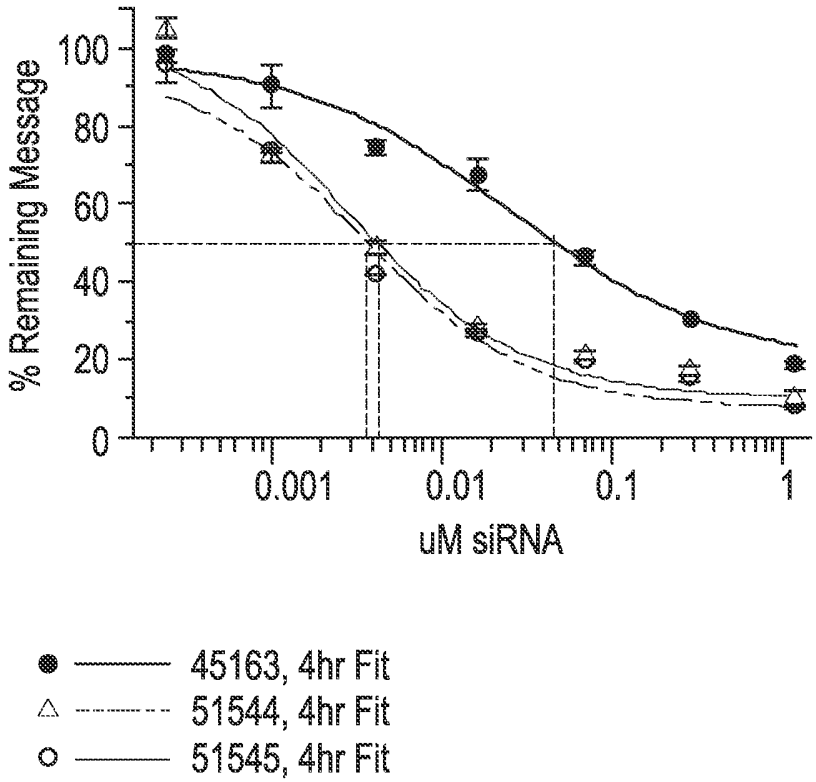

% Remaining Message
100
80
60
40
20
0
0.001
0.01
0.1
1
uM siRNA
45163, 4hr Fit
51544, 4hr Fit
51545, 4hr Fit

FIG. 7

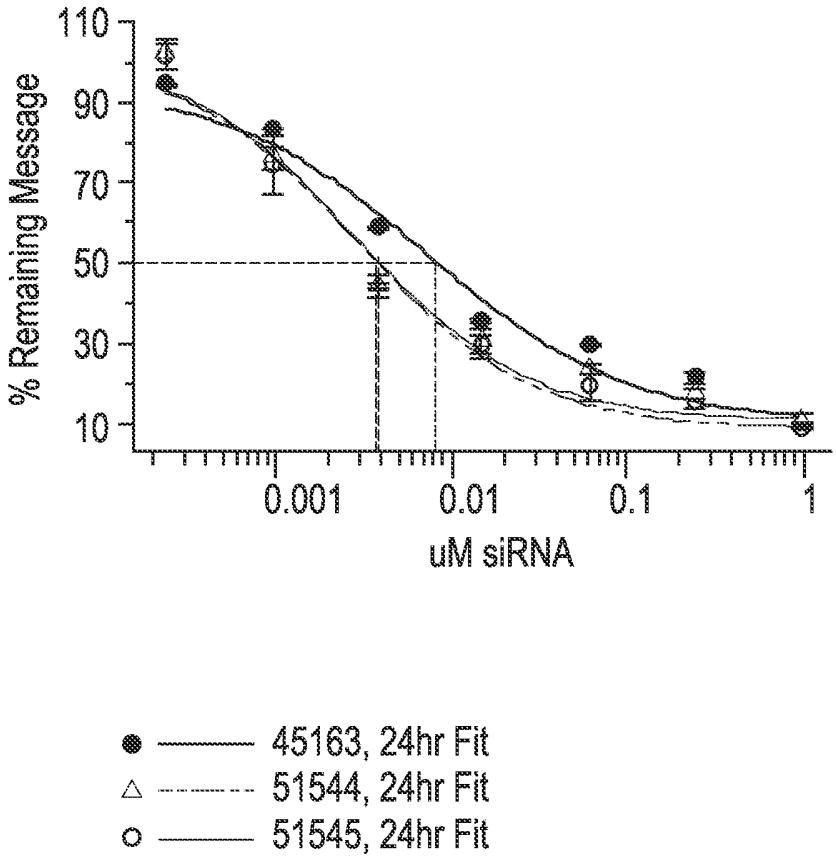

% Remaining Message
110
90
70
50
30
10
0.001
0.01
0.1
1
uM siRNA
45163, 24hr Fit
51544, 24hr Fit
51545, 24hr Fit

FIG. 8

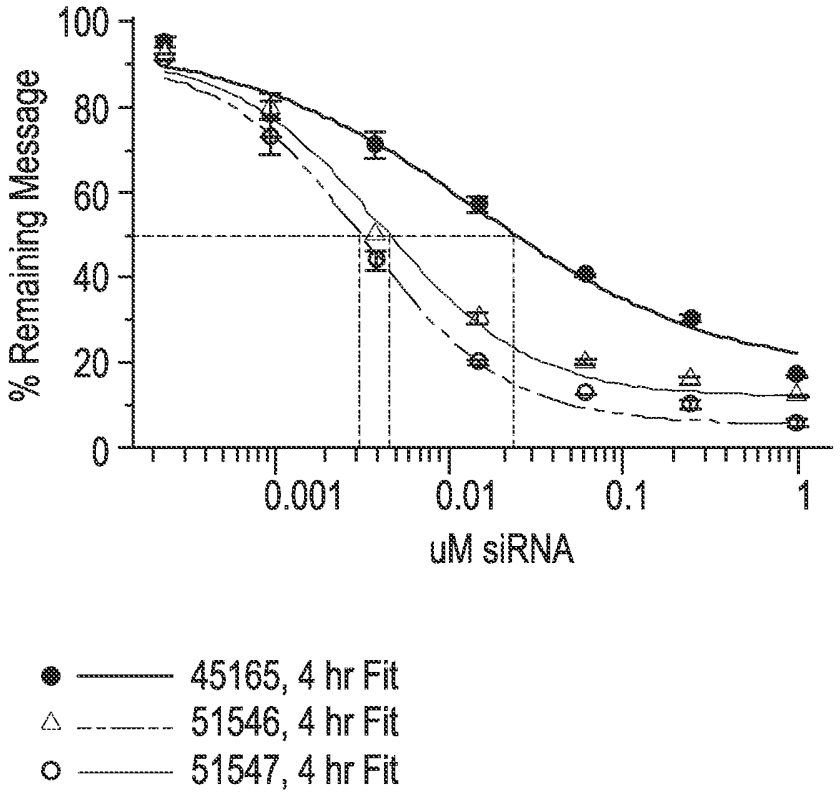

100
80
60
40
20
0
% Remaining Message
0.001
0.01
0.1
1
uM siRNA
45165, 4 hr Fit
51546, 4 hr Fit
51547, 4 hr Fit

FIG. 9

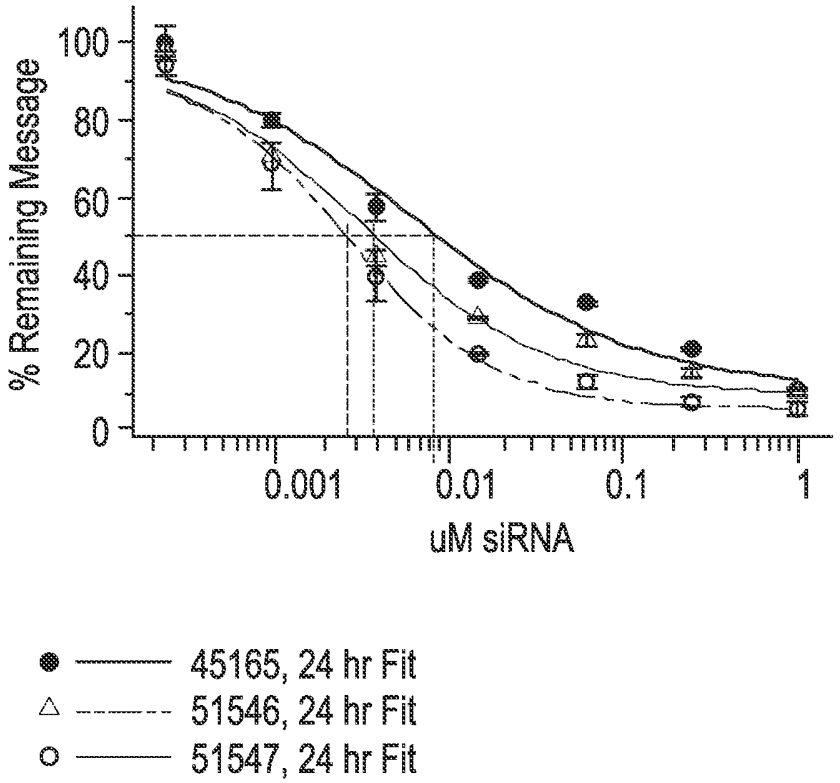

% Remaining Message
100
80
60
40
20
0
0.001
0.01
0.1
1
uM siRNA
45165, 24 hr Fit
51546, 24 hr Fit
51547, 24 hr Fit

FIG. 10A

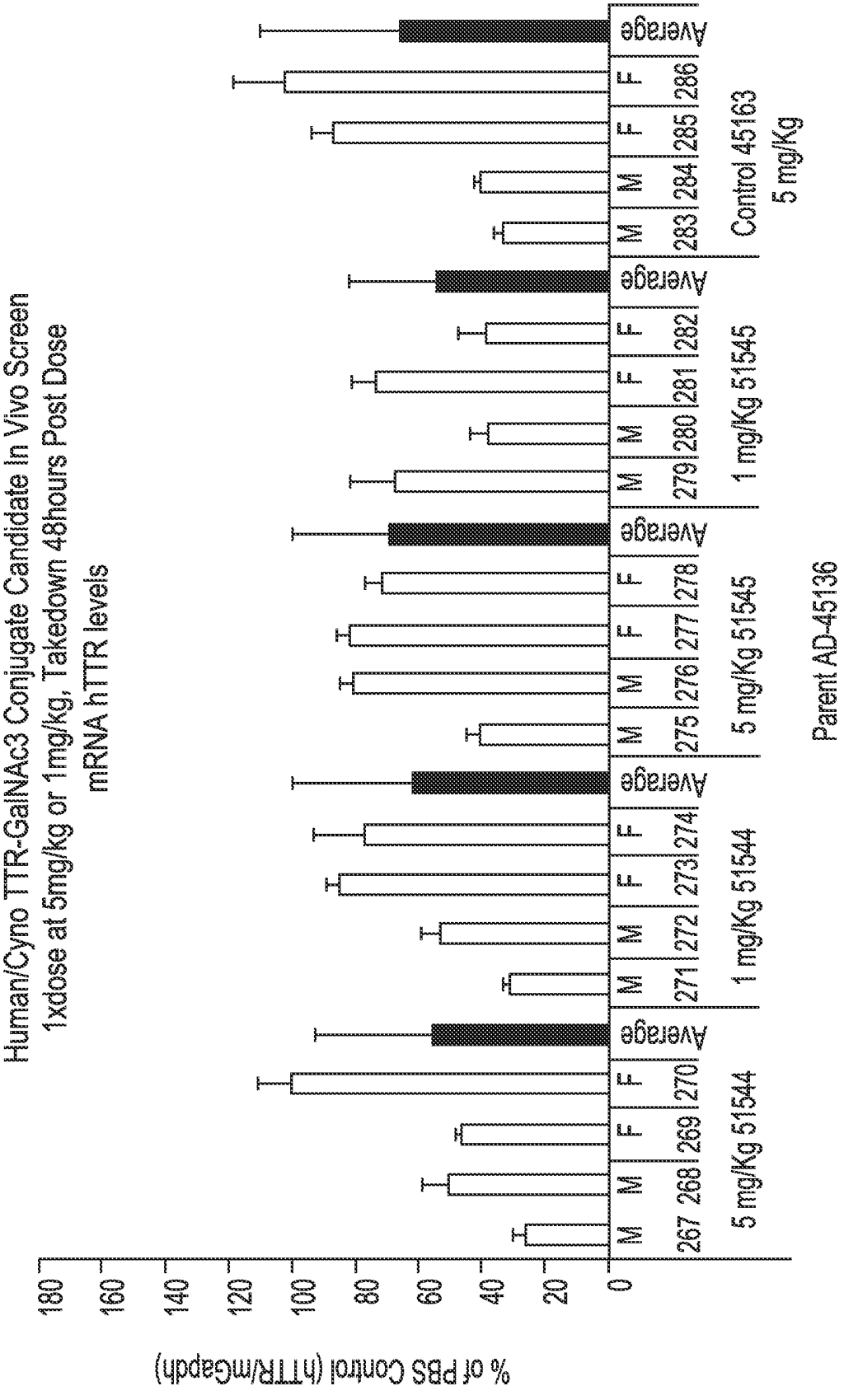

Human/Cyno TTR-GalNAc3 Conjugate Candidate In Vivo Screen
1xdose at 5mg/kg or 1mg/kg, Takedown 48hours Post Dose
mRNA hTTR levels
% of PBS Control (hTTR/mGapdh)
0
20
40
60
80
100
120
140
160
180
M M F F Average
267 268 269 270
5 mg/Kg 51544
M M F F Average
271 272 273 274
1 mg/Kg 51544
M M F F Average
275 276 277 278
5 mg/Kg 51545
M M F F Average
279 280 281 282
1 mg/Kg 51545
M M F F Average
283 284 285 286
Control 45163
5 mg/Kg
Parent AD-45136

FIG. 10B

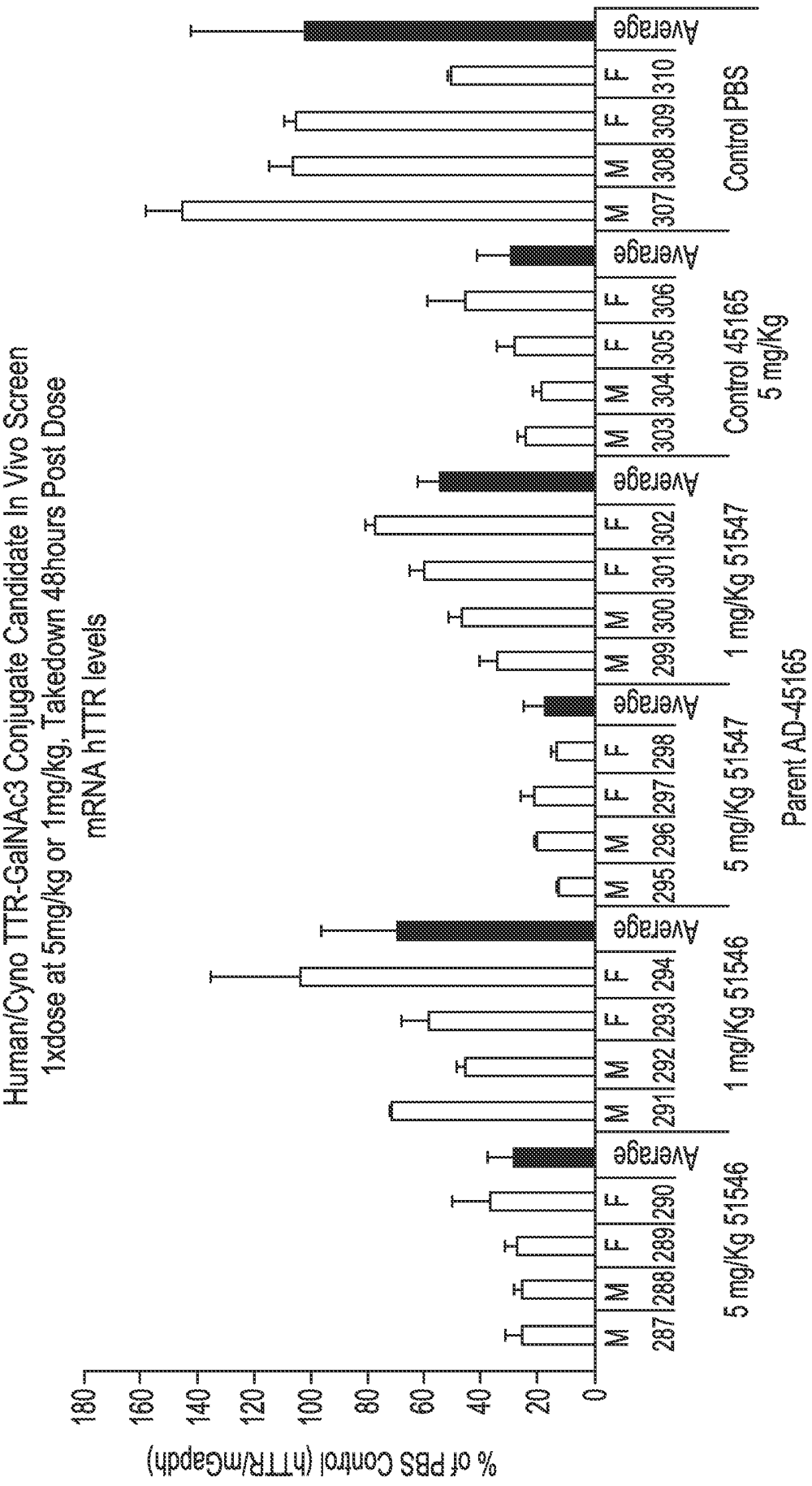

Human/Cyno TTR-GalNAc3 Conjugate Candidate In Vivo Screen
1xdose at 5mg/kg or 1mg/kg, Takedown 48hours Post Dose
mRNA hTTR levels
% of PBS Control (hTTR/mGapdh)
0
20
40
60
80
100
120
140
160
180
M 287
M 288
F 289
F 290
Average
5 mg/Kg 51546
M 291
M 292
F 293
F 294
Average
1 mg/Kg 51546
M 295
M 296
F 297
F 298
Average
5 mg/Kg 51547
M 299
M 300
F 301
F 302
Average
1 mg/Kg 51547
Parent AD-45165
M 303
M 304
F 305
F 306
Average
Control 45165
5 mg/Kg
M 307
M 308
F 309
F 310
Average
Control PBS

FIG. 11

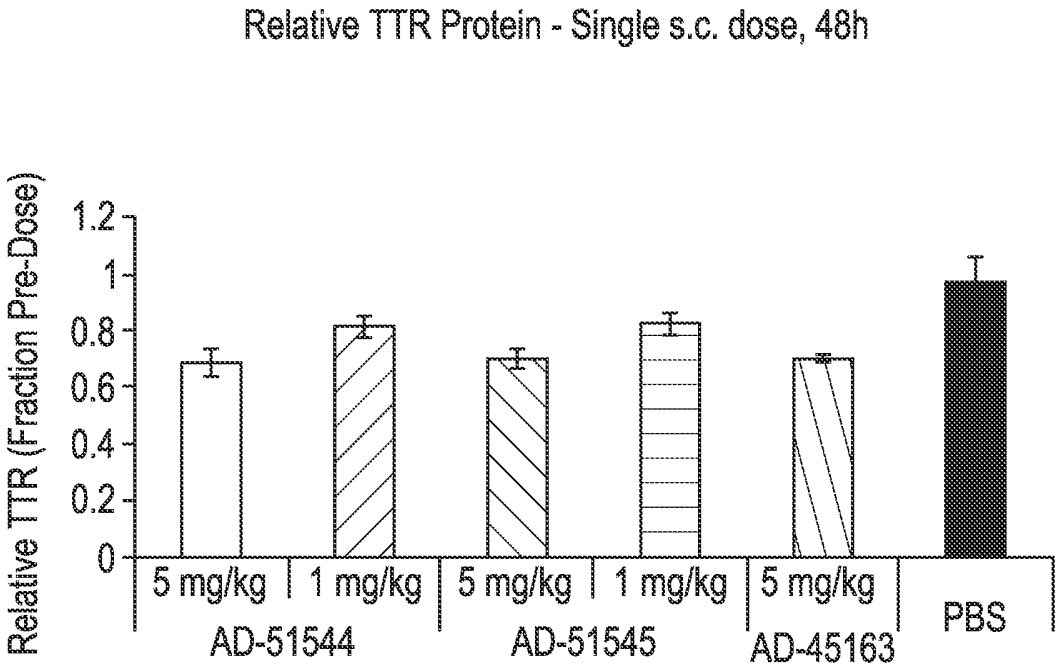

Relative TTR Protein - Single s.c. dose, 48h
Relative TTR (Fraction Pre-Dose)
1.2
1
0.8
0.6
0.4
0.2
0
5 mg/kg
1 mg/kg
5 mg/kg
1 mg/kg
5 mg/kg
PBS
AD-51544
AD-51545
AD-45163

FIG. 12

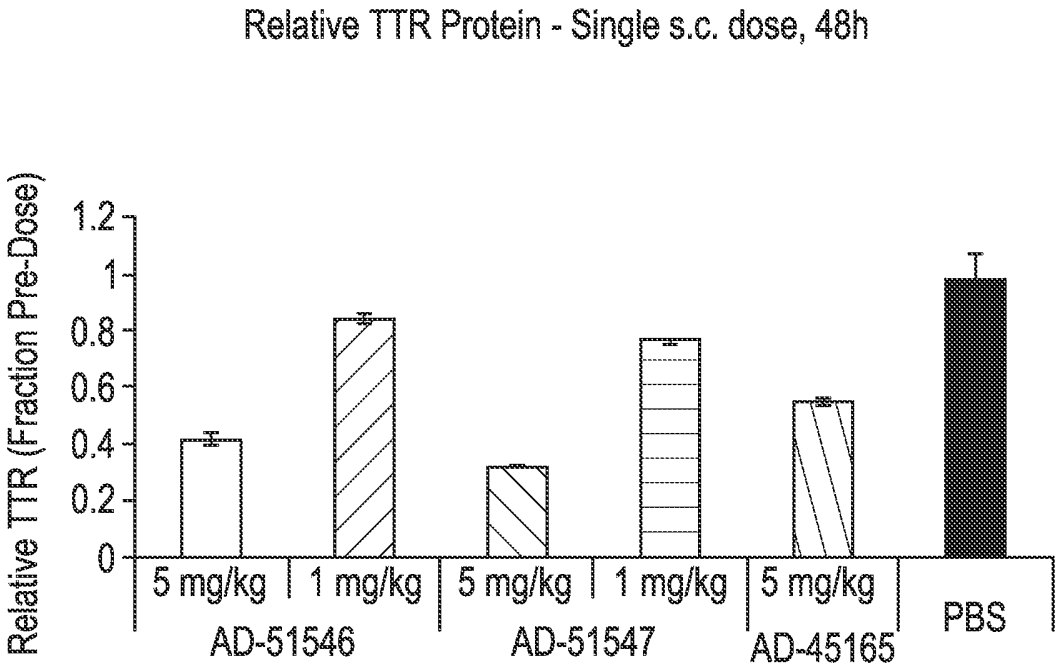

Relative TTR Protein - Single s.c. dose, 48h
Relative TTR (Fraction Pre-Dose)
1.2
1
0.8
0.6
0.4
0.2
0
5 mg/kg
1 mg/kg
5 mg/kg
1 mg/kg
5 mg/kg
PBS
AD-51546
AD-51547
AD-45165

FIG. 13

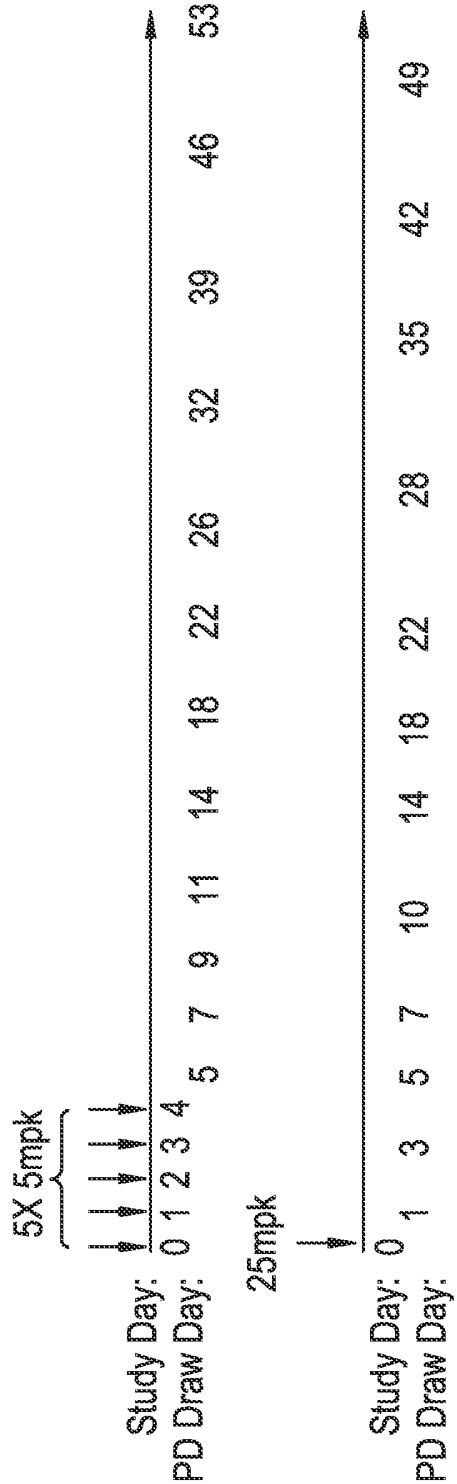
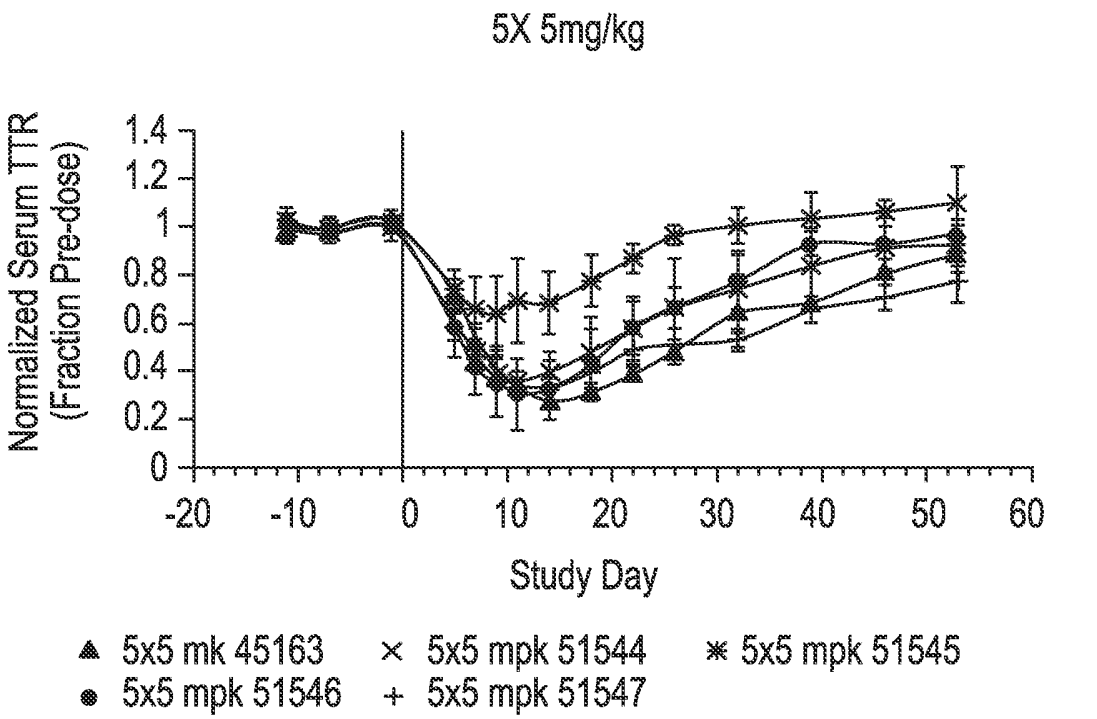

5X 5mpk
Study Day: 0 1 2 3 4 5 7 9 11 14 18 22 26 32 39 46 53
PD Draw Day:
25mpk
Study Day: 0 1 3 5 7 10 14 18 22 28 35 42 49
PD Draw Day:

FIG. 14


5X 5mg/kg
Normalized Serum TTR
(Fraction Pre-dose)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-20
-10
0
10
20
30
40
50
60
Study Day
▲ 5x5 mk 45163
× 5x5 mpk 51544
✳ 5x5 mpk 51545
● 5x5 mpk 51546
+ 5x5 mpk 51547

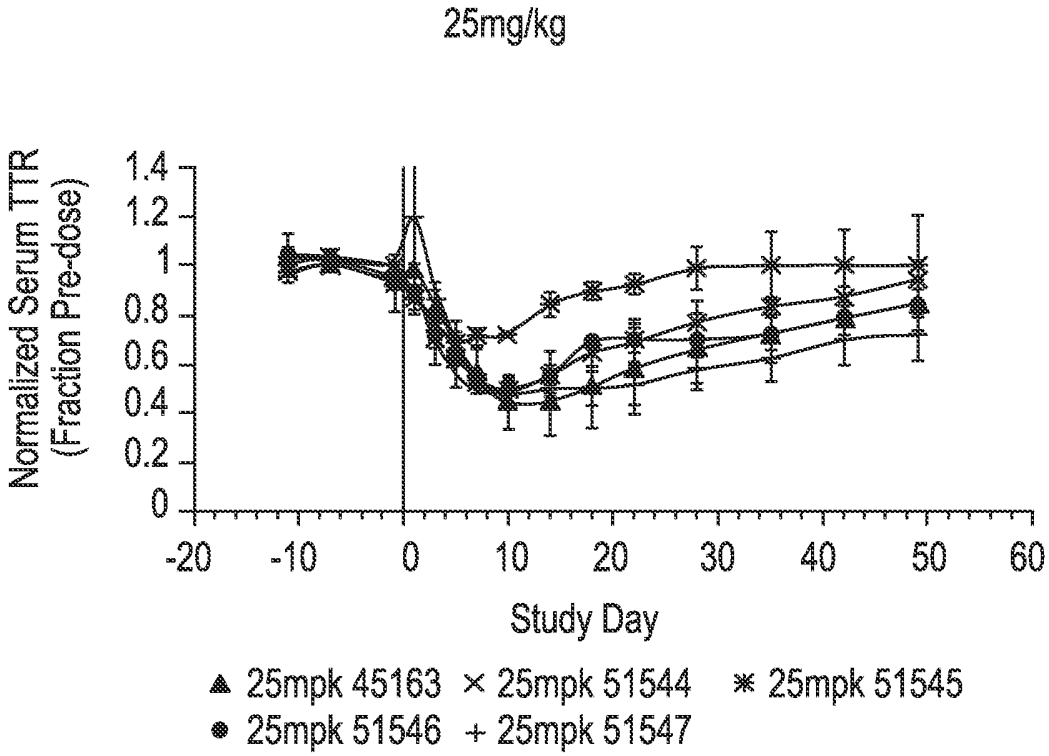
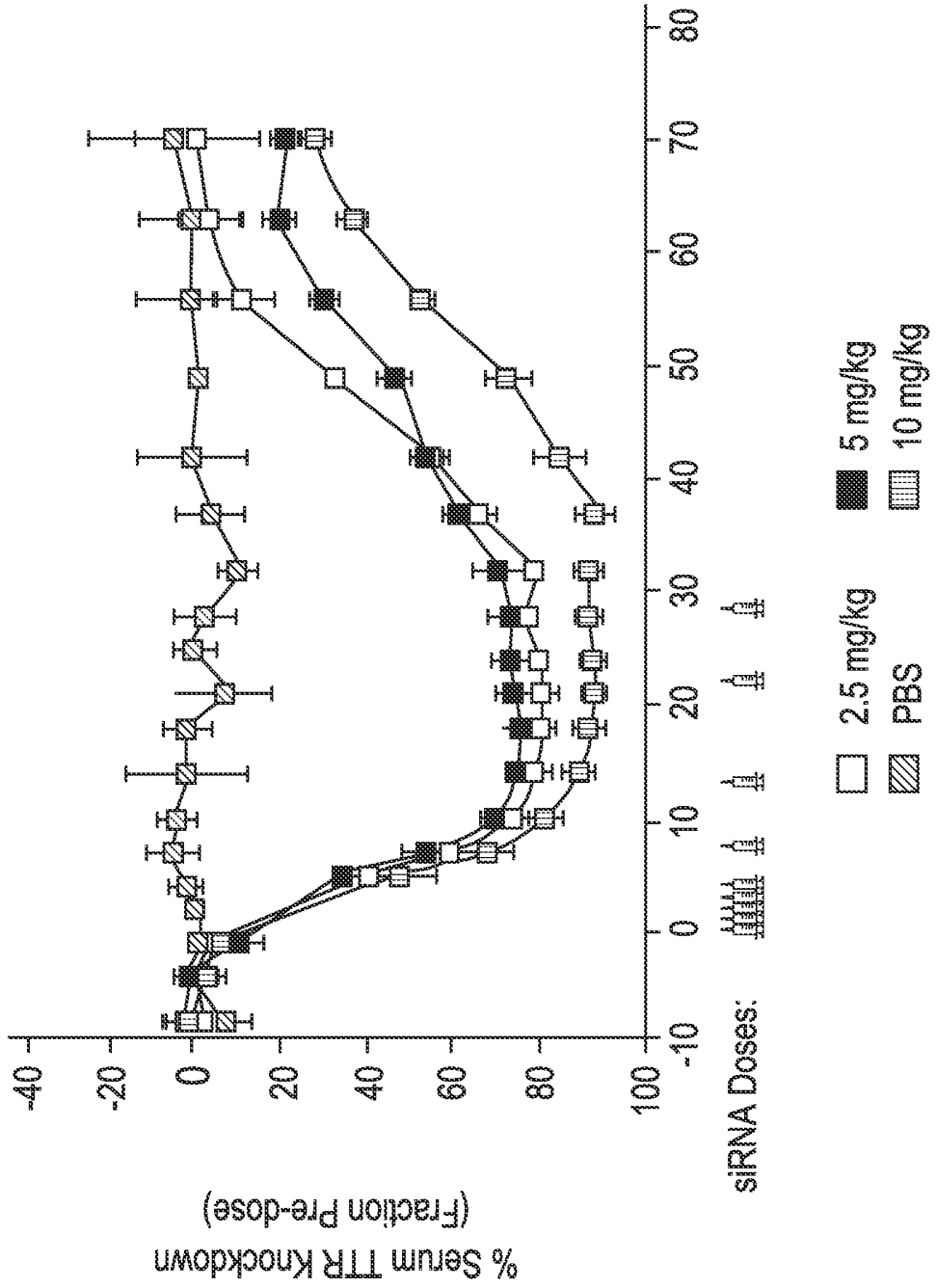

25mg/kg
Normalized Serum TTR
(Fraction Pre-dose)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-20
-10
0
10
20
30
40
50
60
Study Day
▲ 25mpk 45163
× 25mpk 51544
✳ 25mpk 51545
● 25mpk 51546
+ 25mpk 51547

FIG. 15


% Serum TTR Knockdown
(Fraction Pre-dose)
-40
-20
0
20
40
60
80
100
-10
0
10
20
30
40
50
60
70
80
siRNA Doses:
2.5 mg/kg
5 mg/kg
PBS
10 mg/kg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/65691

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/00; C12N 15/113; A61K 31/7088 (2012.01)
USPC - 536/24.5; 514/44A
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07H 21/00; C12N 15/113; A61K 31/7088 (2012.01)
USPC - 536/24.5; 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8) - C07H 21/00; C12N 15/113; A61K 31/7088 (2012.01)
USPC - 536/24.5; 514/44A

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, ScienceDirect, GoogleScholar
Transthyretin (TTR), Ga1NAc, sense, antisense, 2'-O-methyl, 2'-fluoro, phosphorothioate, RNAi,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0239814 A1 (MANOHARAN et al.) 24 September 2009 (24.09.2009) Fig. 2, Fig. 10, para [0070], [0136], [0141], [0200], [0201], [0202], [0240], [0254], [0255], [0323], [0327]-[0328], [0341], [0438], [0316], [0361], [0373], [0844], [0851], [0857]	1, 4-7, 11-23, 23a, 24-30
Y	US 2011/0237646 A1 (SMITH et al.) 29 September 2011 (29.09.2011) para [0070], [0129], [0136], [0138], [0141], [0240], [0255], [0177]	1, 4-7, 11-23, 23a, 24-30
Y	KAWASAKI et al. "Synthesis, Hybridizationk, and Nuclease Resistance Properties of 2'-O-Aminooxyethyl (2'-O-AOE) Modified Oligonucleotides" Tetrahedron Lett.; 1999; Vol. 40; pg. 661-664. Entire document, especially abstract, Table I	1, 4-7, 11-23, 23a, 24-30
Y	MANOHARAN "RNA interference and chemically modified small interfering RNAs" Current Opinion in Chemical Biology; 2004; Vol. 8; pg. 570-579. Abstract	1, 4-7, 11-23, 23a, 24-30
Y	CHEN et al. "Lipophilic siRNAs mediate efficient gene silencing in oligodendrocytes with direct CNS delivery" J. Control. Release; 2010; Vol. 144; pg. 227-232. Abstract	1, 4-7, 11-23, 23a, 24-30
Y	NAKAMURA et al. "Targeted conversion of the transthyretin gene in vitro and in vivo" Gene Therapy; 2004; Vol. 11; 838-846 page 839, Fig. 1 Abstract	1, 4-7, 11-23, 23a, 24-30
Y	GAMBARI et al. "Targeting microRNAs involved in human diseases: A novel approach for modification of gene expression and drug development" Biochem. Pharmacol.; 15 November 2011; Vol. 82, No.10; pg. 1416-1429. Abstract	1, 4-7, 11-23, 23a, 24-30

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 March 2013 (15.03.2013)	01 APR 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/65691

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2011/139917 A1 (MONIA et al.) 10 November 2011 (10.11.2011) entire document	1, 4-7, 11-23, 23a, 24-30
A	US 2011/0256175 A1 (HOPE et al.) 20 October 2011 (20.10.2011) entire document	1, 4-7, 11-23, 23a, 24-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/65691

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 41-105, 112 and 113
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
----------SEE EXTRA SHEET----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 4-7, 11-23, 23a and 24-30

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US 12/65691

Continuation of Box III: Lack of Unity of Invention

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-23, 23a, 24-40, and 106-111, directed to a double stranded RNAi agent comprising a sense strand complementary to an antisense strand, wherein said antisense strand comprises a region complementary to part of an mRNA encoding transthyretin (TTR), wherein each strand has about 14 to about 30 nucleotides, wherein said double stranded RNAi agent is represented by formula (III):

sense: 5' n.sub.p-N.sub.a-(XXX).sub.i-N.sub.b-YYY-N.sub.b-(ZZZ).subb.j-N.sub.a-n.sub.q 3'
antisense: 3' n.sub.p'-N.sub.a'-(X'X'X').sub.k-N.sub.b'-Y'Y'Y'-N.sub.b'-(Z'Z'Z').sub.l-N.sub.a'-n.sub.q' 5' (III), wherein:
i, j, k, and 1 are each independently 0 or 1;
p, p', q, and q' are each independently 0-6;
each N.sub.a and N.sub.a' independently represents an oligonucleotide sequence comprising 0-25 nucleotides which are either modified or unmodified or combinations thereof, each sequence comprising at least two differently modified nucleotides;
each N.sub.b and N.sub.b' independently represents an oligonucleotide sequence comprising 0-10 nucleotides which are either modified or unmodified or combinations thereof;
each n.sub.p, n.sub.p', n.sub.q and n.sub.q' independently represents an overhang nucleotide;
XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides;
modifications on N.sub.b differ from the modification on Y and modifications on N.sub.b' differ from the modification on Y'; and
wherein the sense strand is conjugated to at least one ligand;
wherein the first invention is defined by the first option for each and every optional position, namely:

i, j, k, and 1 are each independently 0; p, p', q, and q' are each independently 0; each N.sub.a and N.sub.a' independently represents an oligonucleotide sequence comprising 0 nucleotides; each N.sub.b and N.sub.b' independently represents an oligonucleotide sequence comprising 0 nucleotides; hence wherein the structure of the first claimed compound comprises:

5' YYY 3'
3' Y'Y'Y' 5'

(claims 1, 4-7, 11-23, 23a and 24-30) (Applicants may opt for additional inhibitory nucleic acid structures to be searched by fully specifying the structure, or by selecting a particular fully specified structure from one of the indicated tables, such as Table 1, and paying an additional invention search fee for each elected inhibitory nucleic acid structure).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of the claims of Groups I+ are based on the particular structural features of individual double-stranded inhibitory nucleic acids directed to transthyretin, and having at least a particular motif comprising a triplet of similarly modified nucleotides in the structure of each of the strands, wherein each subgroup comprises a different invention based on the particular combinations of modifications used in the structure of the strands.

The only common technical element shared by the above groups is that they are related to modified double-stranded oligonucleotides having about 14 to 30 nucleotides directed to transthyretin having at least a motif comprising a triplet of similarly modified nucleotides in each strand, and having at least the sense strand is conjugated to a ligand. These common technical elements do not represent an improvement over the combined prior art of US 2011/0237646 A1 to Smith et al. (hereinafter "Smith") and the article entitled "Synthesis, Hybridizationk, and Nuclease Resistance Properties of 2'-O-Aminooxyethyl (2'-O-AOE) Modified Oligonucleotides" by Kawasaki et al. (hereinafter "Kawasaki"), as follows:

Smith teaches compounds and compositions for modulating the expression of transthyretin (abstract), including modified double-stranded oligonucleotides (para [0177]), which is at least 70% complementary to the human transthyretin nucleic acid, which may be an siRNA (para [0070]) of 12 to 30 linked nucleosides in length (para [0129]). The modified oligonucleotide may comprise at least one modified internucleoside linkage (para [0136]), at least one modified sugar (para [0138]), or a modified base (para [0141]). Smith further teaches wherein the compounds may be linked to one or more moieties or conjugates that enhance the activity, or cellular distribution or uptake of the compounds (para [0254]), and wherein the compounds may have stabilizing groups added to the 5' or 3' ends, such as cap structures, to prevent or reduce exonuclease degradation (para [0255]). Although Smith does not specify wherein the sense strand is conjugated to the ligand, it would have been obvious to a person of ordinary skill in the art to assess the effectiveness of conjugation of either or both of the sense and antisense strands, wherein conjugation of either to a ligand would have been anticipated to be functionally useful, based on the teaching of Smith. Further, although Smith specifies wherein the inhibitory oligonucleotides may comprise modified linkages, sugars, or bases, Smith does not specifically recite wherein the inhibitory oligonucleotides comprise at least a motif of three similarly modified nucleotides in succession. However, in a related disclosure, Kawasaki teaches modified inhibitory oligonucleotides (abstract) including a particular modification, which, when used in repeats of more than three consecutive nucleotides (Table 1) enhances the effect of stabilizing the inhibitory oligonucleotides against degradation (abstract, Table 1). It would have been obvious to a person of ordinary skill in the art to apply the teaching of Kawasaki regarding the enhanced stability of runs of repeated modified nucleotides in an inhibitory oligonucleotide to both strands of a double stranded inhibitory oligonucleotide, as taught by Smith, in order to enhance the stability of the construct and increase its nuclease resistance.

Therefore, the inventions of Groups I+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Continuation of Section 4:

Claims 41-105, 112 and 113 are multiply dependent claims that are not formed according to the second and third sentences of Rule 6.4(a).

摘要

本發明提供了靶向甲狀腺素運載蛋白（TTR）基因的RNAi試劑（例如雙鏈RNAi試劑）和使用這樣的RNAi試劑用於治療或預防TTR相關疾病的方法 。