

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

Related Applications

5 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.

10 **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.

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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
20 the blood and the CSF. Transthyretin is named for its **transport of thyroxine and retinol**. 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
25 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 amyloidogenic transthyretin. *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.

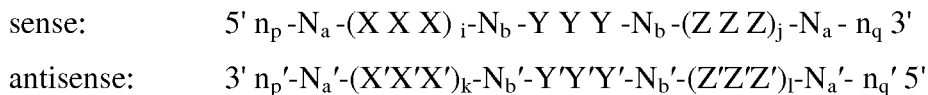
Accordingly, there is a need in the art for effective treatments for TTR-associated diseases.

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
 5 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
 10 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):



15 (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
 20 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
 25 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.

30 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.

- 5 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.

- 10 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.

- 15 In another aspect, the present invention features a double stranded RNAi agent comprising a sense strand complementary to an antisense strand, wherein said antisense strand comprises a sequence that is complementary to nucleotides 504 to 526 of the transthyretin (TTR) gene (SEQ ID NO:1), wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length, wherein said double stranded RNAi agent is represented by formula (III):

- sense: $5' n_p -N_a -(X X X)_i -N_b -Y Y Y -N_b -(Z Z Z)_j -N_a - n_q 3'$
20 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:

$j = 1$; and i, k, and l are 0;

p' is 2; p, q, and q' are 0;

- 25 each N_a and $N_{a'}$ independently represents an oligonucleotide sequence comprising 2-10 nucleotides which are modified nucleotides;

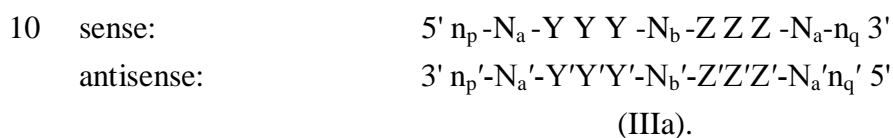
each N_b and $N_{b'}$ independently represents an oligonucleotide sequence comprising 0-7 nucleotides which are modified nucleotides;

n_p' represents an overhang nucleotide;

YYY, ZZZ, and Y'Y'Y', each independently represent one motif of three identical modifications on three consecutive nucleotides, wherein the Y nucleotides contain a 2'-fluoro modification, the Y' nucleotides contain a 2'-O-methyl modification, and the Z nucleotides contain a 2'-O-methyl modification; and

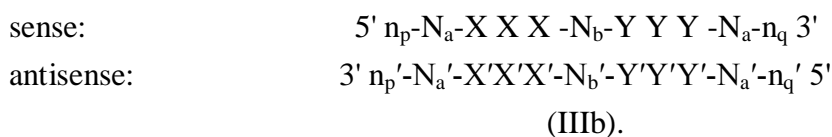
wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

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



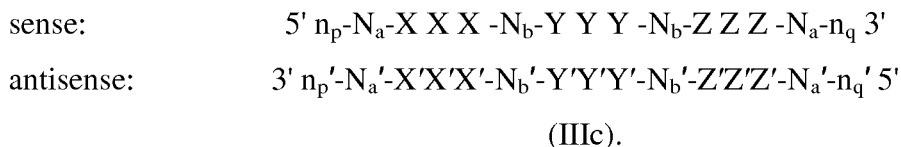
In formula IIIa, each N_b and N_b' independently represents an oligonucleotide sequence including 1-5 modified nucleotides.

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



20 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):



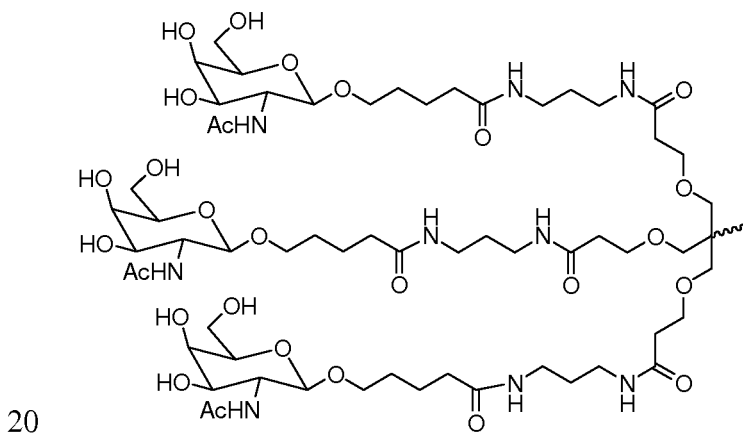
- 5 In formula IIIc, each N_b and $-N_b'$ independently represents an oligonucleotide sequence including 1-5 modified nucleotides and each N_a and N_a' independently represents an oligonucleotide sequence including 2-10 modified nucleotides.

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
 10 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.

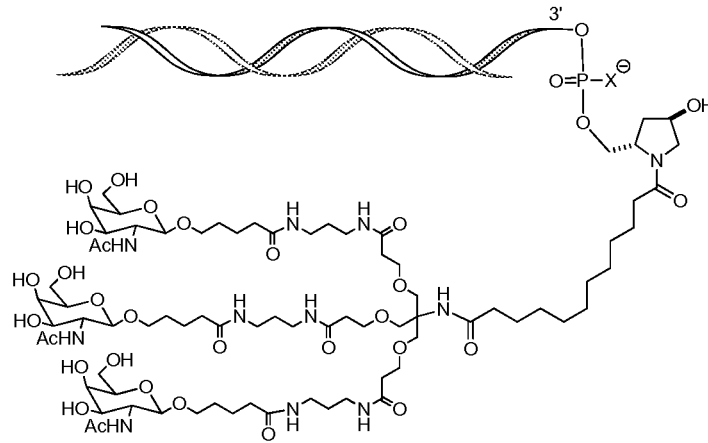
In some embodiments, the modifications on the nucleotides are selected from the group consisting of LNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-alkyl, 2'-O-allyl, 2'-C-
 15 allyl, 2'-fluoro, 2'-deoxy, 2'-hydroxyl, and combinations thereof. In some preferred embodiments, the modifications on the nucleotides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



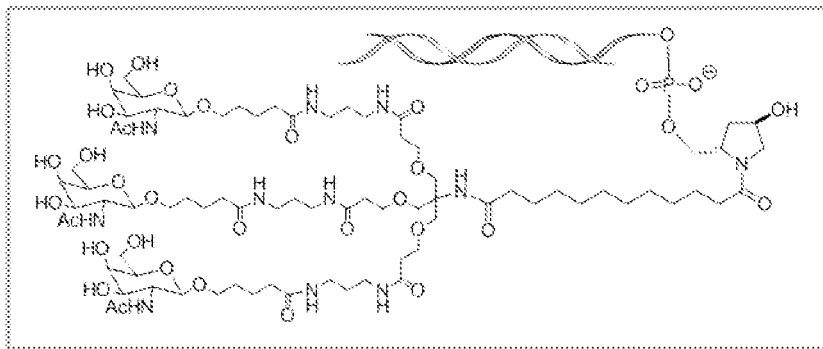
In some embodiments, the ligand is attached to the 3' end of the sense strand.

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



5 wherein X is O or S.

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



10 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.

5 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
 10 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.
 15 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.

20 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
 25 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 GalNAc₃ ligand.

In another aspect, the present invention features a double stranded RNAi agent, comprising a sense strand and an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'-UfgGfgAfuUfuCfAfUfgUfaacCfaAfgAfL96-3' (SEQ ID NO:2) and the antisense strand comprises the nucleotide sequence 5'-uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc-3' (SEQ ID NO:3), wherein a, g, c, and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C, and U; s is a phosphorothioate linkage; and L96 is a GalNAc₃ 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 developing 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),
5 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,
10 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
15 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
20 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
25 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, 5 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, 10 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.

15 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, 20 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 25 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), IL1 β , IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , TNF α , and any combinations 30 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
5 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
10 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,
15 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
20 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.

25 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.

- 5 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, cerebrosplinal, and any combinations thereof. In certain embodiments, the RNAi agent is administered to the subject via subcutaneous or intravenous administration. In preferred
10 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.

- 15 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
20 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
25 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), IL1 β , IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , TNF α , 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 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 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
5 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.

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

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
15 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.

20 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.

25 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.

5 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.

10 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
15 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
20 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
25 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
5 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
10 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
15 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
20 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.

25 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
30 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.

5 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
10 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,
15 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
20 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.

25 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
30 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
5 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
10 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 Hoogsteen base pairing.

The terms “complementary,” “fully complementary” and “substantially
15 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
20 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.

25 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
30 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
5 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
10 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
15 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).

20 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
25 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
30 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 GalNAc₃ 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.

10 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
15 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
20 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,
25 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).
30 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.

5 “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
10 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
15 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
20 other individual characteristics of the patient to be treated.

A “therapeutically-effective amount” or “prophylactically 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
25 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
30 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 to cerebrospinal 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.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each claim of this application.

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 activity 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*.

5 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
5 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
10 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
15 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 GalNAc₃).

In one embodiment, the RNAi agent comprises a sense and an antisense strand,
20 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
25 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
30 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 complementary 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'-
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.
10 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
15 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
20 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 adjacent 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
25 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
30 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
5 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.

10 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.

15 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.

20 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.

25 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
30 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...,”

5 “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

10 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

15 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

20 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 antisense 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.

25 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

30 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
5 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 activity 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
10 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 nucleotides, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the
15 modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, 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
20 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
25 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
30 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.

5 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
10 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 paired nucleotide next to the overhang nucleotide. Preferably, these terminal three nucleotides may be at the 3'-end of the antisense strand.

15 In one embodiment, the RNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch 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
20 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
25 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
30 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,
 5 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):



10

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
 15 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

20

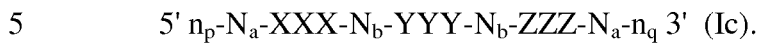
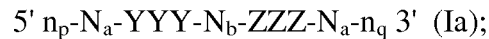
XXX, YYY and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

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

25

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
 30 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:



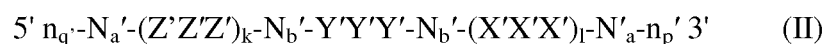
When the sense strand is represented by formula (Ia), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2 or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 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):



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
5 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
10 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:

15 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
20 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
25 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.
30 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.

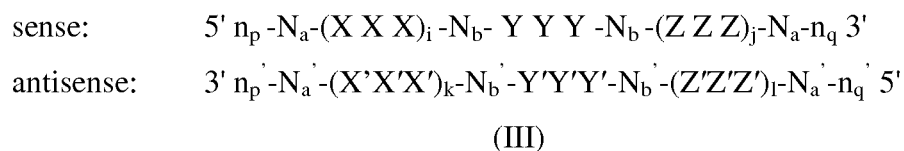
5 Each X, Y, Z, X', Y' and Z', in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

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
 10 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
 15 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
 20 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
 25 an antisense strand, each strand having 14 to 30 nucleotides, the RNAi duplex represented by formula (III):



30 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;

5 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

10 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:

15 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'

20 (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)

25 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.

30 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 0 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 (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 0 modified nucleotides. Each N_a , N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of 5 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. 10 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 15 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 20 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.

25 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 30 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
5 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
10 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, imidazoliny, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl,
15 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,
20 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,
25 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,
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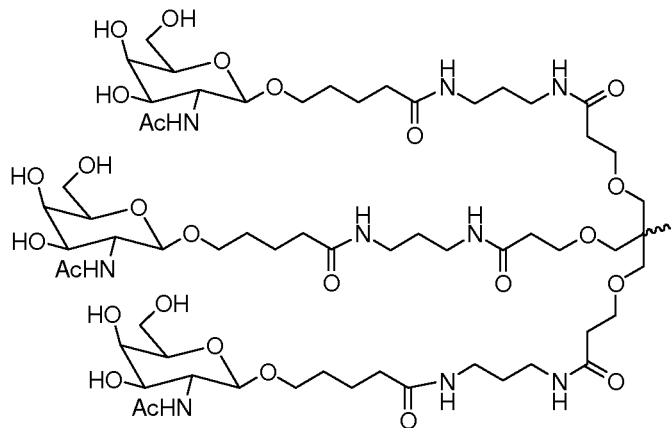
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D1958, D1959, D1960, D1961, D1962, D1963, D1964, D1965, D1966, D1967, D1968,
15 D1969, D1970, D1971, D1972, D1973, D1974, D1975, D1976, D1977, D1978, D1979,
D1980, D1981, D1982, D1983, D1984, D1985, D1986, D1987, D1988, D1989, D1990,
D1991, D1992, 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,
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D2068, D2069, D2070, D2071, D2072, D2073, D2074, D2075, D2076, D2077, D2078,
25 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

30 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 conjugated 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 GalNAc₃:



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.

10 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
15 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
20 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
5 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
10 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
15 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-glycolid) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA),
20 polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic 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,
30 *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, 5 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, 10 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, 15 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 20 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 25 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, 30 an activator of p38 MAP kinase, or an activator of NF- κ B.

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, 5 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.

10 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 15 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 20 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 25 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 30 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
5 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
10 peptide such as tat or antennapedia. 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
15 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.*,
20 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
25 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)
30 (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
5 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
10 (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
15 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
20 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 angiogenesis. Preferred conjugates of this type of ligand target PECAM-1, VEGF, or other cancer gene, *e.g.*, a cancer gene described herein.

25 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.*,
30 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.

5 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, H₂A peptides, *Xenopus* peptides, esculentinis-1, and caerins. A number of factors will preferably be considered

10 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

15 helix. Formation of salt bridges between residues with opposite charges, separated by $i \pm 3$, or $i \pm 4$ 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

20 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

25 sugars such as GalNAc cluster, mannose cluster, galactose cluster, or an aptamer. 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

30 unmodified or have any combination of modifications disclosed herein.

Endosomal release agents include imidazoles, poly or oligoimidazoles, PEIs, peptides, fusogenic peptides, polycarboxylates, 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
5 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. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride,
10 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 backbone are also amenable to the present invention as ligands (*e.g.*, as PK
15 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
20 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
25 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
30 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-(CH₂)_nNH₂ may be incorporated into a growing oligonucleotide 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.

10 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.

15 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.

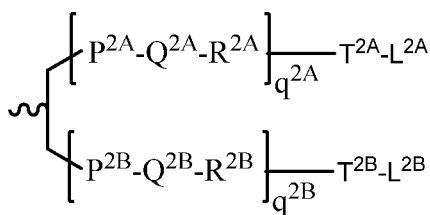
20 In some embodiments, ligand can be conjugated to nucleobases, sugar moieties, or internucleosidic linkages of nucleic acid molecules. Conjugation to purine nucleobases or derivatives thereof can occur at any position including, endocyclic and exocyclic atoms. In some embodiments, the 2-, 6-, 7-, or 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, phosphoramidate, 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.

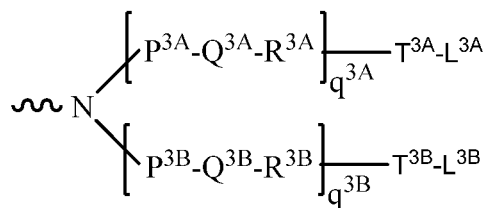
Any suitable ligand in the field of RNA interference may be used, although the ligand is typically a carbohydrate *e.g.* monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, polysaccharide.

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.

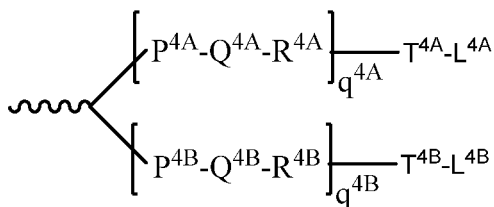
In one embodiment, the dsRNA of the invention is conjugated to a bivalent and trivalent branched linkers include the structures shown in any of formula (IV) – (VII):



Formula (IV)



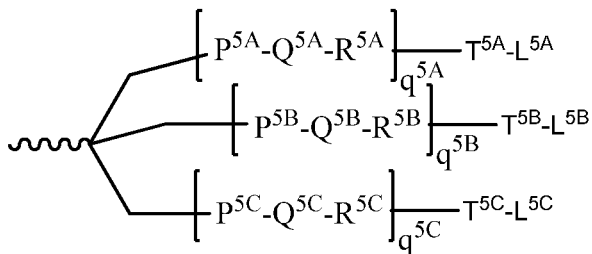
Formula (V)



Formula (VI)

15

, or

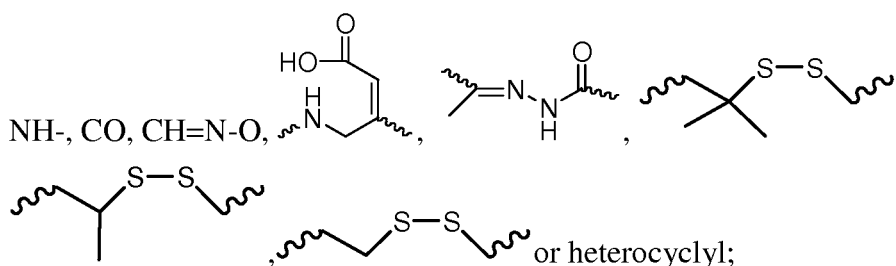


Formula (VII)

;

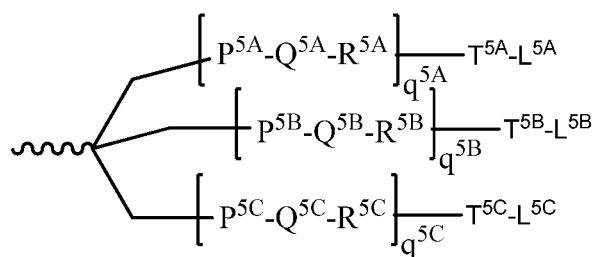
wherein:

- q^{2A} , q^{2B} , q^{3A} , q^{3B} , q^{4A} , 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^{5A} , T^{5B} , T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH₂, CH₂NH or CH₂O;
- 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 wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO₂, 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, CH₂, C(O)O, C(O)NH, NHCH(R^a)C(O), -C(O)-CH(R^a)-



- 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.

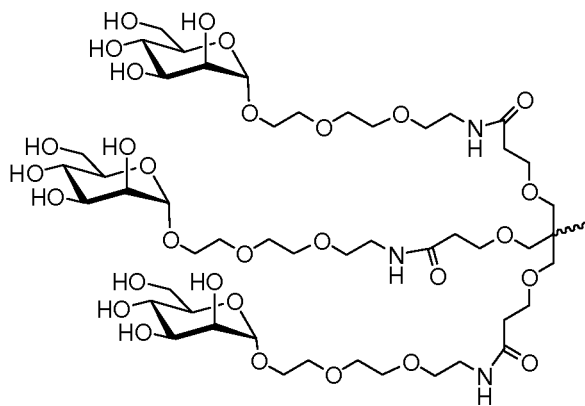
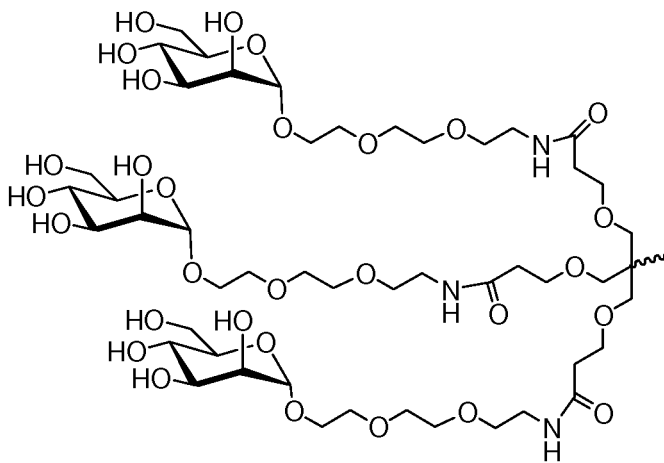
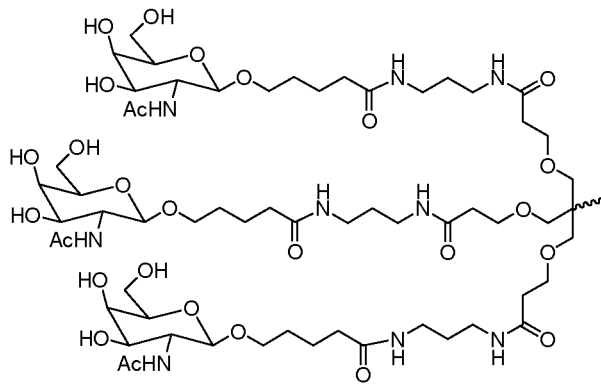
- Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (VII):



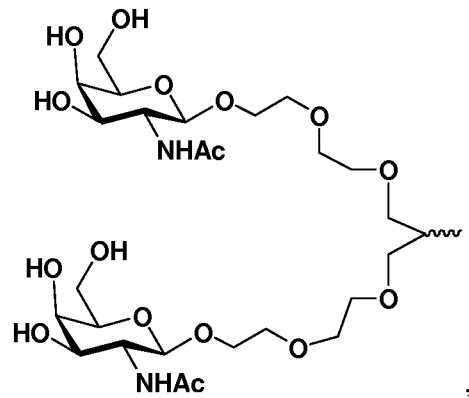
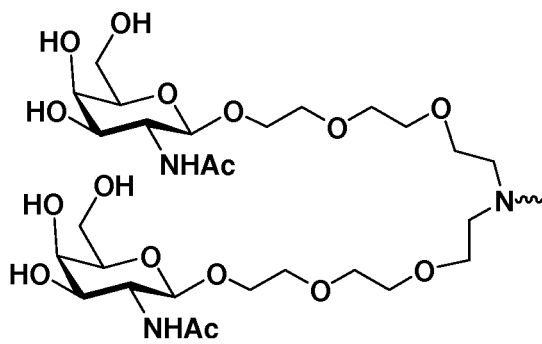
Formula (VII)

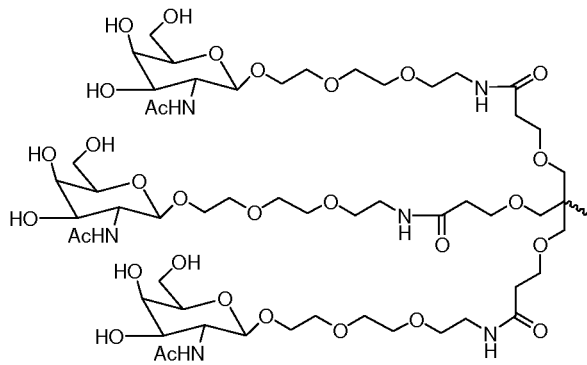
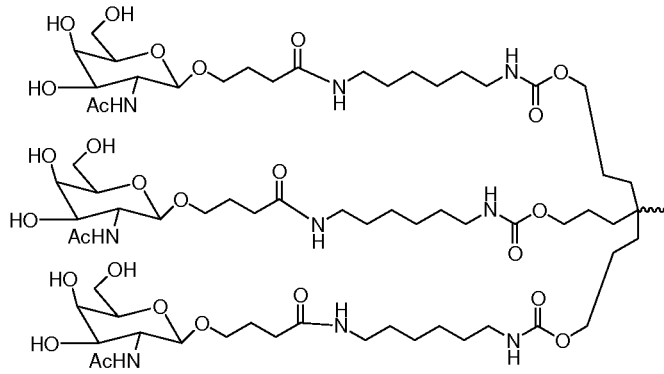
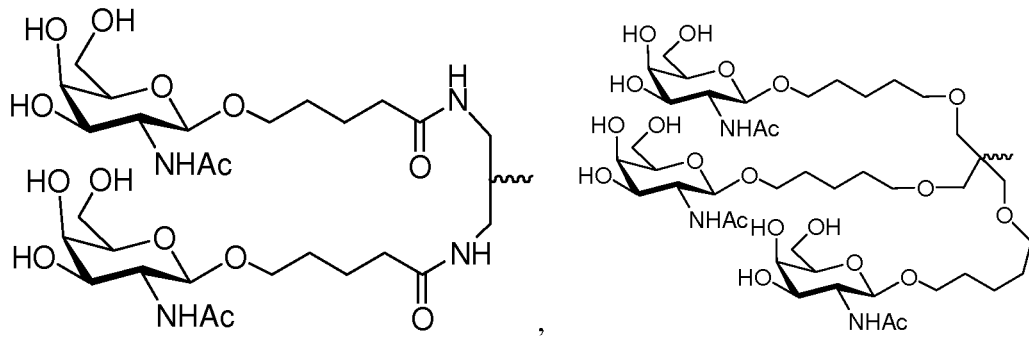
wherein L^{5A} , L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

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



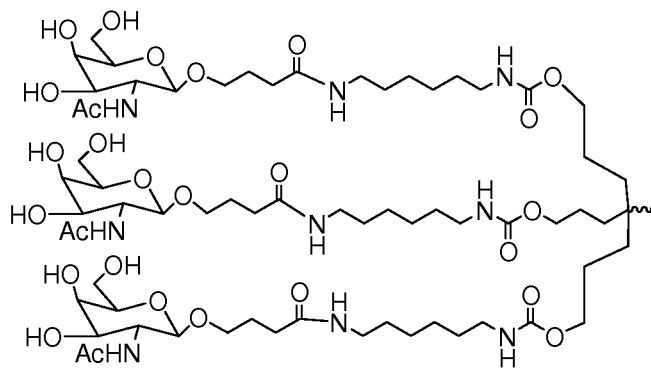
5





5

, or



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.

5 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
10 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
15 saline or in water. Alternatively, the free siRNA may also be administered 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
20 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,
25 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
30 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
5 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-
10 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
15 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
20 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-
25 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 Mg²⁺), salts, RNase
30 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 compositions are 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
5 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 composition and not injurious to the patient.
10 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 stearate, sodium lauryl sulfate and talc;
15 (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
20 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 pharmaceutical compositions.

25 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
30 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
5 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
10 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
15 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
20 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.*
25 *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
30 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.

5 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. 10 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 15 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 20 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.

25 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 dioctaoyleamide ("DOGS") (Transfectam™, Promega, Madison, Wisconsin) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl- 30 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
5 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
10 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
15 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
20 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
25 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
30 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
5 the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transfersomes 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
10 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 transfersomes 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.

15 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
20 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 dsRNA which can be processed into a siRNA, or a DNA which encodes a siRNA or
25 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
30 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
5 membranes may be prepared by mixing an aqueous solution of the siRNA composition, an alkali metal C₈ to C₂₂ 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,
10 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
15 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
20 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
25 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,
30 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-
5 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
10 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
15 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
20 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
25 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 GalNAc₃ ligand, or any other ligand that directs the RNAi
30 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
5 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.

10 “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
15 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-
20 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
25 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
30 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 inhibition 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.*,
5 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
10 *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
15 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.

20 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
25 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
30 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
5 (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
10 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).

15 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
20 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
25 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
30 (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
5 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:*
10 *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,
15 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
20 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
25 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
30 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 asialoglycoprotein receptor).

15 **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.

20 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.

30 A “TTR-associated disease,” as used herein, includes any disease caused by or associated with the formation of amyloid deposits in which the fibril precursors 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. Clinical syndromes associated with TTR aggregation include, for example, 5 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 10 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. 15 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 20 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 25 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 30 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 intravitreal 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
5 “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
10 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
15 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,
20 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,
25 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
30 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
5 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.

10 "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
15 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
20 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.*,
25 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
30 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 "prophylactically effective amount" also
5 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
10 "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
15 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
20 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.

25 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
30 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, 5 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, 10 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 15 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 20 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 25 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 30 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, 5 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 10 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 15 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 20 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.

25 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, 30 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
5 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
10 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.*,
15 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
20 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.

25 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.

30

EXAMPLES**Example 1: Inhibition of TTR with TTR-GalNAc conjugates**

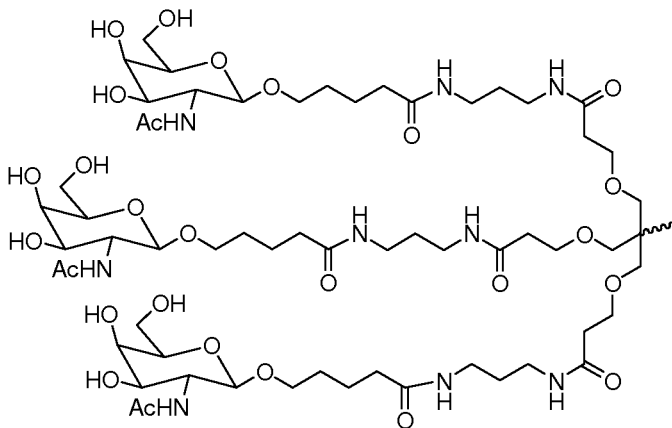
A single dose of the TTR RNAi agent AD-43527 was administered to mice subcutaneously and TTR mRNA levels were determined 72 hours post administration.

- 5 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.

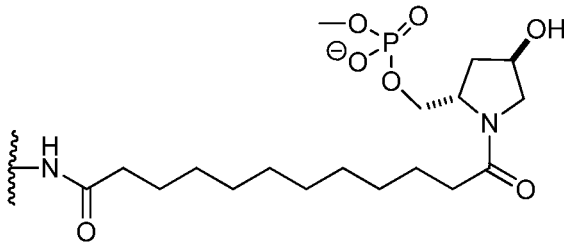
Strand: s= sense; as= antisense

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>i.e.</i> , Af) is a 2'-fluoro nucleotide

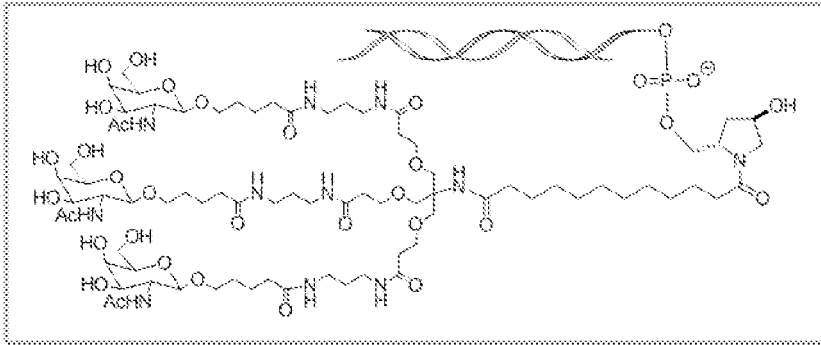
- 10 The ligand used was GalNAc₃:



- 15 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 GalNAc₃ conjugated sense strand is shown in the following schematic:



5

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)

10

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.

15

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 = GalNAc₃; 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.

20

AD-43527 was administered to female C57BL/6 mice (6-10 weeks, 5 per group) via subcutaneous injection at a dose volume of 10 μ l/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.

5 After approximately seventy two hours, mice were anesthetized with 200 μ l 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
10 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 10 μ l of Proteinase-K/ml for a final concentration of 20mg/ml) was incubated at 65 °C for 35 minutes. 5 μ l of liver lysate and 95 μ l of working probe set (TTR probe for gene target and GAPDH for endogenous
15 control) were added into the Capture Plate. Capture Plates were incubated at 53 °C \pm 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. 100 μ l of Amplifier Probe mix per well was added into the Capture Plate, which was sealed with aluminum foil and incubated
20 for 1 hour at 46°C \pm 1°C. Following a 1 hour incubation, the wash step was repeated, then 100 μ l of Label Probe mix per well was added. Capture plates were incubated at 46 °C \pm 1 °C for 1 hour. The plates were then washed with 1X Wash Buffer, dried and 100 μ l 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
25 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
30 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 ED₅₀ 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.

5

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

20 1. Oligonucleotide Synthesis

Oligonucleotides were synthesized on an AKTAoligopilot synthesizer or an ABI 394 synthesizer. 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'-fluoro-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 (CH₃CN) except for guanosine which was used at 0.2M concentration in 10% THF/ANC
5 (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
10 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
15 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
20 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
25 the growing chain under standard phosphoramidite coupling conditions unless otherwise specified. An extended 15 minute coupling of 0.1M solution of phosphoramidite in anhydrous CH₃CN 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-
30 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-difluorotoluy 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

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% CH₃CN (buffer A) and 20 mM sodium phosphate (pH 8.5) in 10% CH₃CN, 1M NaBr (buffer B). Fractions containing full-length oligonucleotides were pooled, desalted, and lyophilized. Approximately 0.15 OD of desalted oligonucleotides were diluted in water to 150 μ l and then pipetted in special vials for CGE and LC/MS analysis. Compounds were finally analyzed by LC-ESMS and CGE.

15

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.

20

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	AfuGfuAfaCfaAfAfGfaGfuAfuUfcCfasU	AS1000	1110	AfUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.1	0.47	0.006
D1001	S1001	19	AfsuGfuAfaCfaAfAfGfaGfuAfuucCfasUf	AS1001	1111	aUfsgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.10	0.49	0.0065
D1002	S1002	20	AfuGfuAfaCfaAfAfGfaGfuAfuucCfasUf	AS1002	1112	aUfgGfAfAfuAfcUfcuuGfgsUfuAfcAfusGfsa	0.04	0.10	0.46	0.0068
D1003	S1003	21	AfuGfuAfaCfaAfAfGfaGfuAfuucCfasUf	AS1003	1113	aUfgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	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	AfuGfuAfaCfaAfAfGfaGfuAfuucCfasUf	AS1005	1115	aUfgGfAfAfuAfcUfcuuGfgsUfsuAfcAfusGfsa	0.06	0.11	0.53	0.0093
D1006	S1006	24	AfuGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1006	1116	aUfgGfaAfuAfcUfcuuGfguuAfcAfusGfsa	0.05	0.16	0.55	0.0095
D1007	S1007	25	AfuGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	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	UfgGfAfAfuUfuCfAfUfgUfaAfcCfaAfAfsaF	AS1009	1119	uCuugGfuUfaCfaugAfaAfuuccCfasUfsc	0.03	0.14	0.56	0.0101
D1010	S1010	28	UfgGfgauUfuCfAfUfgUfaAfcCfaAfgsAf	AS1010	1120	uCfuUfgGfuUfaCfaugAfaAfuCfcCfasUfsc	0.03	0.14	0.65	0.0101
D1011	S1011	29	aUfgGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1011	1121	aUfgGfaAfuAfcUfcuuGfguuAfcAfusGfsa	0.06	0.10	0.55	0.011
D1012	S1012	30	UfgGfgAfuUfuCfAfUfgUfaacCfaAfgsAf	AS1012	1122	uCuUfgGfuUfaCfaugAfaAfuCfcCfasUfsc	0.04	0.13	0.54	0.0114
D1013	S1013	31	auguaaccaadGadGudAudAcdGasu	AS1013	1123	aUfgGfaAfuAfcUfcUfugadGudTadCadTsgsa	0.11	0.19	0.49	0.011
D1014	S1014	32	AfuGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1014	1124	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.04	0.16	0.59	0.013
D1015	S1015	33	AfuguAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1015	1125	dAUdGgdAadTafdCUfcUfuGfgUfuAfcAfusGfsa	0.07	0.15	0.51	0.013
D1016	S1016	34	auGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1016	1126	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.05	0.14	0.64	0.013
D1017	S1017	35	UfgGfgAfuUfuCfAfUfgUfaAfcCfaAfgsAf	AS1017	1127	uCuUfgGfuuaCfaugAfaAfuCfcCfasUfsc	0.09	0.41	0.74	0.0133
D1018	S1018	36	AfuguAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1018	1128	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.03	0.14	0.61	0.014
D1019	S1019	37	AfuGfuAfaCfaAfAfGfaGfuAfuUfcCfasUf	AS1019	1129	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.02	0.2	0.7	0.014
D1020	S1020	38	AfsuGfuAfaCfaAfAfGfaGfuAfuucCfasUf	AS1020	1130	asUfgGfAfAfuAfcUfcuuGfgUfuAfcAfusGfsa	0.04	0.16	0.67	0.0156

D1021	S1021	39	aUfguAfaFccAfAfgagUfaUfuCfcasUf	AS1021	1131	aUfgGfAfaUfaCfaUfcuuGfguuAfcfaUfsgsa	0.11	0.24	0.64	0.016
D1022	S1022	40	dTdGggdAdTuudCdAugTdAacdCqAagsdA	AS1022	1132	udCdTugdGdTuaqCdAugdAdAadCcdCcaasdTsc	0.08	0.27	0.64	0.0161
D1023	S1023	41	AfsuGfuAfaCfcAfAfgaGfuAfuuccFasUf	AS1023	1133	aUfsgGfAfaUfaCfcUfcuuGfgUfuAfcAfcGfsa	0.03	0.19	0.63	0.0163
D1024	S1024	42	UfgGfgAfuUfuCfaUfuguaAfcCfaAfgsAf	AS1024	1134	uCuUfgGfuUfaCfaCfaugAfaAfuCfcCfasUfsc	0.05	0.25	0.69	0.0164
D1025	S1025	43	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1025	1135	uCuUfgGfuuaCfaugAfaAfuCfcCfasUfsc	0.04	0.18	0.75	0.0166
D1026	S1026	44	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1026	1136	uCuUfgGfuUfaCfaCfaugAfaAfuCfcCfasUfsc	0.04	0.19	0.66	0.0178
D1027	S1027	45	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1027	1137	uCuUfgGfuUfaCfaCfaugAfaAfuCfcCfasUfsc	0.04	0.19	0.69	0.018
D1028	S1028	46	dAdTgudAdAccdAdAgadGdTaudTdCcaasdT	AS1028	1138	adTdGgadAdTacdTdCuudGdGuudAdCausdGsa	0.15	0.29	0.72	0.018
D1029	S1029	47	AdTgTdAdACdCAdAGAGdTAGdTudCCdAsU	AS1029	1139	dAUdGGdAAAdTAdCUdCUdTdGdGdAdAdCAdTsGsdA	0.1	0.27	0.61	0.018
D1030	S1030	48	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1030	1140	uCuUfgGfuUfaCfaCfaugAfaAfuCfcCfasUfsc	0.04	0.21	0.64	0.0187
D1031	S1031	49	AfuGfuAfaFccAfAfgaGfuAfuuccAfsu	AS1031	1141	AfUfgGfAfaUfaCfcUfcUfuGfguuAfcAfcGfsa	0.06	0.15	0.62	0.019
D1032	S1032	50	AfsuGfuAfaCfcAfAfgaGfuAfuuccCfasUf	AS1032	1142	asUfgGfAfaUfaCfcUfcuuGfgUfuAfcAfcGfsa	0.09	0.34	0.78	0.021
D1033	S1033	51	UfgGfgAfuUfuCfaUfGfUfaacCfaAfgsAf	AS1033	1143	uCuUfgGfuUfacaUfgAfaAfuCfcCfasUfsc	0.06	0.26	0.57	0.0212
D1034	S1034	52	AfuGfuAfaFccAfaGfaGfuUfuCfcCfasUf	AS1034	1144	aUfgGfaAfuAfcUfcUfuGfguuAfcAfcGfsa	0.11	0.39	0.82	0.0216
D1035	S1035	53	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1035	1145	uCuUfgGfuUfaCfaCfaugAfaAfuCfcCfasUfsc	0.04	0.16	0.56	0.0222
D1036	S1036	54	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1036	1146	uCuugGfuUfaCfaUfgAfaAfuuccCfasUfsc	0.06	0.31	0.78	0.0234
D1037	S1037	55	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1037	1147	uCuUfgGfuUfaCfaCfaugAfaAfuuccCfasUfsc	0.03	0.14	0.62	0.0235
D1038	S1038	56	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1038	1148	uCuUfgGfuuaCfaCfaugAfaAfuCfcCfasUfsc	0.09	0.39	0.78	0.0239
D1039	S1039	57	AfuGfuAfaCfcAfAfgaGfuAfuuccCfasUf	AS1039	1149	aUfgGfAfaUfaCfcUfcuuGfgUfuAfcAfcGfsa	0.03	0.14	0.59	0.025
D1040	S1040	58	AfuGfuAfaCfcAfAfgaGfuAfuUfccasUf	AS1040	1150	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfcGfsa	0.03	0.13	0.56	0.025
D1041	S1041	59	AfsuGfuAfaCfcAfAfgaGfuAfuuccCfasUf	AS1041	1151	asUfgGfAfaUfaCfcUfcuuGfgUfuAfcAfcGfsa	0.06	0.27	0.79	0.0252
D1042	S1042	60	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1042	1152	uCuUfgGfuuaCfaugAfaAfuCfcCfasUfsc	0.05	0.27	0.67	0.0259
D1043	S1043	61	AfuGfuAfaCfcAfAfgaGfuUfuCfcCfasUf	AS1043	1153	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfcGfsa	0.02	0.16	0.63	0.027
D1044	S1044	62	AfsuGfuAfaCfcAfAfgaGfuAfuuccCfasUf	AS1044	1154	asUfgGfAfaUfaCfcUfcuuGfgsUfscAfcAfcGfsa	0.06	0.30	0.81	0.0271
D1045	S1045	63	aUfguAfaFccAfAfgaGfgauUfCfcasUf	AS1045	1155	aUfgGfaAfuUfaCfcUfcuuGfguuAfcfaUfsgsa	0.12	0.29	0.8	0.028
D1046	S1046	64	AfuGfuAfaCfcAfAfgaGfuUfuCfcCfasUf	AS1046	1156	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfcGfsa	0.03	0.15	0.59	0.030
D1047	S1047	65	UfgGfgAfuUfuCfaUfugUfaAfcCfaAfgsAf	AS1047	1157	uCuUfgGfuuaCfaUfgAfaAfuuccCfasUfsc	0.08	0.44	0.83	0.0324

D1048	S1048	66	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1048	1158	aUfgGfaAfuAfcUfcuuGfgUfuAfcAfuGfsa	0.07	0.23	0.67	0.036
D1049	S1049	67	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1049	1159	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	0.08	0.23	0.73	0.037
D1050	S1050	68	UfgGfgAfuUfcCfasUf	AS1050	1160	uCuugGfuUfaCfaUfgAfaAfuCfcCfasUfsc	0.06	0.29	0.78	0.0372
D1051	S1051	69	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1051	1161	aUfgGfaAfuAfcUfcUfcGgdTuAfcAfuGfsa	0.12	0.41	0.86	0.040
D1052	S1052	70	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1052	1162	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.1	0.22	0.72	0.042
D1053	S1053	71	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1053	1163	dAUdGGdAAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.09	0.31	0.69	0.044
D1054	S1054	72	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1054	1164	adTdGGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.1	0.45	0.75	0.047
D1055	S1055	73	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1055	1165	dAUdGGdAAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.12	0.26	0.7	0.049
D1056	S1056	74	AuGuAaCcaAgaGuAuuUcCasU	AS1056	1166	aUgGaAuAcUcuGguUuAcAusGsa	0.08	0.24	0.65	0.050
D1057	S1057	75	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1057	1167	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.14	0.42	0.62	0.051
D1058	S1058	76	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1058	1168	aUfgGfaAfuAfcUfcUfcGgdTuAfcAfuGfsa	0.12	0.36	0.86	0.053
D1059	S1059	77	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1059	1169	dAUdGGdAAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.09	0.27	0.7	0.054
D1060	S1060	78	adTgudAdAccdAdAgagdTadTuUcCcdAsU	AS1060	1170	adTdGgdAadTadCdTdCuudGdGuudAdCadTsgsa	0.11	0.37	0.66	0.056
D1061	S1061	79	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1061	1171	adTdGGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.1	0.31	0.77	0.059
D1062	S1062	80	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1062	1172	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.1	0.27	0.65	0.059
D1063	S1063	81	adTdGuadAdCccdAdGagdTgAuudCdCasU	AS1063	1173	dAdTggdAAuadCdTcuudTgGgdTgAcadTsdGsa	0.12	0.44	0.82	0.064
D1064	S1064	82	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1064	1174	adTdGGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.12	0.32	0.83	0.064
D1065	S1065	83	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1065	1175	dAUdGGdAAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.13	0.34	0.72	0.066
D1066	S1066	84	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1066	1176	adTdGGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.11	0.33	0.72	0.067
D1067	S1067	85	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1067	1177	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.11	0.37	0.62	0.070
D1068	S1068	86	AfuguAfaCfaAfGfaGfuAfuUfcCfasUf	AS1068	1178	dAUdGGdAAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.16	0.33	0.64	0.072
D1069	S1069	87	aUfgGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1069	1179	AfuGfgAfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.14	0.43	0.73	0.074
D1070	S1070	88	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1070	1180	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.08	0.42	0.94	0.075
D1071	S1071	89	UfgGfgAfuUfcCfasUf	AS1071	1181	uCuUfgGfuUfaCfaUfgAfaAfuCfcCfasUfsc	0.14	0.28	0.83	0.0797
D1072	S1072	90	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1072	1182	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.05	0.26	0.8	0.082
D1073	S1073	91	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1073	1183	aUfgGfadAdTAdCUfcUfuGfgUfuAfcAfuGfsa	0.12	0.41	0.73	0.083
D1074	S1074	92	AfuGfuAfaCfaAfGfaGfuAfuUfcCfasUf	AS1074	1184	aUfgGfaAfuAfcUfcUfcGfuUfuAfcAfuGfsa	0.14	0.44	0.75	0.086

D1075	S1075	93	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1075	1185	aUfgGfaAdAdTdAcUfcUfuGfgUfuAfcAfcAfcGfsa	0.1	0.41	0.72	0.088
D1076	S1076	94	AfuGfuAfaCfaCfaGfaGfaGfuAdTdTqCCfasUf	AS1076	1186	aUfgdGdAdAdTAtFcUfcUfuGfgUfuAfcAfcAfcGfsa	0.15	0.45	0.86	0.088
D1077	S1077	95	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasu	AS1077	1187	AfuGfgAfaUfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.08	0.46	0.95	0.092
D1078	S1078	96	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1078	1188	dAudGgdAadTAdTAcUfcUfuGfgUfuAfcAfcAfcGfsa	0.09	0.32	0.76	0.093
D1079	S1079	97	AfuguAfaCfaCfaGfaGfaGfuAdTdudCcdAsu	AS1079	1189	dAudGgdAadTAtFcUfcUfuGfgUfuAfcAfcAfcGfsa	0.14	0.38	0.76	0.095
D1080	S1080	98	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1080	1190	aUfgGfaAfaUfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.05	0.42	0.86	0.099
D1081	S1081	99	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfdCdCqAsdT	AS1081	1191	dAdTdGdGaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.17	0.47	0.9	0.105
D1082	S1082	100	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1082	1192	aUfgGfaAfuAdACudCUfdGGGfudTAdAfcAfcGfsa	0.12	0.44	0.83	0.106
D1083	S1083	101	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1083	1193	adTGGfaAfdTdAcUfcUfuGfgUfuAfcAfcAfcGfsa	0.11	0.34	0.74	0.109
D1084	S1084	102	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1084	1194	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.1	0.45	0.93	0.117
D1085	S1085	103	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1085	1195	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.07	0.42	0.78	0.120
D1086	S1086	104	aUfguAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1086	1196	aUfgGfaAfuAfcUfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.17	0.45	0.83	0.1197
D1087	S1087	105	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasu	AS1087	1197	AfuGfgAfaUfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.05	0.3	0.7	0.120
D1088	S1088	106	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1088	1198	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.11	0.46	0.8	0.120
D1089	S1089	107	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1089	1199	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.14	0.49	0.85	0.122
D1090	S1090	108	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1090	1200	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.1	0.41	0.85	0.125
D1091	S1091	109	AfuguAfaCfaCfaGfaGfaGfuAdTdudCcdAsu	AS1091	1201	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.16	0.38	0.77	0.125
D1092	S1092	110	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasu	AS1092	1202	AfuGfgAfaUfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.05	0.31	0.93	0.126
D1093	S1093	111	auGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1093	1203	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.06	0.33	0.9	0.135
D1094	S1094	112	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfccasUf	AS1094	1204	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.07	0.39	0.85	0.142
D1095	S1095	113	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1095	1205	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.09	0.39	0.76	0.146
D1096	S1096	114	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfccasUf	AS1096	1206	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.06	0.38	0.85	0.147
D1097	S1097	115	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfccasUf	AS1097	1207	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.12	0.47	0.87	0.147
D1098	S1098	116	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfccasUf	AS1098	1208	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.06	0.42	0.85	0.151
D1099	S1099	117	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1099	1209	dAudGgdAadTAdTAdCudCUfdGGGfudTAdAfcAfcGfsa	0.16	0.41	0.85	0.152
D1100	S1100	118	AfuguAfaCfaCfaGfaGfaGfuAfuUfcCfasUf	AS1100	1210	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.15	0.48	0.72	0.152
D1101	S1101	119	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfccasUf	AS1101	1211	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcAfcGfsa	0.06	0.38	0.94	0.158

D1102	S1102	120	AfuGfuAfaccaagaguAfuUfcCfasUf	AS1102	1212	aUfgGfaAfuAfdCuCfdTuGfdGuUfacAfuGfsa	0.21	0.45	0.89	0.162
D1103	S1103	121	AfuGfuAaCfcAfaGfaGfuAfuUfcCfasUf	AS1103	1213	aUfgGfaAfuAfcUfcUfuggUfUfAfcAfuGfsa	0.14	0.49	0.95	0.163
D1104	S1104	122	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1104	1214	aUfgGfaAfuAfcUfcUfGfgUfuAfcAfuGfsa	0.06	0.36	0.92	0.163
D1105	S1105	123	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1105	1215	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.1	0.45	0.84	0.167
D1106	S1106	124	AfuGfuAaCfcAfaGfaGfuAfuUfcCfasUf	AS1106	1216	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.09	0.43	0.91	0.170
D1107	S1107	125	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1107	1217	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.09	0.46	1	0.171
D1108	S1108	126	AfuguAfacAfaGfaGfdTadTudCcdAsu	AS1108	1218	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.11	0.39	0.71	0.176
D1109	S1109	127	AfuGfuAfaCfcAfaGfaGfuAfuUfccasUf	AS1109	1219	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.1	0.43	0.9	0.180
D1110	S1110	128	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1110	1220	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.06	0.42	0.88	0.182
D1111	S1111	129	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1111	1221	dAUdGGdAAuAfcUfcUfuGfgUfuAfcAfuGfsa	0.18	0.49	0.79	0.183
D1112	S1112	130	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1112	1222	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.48	0.85	0.195
D1113	S1113	131	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1113	1223	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.09	0.41	0.85	0.201
D1114	S1114	132	auGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1114	1224	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.05	0.44	0.94	0.201
D1115	S1115	133	AfuguAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1115	1225	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.08	0.41	0.96	0.204
D1116	S1116	134	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1116	1226	adTdGfadAdTAfcUfcUfuGfgUfuAfcAfuGfsa	0.15	0.47	0.79	0.208
D1117	S1117	135	AfuGfuAaCfcAfaGfaGfuAfuUfcCfasUf	AS1117	1227	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.08	0.42	0.92	0.224
D1118	S1118	136	auguaaccaagaguauuccasu	AS1118	1228	AfUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.5	0.87	0.303
D1119	S1119	137	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1119	1229	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.55	0.89	
D1120	S1120	138	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1120	1230	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.63	0.72	
D1121	S1121	139	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1121	1231	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.61	0.91	
D1122	S1122	140	AfuGfuAfaCfcAfaGfaGfuAfuUfccasUf	AS1122	1232	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.54	0.95	
D1123	S1123	141	auGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1123	1233	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.13	0.61	0.97	
D1124	S1124	142	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1124	1234	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.56	0.94	
D1125	S1125	143	AfuGfuAfaCfcaaGfaGfuAfuUfcCfasUf	AS1125	1235	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.21	0.74	0.95	
D1126	S1126	144	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1126	1236	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.2	0.69	0.91	
D1127	S1127	145	AfuguAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1127	1237	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.17	0.7	0.96	
D1128	S1128	146	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfasUf	AS1128	1238	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.62	0.85	

D1129	S1129	147	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1129	1239	aUfggAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.23	0.76	0.98
D1130	S1130	148	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1130	1240	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.21	0.64	0.9
D1131	S1131	149	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1131	1241	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.17	0.7	1.01
D1132	S1132	150	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1132	1242	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.17	0.58	0.87
D1133	S1133	151	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1133	1243	augGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.33	0.89	1.05
D1134	S1134	152	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1134	1244	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.16	0.64	0.96
D1135	S1135	153	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1135	1245	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.12	0.53	0.96
D1136	S1136	154	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1136	1246	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.16	0.58	0.98
D1137	S1137	155	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1137	1247	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.16	0.6	0.91
D1138	S1138	156	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1138	1248	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.1	0.54	0.91
D1139	S1139	157	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1139	1249	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.24	0.68	0.98
D1140	S1140	158	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1140	1250	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.13	0.75	0.9
D1141	S1141	159	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1141	1251	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.15	0.52	1.05
D1142	S1142	160	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1142	1252	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.16	0.66	0.89
D1143	S1143	161	auGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1143	1253	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.12	0.51	0.89
D1144	S1144	162	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1144	1254	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.25	0.71	0.95
D1145	S1145	163	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1145	1255	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.17	0.74	0.98
D1146	S1146	164	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1146	1256	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.11	0.51	0.86
D1147	S1147	165	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1147	1257	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.1	0.52	0.83
D1148	S1148	166	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1148	1258	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.14	0.63	0.98
D1149	S1149	167	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1149	1259	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.13	0.58	0.88
D1150	S1150	168	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1150	1260	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.15	0.62	0.94
D1151	S1151	169	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1151	1261	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.18	0.73	0.94
D1152	S1152	170	auGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1152	1262	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.13	0.53	0.97
D1153	S1153	171	AfuGfuAfaCfaCfaGfaGfaGfuAfuUfCfcfasUf	AS1153	1263	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfcGfsa	0.13	0.53	0.98
D1154	S1154	172	UfgGfgAfuUfCfaUfgUfaAfcCfaAfgsAf	AS1154	1264	uCuUfgGfuUfaCfaUfgAfaAfuUfCfcCfasUfsc	0.09	0.5	0.78
D1155	S1155	173	UfgGfgAfuUfCfaUfgUfaAfcCfaAfgsAf	AS1155	1265	uCuUfgGfuUfaCfaUfgAfaAfuUfCfcCfasUfsc	0.13	0.62	0.89

D1156	S1156	174	UfgGfgAfuuuCfaUfGfUfaAfcCfaAfgsAf	AS1156	1266	uCuUfgGfuUfacaUfgAfaAfuCfcCfasUfsc	0.12	0.65	0.85
D1157	S1157	175	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfgsAf	AS1157	1267	uCuUfgGfuuaCfaUfgAfaAfuCfcCfasUfsc	0.11	0.54	0.85
D1158	S1158	176	UfgGfgAfuuuCfaUfgUfaAfcCfaAfgsAf	AS1158	1268	uCuUfgGfuuaCfaUfgAfaAfuCfcCfasUfsc	0.13	0.53	0.8
D1159	S1159	177	UfgGfgAfuUfuUfcAfuGfuAfaAfcCfaAfgsAf	AS1159	1269	uCuUfgGfuuaAfcAfuGfaAfuCfcCfasUfsc	0.59	0.89	0.81
D1160	S1160	178	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfgsAf	AS1160	1270	uCuUfgGfuuaCfaUfgAfaAfuCfcCfasUfsc	0.16	0.72	0.9
D1161	S1161	179	UfgGfgAfuUfucaUfgUfaAfcCfaAfgsAf	AS1161	1271	uCuUfgGfuUfacaUfgAfaAfuCfcCfasUfsc	0.27	0.69	0.86
D1162	S1162	180	AfuGfuAfaCfaaGfaGfuAfuUfcCfasUf	AS1162	1272	aUfgGfaAfuacUfcUfuGfgUfuAfcAfusGfisa	0.12	0.6	0.95
D1163	S1163	181	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1163	1273	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.05	0.56	1.02
D1164	S1164	182	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1164	1274	aUfgGfaAfuacUfcUfuGfgUfuAfcAfusGfisa	0.13	0.55	1
D1165	S1165	183	AfuGfuAfaCfaaGfaGfuAfuUfcCfasUf	AS1165	1275	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.09	0.6	0.97
D1166	S1166	184	AfuguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1166	1276	aUfgGfaAfuAfcUfcUfuggUfuAfcAfusGfisa	0.15	0.59	0.91
D1167	S1167	185	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1167	1277	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.11	0.59	1
D1168	S1168	186	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1168	1278	aUfgGfaAfuAfcUfcUfuggUfuAfcAfusGfisa	0.13	0.57	0.94
D1169	S1169	187	auGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1169	1279	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.08	0.5	0.9
D1170	S1170	188	AfuguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1170	1280	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.06	0.53	0.91
D1171	S1171	189	auGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1171	1281	aUfggaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.07	0.56	0.89
D1172	S1172	190	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1172	1282	aUfgGfaAfuAfcUfcUfuggUfuAfcAfusGfisa	0.13	0.59	0.98
D1173	S1173	191	AfuGfuAfaCfaaGfaGfuAfuUfcCfasUf	AS1173	1283	aUfgGfaAfuAfcucUfuGfgUfuAfcAfusGfisa	0.2	0.65	1.03
D1174	S1174	192	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1174	1284	aUfgGfaauAfcUfcUfuGfgUfuAfcAfusGfisa	0.07	0.51	0.95
D1175	S1175	193	AfuguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1175	1285	aUfggaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.2	0.53	0.76
D1176	S1176	194	auGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1176	1286	augGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.74	0.98	0.81
D1177	S1177	195	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1177	1287	augGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.43	0.64	0.88
D1178	S1178	196	auguaaccAfaGfaGfuAfuUfcCfasUf	AS1178	1288	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.17	0.49	0.81
D1179	S1179	197	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1179	1289	aUfggaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.22	0.65	0.73
D1180	S1180	198	AfuguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1180	1290	augGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.6	1.09	0.8
D1181	S1181	199	auGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1181	1291	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.3	0.78	0.78
D1182	S1182	200	auguaaccAfaGfaGfuAfuUfcCfasUf	AS1182	1292	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa	0.35	0.73	0.84

D1183	S1183	201	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1183	1293	aUfggaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.6	0.94
D1184	S1184	202	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1184	1294	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.61	1.08	0.8
D1185	S1185	203	auGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1185	1295	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.16	0.52	0.72
D1186	S1186	204	auguaaccaagaGfuAfuUfcCfasUf	AS1186	1296	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.2	0.53	0.74
D1187	S1187	205	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1187	1297	aUfggaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.34	0.66	0.85
D1188	S1188	206	AfuGfuAfacAfaGfaGfuAfuUfcCfasUf	AS1188	1298	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.61	0.98	1.02
D1189	S1189	207	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1189	1299	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.3	0.73	0.85
D1190	S1190	208	auguaaccaagaguuuuccasu	AS1190	1300	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.28	0.69	0.78
D1191	S1191	209	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1191	1301	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.33	0.88	0.64
D1192	S1192	210	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1192	1302	aUfggaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.31	0.64	0.83
D1193	S1193	211	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1193	1303	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.64	0.82	0.92
D1194	S1194	212	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1194	1304	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.21	0.62	0.77
D1195	S1195	213	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1195	1305	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.17	0.7	0.95
D1196	S1196	214	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1196	1306	aUfggaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.71	0.65
D1197	S1197	215	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1197	1307	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.64	0.82	0.93
D1198	S1198	216	auguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1198	1308	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.19	0.65	0.72
D1199	S1199	217	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1199	1309	aUfggaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.15	0.52	0.64
D1200	S1200	218	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1200	1310	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.48	0.74	0.92
D1201	S1201	219	auguAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1201	1311	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.17	0.71	0.77
D1202	S1202	220	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1202	1312	augGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.43	0.69	0.85
D1203	S1203	221	auguaaCfaAfaGfaGfuAfuUfcCfasUf	AS1203	1313	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.61	0.76
D1204	S1204	222	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1204	1314	adTdGgfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.16	0.56	0.89
D1205	S1205	223	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1205	1315	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.13	0.57	0.9
D1206	S1206	224	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1206	1316	adTdGgfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.29	0.73	0.89
D1207	S1207	225	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1207	1317	adTdGgfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.16	0.56	0.78
D1208	S1208	226	AfuGfuAfaCfaAfaGfaGfuAfuUfcCfasUf	AS1208	1318	aUfgGdAdAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.22	0.67	0.89
D1209	S1209	227	AfuguAfacAfaGfaGfuAfuUfcCfasUf	AS1209	1319	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfuGfsa	0.14	0.55	0.78

D1237	S1237	255	augUfaAfccaAfgAfguaUfuCfcasu	AS1237	1347	AfUfGfgAfaUfAfcUfcUfGfgUfUfaCfaUfsgsa	0.29	0.61	0.79
D1238	S1238	256	AfugUfaAfccaAfgAfguaUfuCfcasu	AS1238	1348	AfUfGfgAfaUfAfcUfcUfGfgUfUfaCfaUfsgsa	0.31	0.6	0.88
D1239	S1239	257	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1239	1349	dAUdGGdAauAfcUfcUfGfgUfUfaCfaUfsgsa	0.2	0.67	0.85
D1240	S1240	258	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1240	1350	dAUdGGdAauAfcUfcUfGfgUfUfaCfaUfsgsa	0.23	0.58	0.68
D1241	S1241	259	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1241	1351	dAUdGGdAauAfcUfcUfGfgUfUfaCfaUfsgsa	0.25	0.65	0.78
D1242	S1242	260	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1242	1352	dAUdGGdAadTafCufUfGfgUfUfaCfaUfsgsa	0.18	0.64	0.84
D1243	S1243	261	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1243	1353	dAUdGGdAAfuAfcUfcUfGfgUfUfaCfaUfsgsa	0.19	0.72	0.87
D1244	S1244	262	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1244	1354	dAUdGGdAadTafCufUfGfgUfUfaCfaUfsgsa	0.16	0.55	0.8
D1245	S1245	263	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1245	1355	dAUdGGdAAuAfcUfcUfGfgUfUfaCfaUfsgsa	0.22	0.51	0.9
D1246	S1246	264	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1246	1356	dAUdGGdAadTafCufUfGfgUfUfaCfaUfsgsa	0.27	0.78	0.66
D1247	S1247	265	AfuGfuAfaCfaCfaGfaGfuAfuUfcCfasUf	AS1247	1357	dAdTdGdGaAfuAfcUfcUfGfgUfUfaCfaUfsgsa	0.16	0.57	0.97
D1248	S1248	266	AfacaAfguUfcUfuGfdCUdCudAudAsa	AS1248	1358	dTUdAudAgdAGfcAfaGfaAfcAfcUfgUfUfsu	0.06	0.09	0.36
D1249	S1249	267	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1249	1359	UfUfaUfaGfagcAfaGfaAfcAfcUfgUfUfsu	0.06	0.10	0.47
D1250	S1250	268	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1250	1360	uUfaUfaGfcAfaGfaAfcAfcUfgUfUfsu	0.07	0.14	0.55
D1251	S1251	269	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1251	1361	uUfaUfaGfcAfaGfaAfcAfcUfgUfUfsu	0.07	0.14	0.49
D1252	S1252	270	cAGuGuuuuuGccucuAuaAdTd	AS1252	1362	UuuAGAGcAAGAAcACUGdTd			0.006
D1253	S1253	271	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1253	1363	uUfaUfagaGfcAfaGfaAfcAfcUfgUfUfsu	0.05	0.12	0.43
D1254	S1254	272	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1254	1364	UfUfaUfaGfaGfcAfaGfaAfcAfcUfgUfUfsu	0.06	0.13	0.39
D1255	S1255	273	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1255	1365	UfUfaUfagaGfcAfaGfaAfcAfcUfgUfUfsu	0.08	0.17	0.48
D1256	S1256	274	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1256	1366	UfUfaUfaGfaGfcaaGfaAfcAfcUfgUfUfsu	0.08	0.14	0.40
D1257	S1257	275	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1257	1367	uUfaUfagaGfcAfaGfaAfcAfcUfgUfUfsu	0.07	0.12	0.40
D1258	S1258	276	AfaCfaguGfuUfcUfuGfcUfUfaUfasa	AS1258	1368	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfUfsu	0.08	0.13	0.41
D1259	S1259	277	AfaCfAGfuUfcUfuGfcUfUfaUfasa	AS1259	1369	uUfaUfaGfAGfcAfaGfaAfcAfcUfgUfUfsu	0.05	0.11	0.35
D1260	S1260	278	AfacaGfuUfcUfuGfcUfUfaUfasa	AS1260	1370	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfUfsu	0.06	0.12	0.40
D1261	S1261	279	AfacaGfuUfcUfuGfcUfUfaUfasa	AS1261	1371	uUfaUfagaGfcAfaGfaAfcAfcUfgUfUfsu	0.06	0.13	0.42
D1262	S1262	280	AfaCfaGfuUfcUfuGfcUfUfaUfasa	AS1262	1372	uUfaUfaGfAGfcAfaGfaAfcAfcUfgUfUfsu	0.06	0.13	0.37
D1263	S1263	281	cAGuGuuuuuGccucuAuaAdTd	AS1263	1373	UuuAGAGcAAGAAcACUGdTd			0.008

D1264	S1264	282	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1264	1374	uUfaUfaGfagcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.12	0.50	0.008
D1265	S1265	283	AfaCfaGfugUfcUfuGfcUfcUfaUfasAf	AS1265	1375	uUfaUfaGfaGfcAfagaAfcAfcUfgUfusUfsu	0.12	0.13	0.48	0.009
D1266	S1266	284	AfacaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1266	1376	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.51	0.009
D1267	S1267	285	AfacaFfuguUfcUfuGfdCudCudAudAsa	AS1267	1377	dTudAudAgdAGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.14	0.48	0.0088
D1268	S1268	286	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1268	1378	uUfaUfaGfAfGfcAfagaAfcAfcUfgUfusUfsu	0.05	0.09	0.35	0.009
D1269	S1269	287	cAGuGuuuuuGccuuAuAAAdTd	AS1269	1379	UuuAuAGAcAAGAAACACUGdTd				0.009
D1270	S1270	288	aaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1270	1380	uUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.49	0.009
D1271	S1271	289	AfaCfaGfUfcUfuGfcUfcUfaUfasAf	AS1271	1381	uUfaUfaGfAfGfcAfaGfaAfcacUfgUfusUfsu	0.06	0.10	0.36	0.009
D1272	S1272	290	cAGuGuuuuuGccuuAuAAAdTd	AS1272	1382	UuuAuAGAcAAGAAACACUGdTd				0.009
D1273	S1273	291	AfaCfaGfUfcUfuGfcUfcUfaUfasAf	AS1273	1383	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.51	0.009
D1274	S1274	292	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1274	1384	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.12	0.46	0.010
D1275	S1275	293	cAGuGuuuuuGccuuAuAAAdTd	AS1275	1385	UuuAuAGAcAAGAAACACUGdTd				0.010
D1276	S1276	294	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1276	1386	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.14	0.47	0.010
D1277	S1277	295	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1277	1387	uUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.50	0.010
D1278	S1278	296	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1278	1388	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.43	0.010
D1279	S1279	297	cAGuGuuuuuGccuuAuAAAdTd	AS1279	1389	UuuAuAGAcAAGAAACACUGdTd				0.010
D1280	S1280	298	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1280	1390	UfUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.06	0.14	0.45	0.010
D1281	S1281	299	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1281	1391	UfUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.07	0.18	0.46	0.011
D1282	S1282	300	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1282	1392	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.07	0.15	0.55	0.011
D1283	S1283	301	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1283	1393	uUfaUfaGfAfGfcAfaGfaAfcAfcUfgUfususu	0.07	0.12	0.45	0.011
D1284	S1284	302	AfacaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1284	1394	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.48	0.011
D1285	S1285	303	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1285	1395	uUfaUfaGfAfGfcAfaGfaAfcAfcUfguuusUfsu	0.06	0.11	0.40	0.011
D1286	S1286	304	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1286	1396	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.16	0.47	0.011
D1287	S1287	305	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1287	1397	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.07	0.19	0.46	0.012
D1288	S1288	306	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1288	1398	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.17	0.46	0.012
D1289	S1289	307	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1289	1399	uUfaUfaGfAfGfcaaaGfaAfcAfcUfgUfusUfsu	0.05	0.09	0.31	0.012
D1290	S1290	308	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1290	1400	UfUfaUfaGfaGfcAfaGfaAfcAfcUfguuusUfsu	0.06	0.16	0.49	0.013

D1291	S1291	309	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1291	1401	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.06	0.11	0.32	0.013
D1292	S1292	310	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1292	1402	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.06	0.14	0.44	0.013
D1293	S1293	311	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasA	AS1293	1403	UfUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.16	0.39	0.013
D1294	S1294	312	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1294	1404	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.18	0.41	0.014
D1295	S1295	313	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1295	1405	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	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	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1297	1407	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.06	0.15	0.50	0.016
D1298	S1298	316	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1298	1408	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.08	0.17	0.50	0.016
D1299	S1299	317	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1299	1409	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.16	0.50	0.018
D1300	S1300	318	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1300	1410	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.06	0.12	0.43	0.020
D1301	S1301	319	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1301	1411	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.17	0.45	0.021
D1302	S1302	320	AfaCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1302	1412	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.06	0.14	0.49	0.021
D1303	S1303	321	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1303	1413	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.24	0.51	0.022
D1304	S1304	322	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1304	1414	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.09	0.27	0.47	0.033
D1305	S1305	323	aadCdAgudGdTuccdTgCdTgCdCuadTdAasa	AS1305	1415	udTadTdAgadGdCaadGcaadAdCugdTdTtsu	0.19	0.36	0.86	0.045
D1306	S1306	324	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1306	1416	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.08	0.22	0.61	
D1307	S1307	325	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1307	1417	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.13	0.39	0.84	
D1308	S1308	326	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1308	1418	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.09	0.13	0.48	
D1309	S1309	327	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1309	1419	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.13	0.58	
D1310	S1310	328	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1310	1420	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.14	0.55	
D1311	S1311	329	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1311	1421	dTdTadTdAfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.10	0.30	0.66	
D1312	S1312	330	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1312	1422	dTUdAUdAGfaGfcAfaGfaAfcUfgUfusUfsUf	0.09	0.13	0.48	
D1313	S1313	331	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1313	1423	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.14	0.38	0.74	
D1314	S1314	332	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1314	1424	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.19	0.54	
D1315	S1315	333	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1315	1425	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.15	0.55	
D1316	S1316	334	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1316	1426	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.16	0.53	
D1317	S1317	335	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1317	1427	uUfaUfaGfaGfcAfaGfaAfcUfgUfusUfsUf	0.07	0.16	0.55	

D1318	S1318	336	AfAfCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1318	1428	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	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	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1321	1431	uUfaUfagaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.14	0.58	
D1322	S1322	340	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1322	1432	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.15	0.49	0.84	
D1323	S1323	341	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1323	1433	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.07	0.20	0.62	
D1324	S1324	342	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1324	1434	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.08	0.25	0.78	
D1325	S1325	343	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1325	1435	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.08	0.18	0.80	
D1326	S1326	344	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1326	1436	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.21	0.66	
D1327	S1327	345	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1327	1437	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.10	0.31	0.70	
D1328	S1328	346	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1328	1438	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.07	0.15	0.55	
D1329	S1329	347	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1329	1439	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.71	
D1330	S1330	348	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1330	1440	uuUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.27	0.76	
D1331	S1331	349	AfaCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1331	1441	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.21	0.65	
D1332	S1332	350	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1332	1442	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.07	0.17	0.53	
D1333	S1333	351	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1333	1443	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.25	0.73	
D1334	S1334	352	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1334	1444	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.18	0.54	
D1335	S1335	353	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1335	1445	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.14	0.38	0.57	
D1336	S1336	354	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1336	1446	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.16	0.50	0.96	
D1337	S1337	355	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1337	1447	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.54	
D1338	S1338	356	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1338	1448	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.08	0.20	0.69	
D1339	S1339	357	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1339	1449	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.16	0.55	
D1340	S1340	358	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1340	1450	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.17	0.57	
D1341	S1341	359	AfaCfaGfuguUfcUfuGfcUfcUfaUfasAf	AS1341	1451	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfususu	0.08	0.22	0.63	
D1342	S1342	360	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1342	1452	uUfaUfaGfaGfcAfaGfaAfcAfcUfguuUfsu	0.21	0.56	0.86	
D1343	S1343	361	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1343	1453	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.14	0.37	0.73	
D1344	S1344	362	AfaCfaGfuGfuuUfcUfuGfcUfcUfaUfasAf	AS1344	1454	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.20	0.66	

D1345	S1345	363	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1345	1455	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.12	0.34	0.73
D1346	S1346	364	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1346	1456	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.16	0.42	0.90
D1347	S1347	365	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1347	1457	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.17	0.43	0.85
D1348	S1348	366	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1348	1458	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.21	0.58
D1349	S1349	367	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1349	1459	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.21	0.39	0.88
D1350	S1350	368	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1350	1460	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.52
D1351	S1351	369	AfaCfaGfugfuUfcUfuGfcUfcUfaUfasAf	AS1351	1461	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.21	0.58
D1352	S1352	370	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1352	1462	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.18	0.49	0.84
D1353	S1353	371	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1353	1463	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.11	0.25	0.68
D1354	S1354	372	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1354	1464	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.52
D1355	S1355	373	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1355	1465	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.10	0.26	0.63
D1356	S1356	374	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1356	1466	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.16	0.33	0.79
D1357	S1357	375	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1357	1467	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.19	0.51
D1358	S1358	376	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1358	1468	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.22	0.48	0.71
D1359	S1359	377	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1359	1469	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.10	0.17	0.61
D1360	S1360	378	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1360	1470	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.14	0.40	0.87
D1361	S1361	379	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1361	1471	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.52
D1362	S1362	380	aaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1362	1472	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.10	0.28	0.81
D1363	S1363	381	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1363	1473	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.16	0.68
D1364	S1364	382	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1364	1474	uuuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.26	0.67
D1365	S1365	383	aaCaguuuuuugcuuaasa	AS1365	1475	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.20	0.59	0.95
D1366	S1366	384	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1366	1476	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.13	0.53
D1367	S1367	385	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1367	1477	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.16	0.53
D1368	S1368	386	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1368	1478	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.54
D1369	S1369	387	AfaCfaGfuGfuUfcuuGfcUfcUfaUfasAf	AS1369	1479	uuuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.23	0.56	0.89
D1370	S1370	388	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1370	1480	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.12	0.55
D1371	S1371	389	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1371	1481	uUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.18	0.58

D1372	S1372	390	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1372	1482	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.15	0.56	
D1373	S1373	391	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1373	1483	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.21	0.51	0.89	
D1374	S1374	392	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1374	1484	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.21	0.64	
D1375	S1375	393	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1375	1485	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.15	0.40	0.94	
D1376	S1376	394	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1376	1486	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.13	0.40	0.96	
D1377	S1377	395	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1377	1487	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.17	0.64	
D1378	S1378	396	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1378	1488	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.18	0.50	0.97	
D1379	S1379	397	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1379	1489	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.24	0.79	
D1380	S1380	398	aaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1380	1490	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.14	0.58	
D1381	S1381	399	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1381	1491	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.11	0.34	0.96	
D1382	S1382	400	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1382	1492	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.18	0.69	
D1383	S1383	401	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1383	1493	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.14	0.38	0.85	
D1384	S1384	402	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1384	1494	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.16	0.54	
D1385	S1385	403	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1385	1495	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.20	0.75	
D1386	S1386	404	aacaguguUfcUfuGfcUfcUfaUfasAf	AS1386	1496	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.25	0.56	0.90	
D1387	S1387	405	AfaCfaguGfuUfcUfuGfcUfcUfaUfasAf	AS1387	1497	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.70	
D1388	S1388	406	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1388	1498	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.14	0.60	
D1389	S1389	407	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1389	1499	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.19	0.62	
D1390	S1390	408	aaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1390	1500	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.08	0.27	0.76	
D1391	S1391	409	aacaguguUfcUfuGfcUfcUfaUfasAf	AS1391	1501	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.18	0.36	0.81	
D1392	S1392	410	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1392	1502	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.17	0.55	
D1393	S1393	411	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1393	1503	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.07	0.15	0.57	
D1394	S1394	412	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1394	1504	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.26	0.68	1.06	
D1395	S1395	413	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1395	1505	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.06	0.18	0.58	
D1396	S1396	414	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1396	1506	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.09	0.27	0.73	
D1397	S1397	415	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1397	1507	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.20	0.51	0.73	
D1398	S1398	416	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasAf	AS1398	1508	uuUfaUfaGfaGfcAfaGfaAfcAfcUfgUfusUfsu	0.13	0.34	0.86	

D1399	S1399	417	dAacadGugudTcuudGcucdTauasda	AS1399	1509	udTAdAdTadGdAdGcdAdAdGadAdCdaCdTdTsdTtsu	0.24	0.42	0.82
D1400	S1400	418	AfaCfaAfuGfuUfcUfuGfdCdAcCdTtaUfasAf	AS1400	1510	uUfaUfdAdGdAdGcAfaGfaGfcAfaAfgUfusUfsu	0.49	0.85	0.78
D1401	S1401	419	AfaCfaAfuGfuUfcUfuGfdCdAdCUfaUfasAf	AS1401	1511	uUfaUfadGdAdGdCfaGfaGfcAfaAfgUfusUfsu	0.67	0.83	0.85
D1402	S1402	420	aaCfaAfgUGfuUfcUfuGfcUfcuaUfAfsa	AS1402	1512	uUfaUfAfgaGfCfaaGfAfacAfcUfgUfusUfsu	0.18	0.47	0.80
D1403	S1403	421	AfaCfaAfuGfuUfcUfuGfdAcCufadTdAsAf	AS1403	1513	udTdaUfadGdAGfcAfaGfaGfcAfaAfgUfusUfsu	0.73	0.89	0.77
D1404	S1404	422	aacAgugUjucUjgcuCuauAsa	AS1404	1514	uUaUAgaAGCaAGaAcACaCuGUUsusu	0.12	0.39	0.79
D1405	S1405	423	AacaGuguUcuuGcucUauasA	AS1405	1515	uUAUaGAGcAAGaAcAcUGUUsusu	0.12	0.37	0.77
D1406	S1406	424	AfaCfaAfuGfuUfcUfuGfdGdCafCufadTdAsAf	AS1406	1516	udTdaUfaGfadGdCafaGfaGfcAfaAfgUfusUfsu	0.59	0.93	0.89
D1407	S1407	425	aACagUGuuCUugCUcuUUsa	AS1407	1517	UUauAGagCAagAAcaCUguUUsusu	0.09	0.16	0.55
D1408	S1408	426	AfaCfaAfuGfuUfcUfuGfcAfcdTdAdTdAsAf	AS1408	1518	udTdaAdTdAGfaGfcAfaGfaGfcAfaAfgUfusUfsu	0.22	0.64	0.86
D1409	S1409	427	aaCaguGUucUugcUcuaUAsa	AS1409	1519	uUaUAgaGCaAGaAcACugUUUsusu	0.13	0.31	0.76
D1410	S1410	428	AfaCfaAfuGfuUfcUfuGfcAfdCdTdAdTdAsAf	AS1410	1520	udTdaAdTdAdGgaGfcAfaGfaGfcAfaAfgUfusUfsu	0.77	0.94	0.93
D1411	S1411	429	aacAfgugUfucuUfgcuCfuuaUfsa	AS1411	1521	uUfaUfAfgAfgCfaAfgAfaCfaCfaCfuGfuUfusUfsu	0.23	0.53	1.04
D1412	S1412	430	aacdAgugdTucudTgcudCuauAsa	AS1412	1522	udTadTadAgdAdGdCadAdGdAdCdAdCudGdTdTtsusu	0.30	0.64	0.90
D1413	S1413	431	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1413	1523	UfUfaUfaGfaGfcAfaGfaAfaAfcUfgUfusUfsu	0.09	0.19	0.63
D1414	S1414	432	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1414	1524	UfUfaUfaGfaGfcAfaGfaaacAfcUfgUfusUfsu	0.11	0.28	0.66
D1415	S1415	433	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1415	1525	UfUfaUfaGfagcAfaGfaAfaAfcUfgUfusUfsu	0.06	0.13	0.53
D1416	S1416	434	aacaguuuuugcucuauasa	AS1416	1526	UfUfaUfAfgAfgCfaAfaGfaAfaAfcUfgUfusUfsu	0.20	0.53	0.99
D1417	S1417	435	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1417	1527	UfUfaUfaGfaGfcAfaGfaAfaAfcUfgUfusUfsu	0.07	0.17	0.53
D1418	S1418	436	aAfcfagUfgfuUfcUfuGfcUfcUfaUfasa	AS1418	1528	UfUfaUfAfgCfaAfaGfaAfaAfcUfgUfusUfsu	0.08	0.20	0.70
D1419	S1419	437	AfaCfaGfuGfuUfcUfuGfcUfcUfaUfasa	AS1419	1529	uUfaUfaGfaGfcAfaGfaAfaAfcUfgUfusUfsu	0.08	0.20	0.70
D1420	S1420	438	GfaCfuUfcUfcUfcUfcAfgGfaCfcUfl96	AS1420	1530	aGfgUfcCfaCfuGfgagGfaGfaAfgUfcsCfsc			
D1421	S1421	439	GfaCfuUfcUfcUfcUfcAfgUfgGfaCfcUfl96	AS1421	1531	aGfgUfccuCuGfgagGfaGfaAfgUfcsCfsc			
D1422	S1422	440	AfcUfcUfcUfcUfcCfaGfuggAfcUfcUfl96	AS1422	1532	cAfgGfuCfcAfcUfuggaGfgAfgAfaGfusCfsc			
D1423	S1423	441	AfcUfcUfcUfcUfcCfaGfGfAfcUfcUfl96	AS1423	1533	cAfgGfuccAfcUfuggaGfgAfgAfaGfusCfsc			
D1424	S1424	442	CfuUfcUfcUfcUfcCfaGfUfggaCfcUfgAfl96	AS1424	1534	uCfaGfgUfcCfaCfuggAfgGfaGfaAfgUfcs			
D1425	S1425	443	CfuUfcUfcUfcUfcCfaGfAfcUfgAfl96	AS1425	1535	uCfaGfgucCfaCfuggAfgGfaGfaAfgUfcs			

D1426	S1426	444	UfuCfuCfcUfcCfAfGfUgFgacCfuGfaAfl96	AS1426	1536	uUfAfGfUfCfCfAfCugGfaGfGfAfGfAfGfGfsu		
D1427	S1427	445	UfuCfuCfcUfcCfAfGfUgFgAfCfCfuGfaAfl96	AS1427	1537	uUfAfGgguCfcAfCugGfaGfGfAfGfAfGfGfsu		
D1428	S1428	446	UfcUfcCfuCfcAfGfUgFgGfaccUfgAfaGfl96	AS1428	1538	cUfuCfaGfGfUfcCfacuGfgAfGfGfaGfasAfsG		
D1429	S1429	447	UfcUfcCfuCfcAfGfUgFgGfCfCfuGfaGfl96	AS1429	1539	cUfuCfaggUfcCfacuGfgAfGfGfaGfasAfsG		
D1430	S1430	448	CfuCfcUfcCfaGfUgFgAfGfAfcuGfaAfgGfl96	AS1430	1540	cCfuUfcAfGfGfUfcCcacUfgGfaGfGfAfGfAfsa		
D1431	S1431	449	CfuCfcUfcCfaGfUgFgAfCfCfuGfaAfgGfl96	AS1431	1541	cCfuUfcagGfuCfcaUfgGfaGfGfAfGfAfsa		
D1432	S1432	450	UfcCfuCfcAfGfUgFgGfCfCugAfaGfAfl96	AS1432	1542	uCfcUfuCfAfGfUfccacUfccacUfgGfGfGfGfGfsa		
D1433	S1433	451	UfcCfuCfcAfGfUgFgGfCfCfuGfaAfgAfl96	AS1433	1543	uCfcUfucaGfGfUfccacUfccacUfgGfGfGfGfGfsa		
D1434	S1434	452	CfcUfcCfaGfUgFgAfCfCfugaAfgGfAfl96	AS1434	1544	gUfcCfuUfcAfGfGfuccAfcUfgGfaGfGfAfsG		
D1435	S1435	453	CfcUfcCfaGfUgFgAfCfCfuGfaAfgGfAfl96	AS1435	1545	gUfcCfuucAfgGfuccAfcUfgGfaGfGfAfsG		
D1436	S1436	454	CfuCfcAfGfUgFgAfCfCfuGfaAfgGfAfl96	AS1436	1546	cGfuCfcUfUfcCfaGfGfucCfaCfuGfGfAfsGfsa		
D1437	S1437	455	CfuCfcAfGfUgFgAfCfCfuGfaAfgAfl96	AS1437	1547	cGfuCfcuucCfaGfGfucCfaCfuGfGfAfsGfsa		
D1438	S1438	456	UfcCfaGfUgFgAfCfCfuGfaAfgAfl96	AS1438	1548	uCfGfUfcCfuUfcAfGgucCfaCfuGfGfAfsGfsg		
D1439	S1439	457	UfcCfaGfUgFgAfCfCfuGfaAfgGfAfl96	AS1439	1549	uCfGfUfcuUfcAfGgucCfaCfuUfgGfGfGfsG		
D1440	S1440	458	CfcAfGfUgFgAfCfCfuGfaAfgGfAfl96	AS1440	1550	cUfcGfuCfcUfuCfaggUfcCfaCfuGfGfAfsG		
D1441	S1441	459	CfcAfGfUgFgAfCfCfuGfaAfgGfAfl96	AS1441	1551	cUfcGfuUfcCfaggUfcCfaCfuGfGfAfsG		
D1442	S1442	460	CfaGfuGfAfcCfuGfaAfgGfAfl96	AS1442	1552	cCfuCfGfUfcCfuUfcagGfuCfcaUfGfGfGfsa		
D1443	S1443	461	CfaGfuGfAfcCfuGfaAfgGfAfl96	AS1443	1553	cCfuCfGgucCfuUfcagGfuCfcaUfGfGfGfsa		
D1444	S1444	462	AfgUfgGfAfcUfgGfAfaGfGfGfAfl96	AS1444	1554	cCfcUfcGfUfcUfcaGfGfUfcCfaCfusGfsg		
D1445	S1445	463	AfgUfgGfAfcUfgGfAfaGfGfGfAfl96	AS1445	1555	cCfcUfcgucCfuUfcaGfGfUfcCfaCfusGfsg		
D1446	S1446	464	GfuGfAfcCfuGfAfaGfGfAfaGfGfAfl96	AS1446	1556	uCfcCfuCfGfUfcCfuucAfgGfuCfcaUfGfGfGfsG		
D1447	S1447	465	GfuGfAfcCfuGfAfaGfGfAfaGfGfAfl96	AS1447	1557	uCfcCfucgUfcCfuucAfgGfuCfcaUfGfGfGfsG		
D1448	S1448	466	UfgGfAfcUfgAfaGfGfAfaGfGfAfl96	AS1448	1558	aUfcCfcUfcGfUfcCfuucCfaGfGfUfcCfasCfsu		
D1449	S1449	467	UfgGfAfcUfgAfaGfGfAfaGfGfAfl96	AS1449	1559	aUfcCfcucGfuCfuuCfaGfGfUfcCfasCfsu		
D1450	S1450	468	GfgAfcCfuGfaAfgGfAfaGfGfAfl96	AS1450	1560	cAfuCfcCfuCfGfUfcuUfcAfgGfuCfcaUfGfGfGfsG		
D1451	S1451	469	GfgAfcCfuGfaAfgGfAfaGfGfAfl96	AS1451	1561	cAfuCfcuucCfGfUfcuUfcAfgGfuCfcaUfGfGfGfsG		
D1452	S1452	470	GfaCfuGfAfaGfAfaGfGfAfaGfGfAfl96	AS1452	1562	cCfaUfcCfcUfcGfUfcuUfcAfgGfuCfcaUfGfGfGfsG		

D1453	S1453	471	GfaCfcUfgAfaGfGfAfcCfaGfGfGfaUfgGfl96	AS1453	1563	cfaUfcccUfcGfuUfcCfaGfgUfcsCfsa		
D1454	S1454	472	AfcCfuGfaAfgGfAfcGfAfggAfuGfgGfl96	AS1454	1564	cCfAfuCfcCfuCfugCfuUfcAfgGfusCfsc		
D1455	S1455	473	AfcCfuGfaAfgGfAfcGfAfgGfAfuGfgGfl96	AS1455	1565	cCfAfuCfcCfuCfugCfuUfcAfgGfusCfsc		
D1456	S1456	474	CfcUfgAfaGfGfAfcGfGfGfgaUfgGfAfl96	AS1456	1566	uCfcCfaUfcCfcUfcguCfcUfcCfaGfGfUfsc		
D1457	S1457	475	CfcUfgAfaGfGfAfcGfGfGfAfuUfgGfAfl96	AS1457	1567	uCfcCfaucCfcUfcguCfcUfcCfaGfGfUfsc		
D1458	S1458	476	CfuGfaAfgGfaCfGfAfgGfGauGfgGfaUfl96	AS1458	1568	aUfcCfaUfcCfcCfuegUfcCfuUfcAfgGfsu		
D1459	S1459	477	CfuGfaAfgGfaCfGfAfgGfGfAfuUfgGfAfl96	AS1459	1569	aUfcCfcauCfcCfuegUfcCfuUfcAfgGfsu		
D1460	S1460	478	UfgAfaGfgAfcGfAfcGfGfGfAfuUfl96	AS1460	1570	aAfuCfcCfAfuUfcCfcucGfuCfcUfcCfasGfsg		
D1461	S1461	479	UfgAfaGfgAfcGfAfcGfGfGfAfuUfl96	AS1461	1571	aAfuCfcaUfcCfcucGfuCfcUfcCfasGfsg		
D1462	S1462	480	GfaAfgGfaCfGfAfcGfGfGfAfuUfl96	AS1462	1572	aAfaUfcCfcAfuCfccuCfGufcCfuUfcsAfg		
D1463	S1463	481	GfaAfgGfaCfGfAfcGfGfGfAfuUfl96	AS1463	1573	aAfaUfcccAfuCfccuCfGufcCfuUfcsAfg		
D1464	S1464	482	AfaGfAfcGfaGfGfGfAfuUfGfgAfuUfcl96	AS1464	1574	gAfaAfuCfcCfAfuUfcccUfcGfuCfcUfusCfsa		
D1465	S1465	483	AfaGfAfcGfaGfGfGfAfuUfGfgAfuUfcl96	AS1465	1575	gAfaAfuCfcCfAfuUfcccUfcGfuCfcUfusCfsa		
D1466	S1466	484	AfgGfaCfGfAfcGfAfuUfGfgaUfuUfcafl96	AS1466	1576	uGfaAfaUfcCfcAfuCfccuCfGufcCfusUfsc		
D1467	S1467	485	AfgGfaCfGfAfcGfAfuUfGfgAfuUfcafl96	AS1467	1577	uGfaAfaucCfcAfuCfccuCfGufcCfusUfsc		
D1468	S1468	486	GfgAfcGfaGfGfAfuUfGfgaUfuCfaUfl96	AS1468	1578	aUfgAfaUfcCfcCfaucCfcUfcGfuCfcsUfsu		
D1469	S1469	487	GfgAfcGfaGfGfAfuUfGfgAfuUfcaUfl96	AS1469	1579	aUfgAfaucCfcCfaucCfcUfcGfuCfcsUfsu		
D1470	S1470	488	GfaCfGfGfAfuUfGfgGfaUfcAfuGfl96	AS1470	1580	cAfuGfaAfaUfcCfcauUfcCfcUfcsCfsu		
D1471	S1471	489	GfaCfGfGfAfuUfGfgGfaUfuUfcafuGfl96	AS1471	1581	cAfuGfaaaUfcCfcauUfcCfcUfcsCfsu		
D1472	S1472	490	AfcGfaGfGfAfuUfGfgGfAfuUfcaUfl96	AS1472	1582	aCfaUfgAfaUfcCfcaUfcCfcUfcGfusCfsc		
D1473	S1473	491	AfcGfaGfGfAfuUfGfgGfAfuUfcaUfl96	AS1473	1583	aCfaUfgaaAfuCfcaUfcCfcUfcGfusCfsc		
D1474	S1474	492	CfgAfgGfAfuGfGfGfAfuUfuUfgAfuUfl96	AS1474	1584	uAfcAfuGfAfaUfcccAfuCfcUfcCfcsUfsc		
D1475	S1475	493	CfgAfgGfAfuGfGfGfAfuUfcafuGfAfl96	AS1475	1585	uAfcAfuGfAfaUfcccAfuCfcUfcCfcsUfsc		
D1476	S1476	494	GfaGfgGfaUfgGfGfAfuUfcaUfl96	AS1476	1586	uUfaCfaUfgGfAfaUfcccCfaUfcCfcUfcsGfsu		
D1477	S1477	495	GfaGfgGfaUfgGfGfAfuUfcaUfl96	AS1477	1587	uUfaCfaugAfaAfaUfcccCfaUfcCfcUfcsGfsu		
D1478	S1478	496	AfgGfgAfuGfgGfAfuUfcaUfcaUfcaUfl96	AS1478	1588	gUfuAfaUfgGfAfaUfcccCfaUfcCfcUfcsCfsg		
D1479	S1479	497	AfgGfgAfuGfgGfAfuUfcaUfcaUfcaUfl96	AS1479	1589	gUfuAfaucGfaAfaUfcccCfaUfcCfcUfcsCfsg		

D1480	S1480	498	GfgGfaUfgGfgAfuUfuCfaugUfaAfcCfl96	AS1480	1590	gGfuUfaCfaUfgAfaauCfcCfaUfcCfcsUfsc		
D1481	S1481	499	GfgGfaUfgGfgAfuUfuCfaUfgUfaAfcCfl96	AS1481	1591	gGfuUfacaUfgAfaauCfcCfaUfcCfcsUfsc		
D1482	S1482	500	GfgAfuGfgGfaUfuUfcAfuGfuAfaCfaAfl96	AS1482	1592	uGfgUfuAfcAfuGfaaaUfcCfcAfuCfcsCfsu		
D1483	S1483	501	GfgAfuGfgGfaUfuUfcAfuGfuAfaCfaAfl96	AS1483	1593	uGfgUfuacAfuGfaaaUfcCfcAfuCfcsCfsu		
D1484	S1484	502	GfaUfgGfgAfuUfuCfaUfguaAfcCfaAfl96	AS1484	1594	uUfgGfuUfaCfaUfgaaAfuCfcCfaUfcsCfsc		
D1485	S1485	503	GfaUfgGfgAfuUfuCfaUfgUfaAfcCfaAfl96	AS1485	1595	uUfgGfuuaCfaUfgaaAfuCfcCfaUfcsCfsc		
D1486	S1486	504	AfuGfgGfaUfuUfcAfuGfuuaCfaAfaGfl96	AS1486	1596	cUfuGfgUfuUfaCfaAfuAfuCfcCfaUfcsCfsc		
D1487	S1487	505	AfuGfgGfaUfuUfcAfuGfuAfaCfaAfaGfl96	AS1487	1597	cUfuGfguuAfcAfuAfuAfuCfcCfaUfcsCfsc		
D1488	S1488	506	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfaGfl96	AS1488	1598	uCfuUfgGfuUfaCfaugAfaAfuCfcCfasUfsc		
D1489	S1489	507	UfgGfgAfuUfuCfaUfgUfaAfcCfaAfaGfl96	AS1489	1599	uCfuUfgguUfaCfaugAfaAfuCfcCfasUfsc		
D1490	S1490	508	GfgGfaUfuUfcAfuGfuAfaCfaAfaGfl96	AS1490	1600	cUfuUfuGfgUfuAfcuGfaAfaUfcCfcsAfsu		
D1491	S1491	509	GfgGfaUfuUfcAfuGfuAfaCfaAfaGfaGfl96	AS1491	1601	cUfuUfuggUfuAfcuGfaAfaUfcCfcsAfsu		
D1492	S1492	510	GfgAfuUfuCfaUfgUfaAfcAfaAfgUfl96	AS1492	1602	aCfuCfuUfgGfuUfacaUfgAfaAfuCfcsCfsa		
D1493	S1493	511	GfgAfuUfuCfaUfgUfaAfcCfaAfaAfgUfl96	AS1493	1603	aCfuCfuugGfuUfacaUfgAfaAfuCfcsCfsa		
D1494	S1494	512	GfaUfuUfcAfuGfuAfaCfaaGfaAfaUfl96	AS1494	1604	uAfcUfcUfuUfgUfuacAfuGfaAfaUfcsCfsc		
D1495	S1495	513	GfaUfuUfcAfuGfuAfaCfaAfaGfaGfuAfl96	AS1495	1605	uAfcUfcuuGfgUfuacAfuGfaAfaUfcsCfsc		
D1496	S1496	514	AfuUfuCfaUfgUfaAfcCfaaGfaUfaUfl96	AS1496	1606	aUfaCfuCfuUfgUfgGfuuaCfaUfgAfaAfuCfsc		
D1497	S1497	515	AfuUfuCfaUfgUfaAfcCfaAfaGfaUfaUfl96	AS1497	1607	aUfaCfuuUfgGfuuaCfaUfgAfaAfuCfsc		
D1498	S1498	516	UfuUfcAfuGfuAfaCfaAfaGfaGfuAfuUfl96	AS1498	1608	aAfuAfcUfcUfuGfguuAfcAfuGfaAfaUfsc		
D1499	S1499	517	UfuUfcAfuGfuAfaCfaAfaGfaGfuAfuUfl96	AS1499	1609	aAfuAfcuuUfuGfguuAfcAfuGfaAfaUfsc		
D1500	S1500	518	UfuCfaUfgUfaAfcCfaAfaGfaUfuCfl96	AS1500	1610	gAfaUfaCfuUfcUfuUfgguUfaCfaUfgAfaAfu		
D1501	S1501	519	UfuCfaUfgUfaAfcCfaAfaGfaUfuCfl96	AS1501	1611	gAfaUfacaCfuUfgguUfaCfaUfgAfaAfu		
D1502	S1502	520	UfcAfuGfuAfaCfaAfaGfaGfaUfuCfl96	AS1502	1612	gGfaAfaUfcUfcUfuUfgguUfuAfcAfuGfasAfsa		
D1503	S1503	521	UfcAfuGfuAfaCfaAfaGfaGfaUfuUfcCfl96	AS1503	1613	gGfaAfuacUfcUfuUfgguUfuAfcAfuGfasAfsa		
D1504	S1504	522	CfaUfgUfaAfcCfaAfaGfaUfuCfaAfl96	AS1504	1614	uGfgAfaUfaAfcUfcUfuUfgguUfaCfaUfgsAfsa		
D1505	S1505	523	CfaUfgUfaAfcCfaAfaGfaUfuCfaAfl96	AS1505	1615	uGfgAfaaaCfuCfuugGfuUfaCfaUfgsAfsa		
D1506	S1506	524	AfuGfuAfaCfaAfaGfaGfaUfuCfaUfl96	AS1506	1616	aUfgGfaUfaAfcUfcuuGfgUfuAfcAfuGfasAfsa		

D1507	S1507	525	AfuGfuAfaCfaAfGfaGfuAfuUfCfaUfl96	AS1507	1617	aUfgGfaauAfcUfcuuGfgUfuAfcAfasGfsa	
D1508	S1508	526	UfgUfaAfcCfaAfGfaUfaUfaUfcAfuUfl96	AS1508	1618	aAfuGfgAfAfuAfuCfuuUfgGfuUfaCfasUfsg	
D1509	S1509	527	UfgUfaAfcCfaAfGfaUfaUfCfcAfuUfl96	AS1509	1619	aAfuGfgaaUfaCfuuUfgGfuUfaCfasUfsg	
D1510	S1510	528	GfuAfaCfaAfaGfaGfaUfaUfaUfl96	AS1510	1620	aAfaUfgGfaAfaAfcuUfuGfgUfaAfcAfsu	
D1511	S1511	529	GfuAfaCfaAfaGfaGfaUfaUfCfaUfuUfl96	AS1511	1621	aAfaUfggaAfuAfcuUfuGfgUfaAfcAfsu	
D1512	S1512	530	UfaAfcCfaAfaGfaUfaUfuccAfuUfuUfl96	AS1512	1622	aAfaAfuUfgGfaAfaUfacuUfuUfgGfuUfasCfsa	
D1513	S1513	531	UfaAfcCfaAfaGfaUfaUfCfaUfuUfl96	AS1513	1623	aAfaAfuUfgGfaAfaUfacuUfuUfgGfuUfasCfsa	
D1514	S1514	532	AfaCfaAfaGfaUfaUfcaUfuUfl96	AS1514	1624	aAfaAfuUfgGfaAfuacUfuUfgUfusAfc	
D1515	S1515	533	AfaCfaAfaGfaUfaUfCfaUfuUfl96	AS1515	1625	aAfaAfaugGfaAfuacUfuUfgUfusAfc	
D1516	S1516	534	AfcCfaAfaGfaUfaUfcauUfuUfl96	AS1516	1626	uAfaAfaUfgGfaAfaUfaUfcuUfgGfusUfsa	
D1517	S1517	535	AfcCfaAfaGfaUfaUfcaUfuUfl96	AS1517	1627	uAfaAfaUfgGfaAfaUfaUfcuUfgGfusUfsa	
D1518	S1518	536	CfaAfaGfaUfaUfCfaUfuUfaCfl96	AS1518	1628	gUfaAfaAfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1519	S1519	537	CfaAfaGfaUfaUfCfaUfuUfaCfl96	AS1519	1629	gUfaAfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1520	S1520	538	CfaAfaGfaUfaUfCfaUfuUfaCfl96	AS1520	1630	aGfuAfaAfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1521	S1521	539	CfaAfaGfaUfaUfCfaUfuUfaCfl96	AS1521	1631	aGfuAfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1522	S1522	540	AfaGfaGfuAfuUfCfaUfuUfaCfl96	AS1522	1632	uAfgUfaAfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1523	S1523	541	AfaGfaGfuAfuUfCfaUfuUfaCfl96	AS1523	1633	uAfgUfaUfgGfaUfaUfcUfuUfgGfusUfsu	
D1524	S1524	542	AfgAfgUfaUfCfaUfuUfaUfaCfl96	AS1524	1634	uUfaGfuAfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1525	S1525	543	AfgAfgUfaUfCfaUfuUfaUfaCfl96	AS1525	1635	uUfaGfuAfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1526	S1526	544	GfaGfuAfuUfCfaUfuUfaUfaCfl96	AS1526	1636	uUfaAfgUfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1527	S1527	545	GfaGfuAfuUfCfaUfuUfaUfaCfl96	AS1527	1637	uUfaAfgUfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1528	S1528	546	AfgUfaUfCfaUfuUfaUfaUfaCfl96	AS1528	1638	cUfuUfaGfuAfaUfaUfgGfaUfaCfuCfusUfsg	
D1529	S1529	547	AfgUfaUfCfaUfuUfaUfaUfaCfl96	AS1529	1639	cUfuUfaGfuAfaUfaUfgGfaUfaCfuCfusUfsg	
D1530	S1530	548	GfuAfuUfCfaUfuUfaUfaUfaCfl96	AS1530	1640	gCfuUfaUfgUfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1531	S1531	549	GfuAfuUfCfaUfuUfaUfaUfaCfl96	AS1531	1641	gCfuUfuUfgUfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1532	S1532	550	UfaUfuCfaUfuUfuUfaUfaUfaCfl96	AS1532	1642	uGfcUfuUfAfgUfaAfaUfaUfgGfaUfaCfuCfusUfsg	
D1533	S1533	551	UfaUfuCfaUfuUfuUfaUfaUfaCfl96	AS1533	1643	uGfcUfuUfaUfaUfaUfgGfaUfaCfuCfusUfsg	

D1534	S1534	552	AfuUfcCfaUfuUfuUfaCfuuaaAfgCfaGfl96	AS1534	1644	cUfgCfuUfuUfaUfgUfaaaaAfaUfgGfaAfasAfc	
D1535	S1535	553	AfuUfcCfaUfuUfuUfaCfuUfaFAfAfgCfaGfl96	AS1535	1645	cUfgCfuUfuUfaUfgUfaaaaAfaUfgGfaAfasAfc	
D1536	S1536	554	UfuCfcAfuUfuUfuUfaCfuUfaaaGfcAfgUfl96	AS1536	1646	aCfuGfcUfuUfuUfaGfuuaAfaAfuGfgAfasUfsa	
D1537	S1537	555	UfuCfcAfuUfuUfuUfaCfuUfaFAfAfgCfaGfl96	AS1537	1647	aCfuGfcuUfaGfuuaAfaAfuGfgAfasUfsa	
D1538	S1538	556	UfcCfaUfuUfuUfaCfuUfaaGfcAfgUfl96	AS1538	1648	aCfuUfgCfuUfuUfaGfuuaAfaAfuUfgGfasAfsu	
D1539	S1539	557	UfcCfaUfuUfuUfaCfuUfaAfgCfaGfuGfl96	AS1539	1649	aCfuUfgcuUfuUfaGfuuaAfaAfuUfgGfasAfsu	
D1540	S1540	558	CfcAfuUfuUfaCfuUfaAfgCfaGfuGfl96	AS1540	1650	aCfaCfuGfcUfuUfaguAfaAfaAfuGfgsAfsa	
D1541	S1541	559	CfcAfuUfuUfaCfuUfaAfaGfAfgUfl96	AS1541	1651	aCfaCfugcUfuUfaguAfaAfaAfuGfgsAfsa	
D1542	S1542	560	CfaUfuUfuUfaCfuUfaAfgCfaGfuGfl96	AS1542	1652	aAfaCfuUfgCfuUfuUfuUfaAfaAfuUfgsGfsa	
D1543	S1543	561	CfaUfuUfuUfaCfuUfaAfgCfaGfuGfl96	AS1543	1653	aAfaCfugCfuUfuUfuUfaAfaAfuUfgsGfsa	
D1544	S1544	562	AfuUfuUfaCfuUfaAfaGfcagUfgUfl96	AS1544	1654	aAfaCfaCfuUfgCfuUfuuaGfuAfaAfaAfuGfsg	
D1545	S1545	563	AfuUfuUfaCfuUfaAfaGfcAfgUfgUfl96	AS1545	1655	aAfaCfacuGfcUfuuaGfuAfaAfaAfuGfsg	
D1546	S1546	564	UfuUfuUfaCfuUfaAfaGfcagUfgUfl96	AS1546	1656	aAfaAfcUfgCfuUfuUfaAfaAfuUfgsUfsg	
D1547	S1547	565	UfuUfuUfaCfuUfaAfaGfcUfgUfgUfl96	AS1547	1657	aAfaAfcacUfgCfuUfuUfaAfaAfuUfgsUfsg	
D1548	S1548	566	UfuUfuUfaCfuUfaAfaGfcAfgUfuUfl96	AS1548	1658	gAfaAfaCfaCfuGfcuUfaGfuAfaAfasAfsu	
D1549	S1549	567	UfuUfuUfaCfuUfaAfaGfcAfgUfgUfl96	AS1549	1659	gAfaAfaCfuGfcuUfaGfuAfaAfasAfsu	
D1550	S1550	568	UfuUfaCfuUfaAfaGfcAfgUfuUfl96	AS1550	1660	uGfaAfaAfcAfcUfgcuUfuUfaAfaAfasAfsa	
D1551	S1551	569	UfuUfaCfuUfaAfaGfcAfgUfuUfl96	AS1551	1661	uGfaAfaaCfaCfugcUfuUfaAfaAfasAfsa	
D1552	S1552	570	UfuAfcUfaAfaGfcAfgUfguuUfuCfaCfl96	AS1552	1662	gUfgAfaAfaCfaCfugcUfuUfaGfuAfasAfsa	
D1553	S1553	571	UfuAfcUfaAfaGfcAfgUfgUfuCfaCfl96	AS1553	1663	gUfgUfaaaCfaCfugcUfuUfaGfuAfasAfsa	
D1554	S1554	572	UfaCfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1554	1664	gGfuGfaAfaAfaCfaCfugCfuUfaUfgUfasAfsa	
D1555	S1555	573	UfaCfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1555	1665	gGfuGfaaaAfaCfaCfugCfuUfaUfgUfasAfsa	
D1556	S1556	574	AfaCfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1556	1666	aGfgUfgAfaAfaCfaCfugCfuUfaGfuAfasAfsa	
D1557	S1557	575	AfaCfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1557	1667	aGfgUfaaaAfaCfaCfugCfuUfaGfuAfasAfsa	
D1558	S1558	576	CfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1558	1668	gAfgGfuGfaAfaAfaCfaCfugCfuUfaUfgsUfsa	
D1559	S1559	577	CfuAfaAfgCfaGfuUfuUfaCfaCfl96	AS1559	1669	gAfgGfugaAfaAfaCfaCfugCfuUfaUfgsUfsa	
D1560	S1560	578	UfaAfaGfcAfgUfgUfuUfuUfaCfaCfl96	AS1560	1670	uGfaGfgUfgAfaAfaCfaCfugCfuUfaUfasGfsa	

D1561	S1561	579	UfaAfaGfcAfgUfgGfuUfuUfcFAfcfcUfcaAfl96	AS1561	1671	uGfaGfgugAfaAfacacFcuGfcUfuUfasGfsu		
D1562	S1562	580	AfaAfgCfaGfuGfuUfuUfcacCfuCfaUfl96	AS1562	1672	aUfgAfgGfuUfgAfaAaacAfcUfgCfuUfusAfsG		
D1563	S1563	581	AfaAfgCfaGfuGfuUfuUfcAfcCfuCfaUfl96	AS1563	1673	aUfgAfgGfuGfaAfaaacAfcUfgCfuUfusAfsG		
D1564	S1564	582	AfaGfcAfgUfgUfuUfuUfcaccUfcAfuAfl96	AS1564	1674	uAfuGfaGfgUfgAfaaaaCfaCfuGfcUfusUfsa		
D1565	S1565	583	AfaGfcAfgUfgUfuUfuUfcAfcCfuCfaUfl96	AS1565	1675	uAfuGfaggUfgAfaaaCfaCfuGfcUfusUfsa		
D1566	S1566	584	AfgCfaGfuGfuUfuUfcAfcCfuCfaUfl96	AS1566	1676	aUfaUfgAfgGfuGfaaaaAfcAfcUfgCfusUfsu		
D1567	S1567	585	AfgCfaGfuGfuUfuUfcAfcCfuCfaUfl96	AS1567	1677	aUfaUfgagGfuGfaaaaAfcAfcUfgCfusUfsu		
D1568	S1568	586	GfcAfgUfgUfuUfcAfcCfuCfaUfl96	AS1568	1678	cAfuAfuGfAfgUfgaaAfaCfaCfuGfcsUfsu		
D1569	S1569	587	GfcAfgUfgUfuUfcAfcCfuCfaUfl96	AS1569	1679	cAfuAfuGfGfgUfgaaAfaCfaCfuGfcsUfsu		
D1570	S1570	588	CfaGfuGfuUfuUfcAfcCfuCfaUfl96	AS1570	1680	gCfaUfaUfgAfgGfugaAfaAfcAfcUfgsCfsu		
D1571	S1571	589	CfaGfuGfuUfuUfcAfcCfuCfaUfl96	AS1571	1681	gCfaUfaugAfgGfugaAfaAfcAfcUfgsCfsu		
D1572	S1572	590	AfgUfgUfuUfcAfcCfuCfaUfl96	AS1572	1682	aGfcAfuUfgGfaGfgugAfaAfaCfaCfusGfsc		
D1573	S1573	591	AfgUfgUfuUfcAfcCfuCfaUfl96	AS1573	1683	aGfcAfuauGfaGfgugAfaAfaCfaCfusGfsc		
D1574	S1574	592	GfuGfuUfuUfcAfcCfuCfaUfl96	AS1574	1684	uAfgCfaUfaUfgAfgGfuGfaAfaAfcAfcUfgsGfsc		
D1575	S1575	593	GfuGfuUfuUfcAfcCfuCfaUfl96	AS1575	1685	uAfgCfaauUfgAfgGfuGfaAfaAfcAfcUfgsGfsc		
D1576	S1576	594	UfgUfuUfuUfcAfcCfuCfaUfl96	AS1576	1686	aUfaGfcAfuUfaUfgGfaggUfgAfaAfaCfasCfsu		
D1577	S1577	595	UfgUfuUfuUfcAfcCfuCfaUfl96	AS1577	1687	aUfaGfcAfuUfgGfaggUfgAfaAfaCfasCfsu		
D1578	S1578	596	GfuUfuUfcAfcCfuCfaUfl96	AS1578	1688	cAfuAfgCfaUfaUfgagGfuGfaAfaAfcAfcUfgsGfsc		
D1579	S1579	597	GfuUfuUfcAfcCfuCfaUfl96	AS1579	1689	cAfuAfgcaUfaUfgagGfuGfaAfaAfcAfcUfgsGfsc		
D1580	S1580	598	UfuUfuCfaCfcUfcAfuAfuUfgUfl96	AS1580	1690	aCfaUfaGfcAfuAfuAfuGfgUfgAfaAfasCfsa		
D1581	S1581	599	UfuUfuCfaCfcUfcAfuAfuUfgUfl96	AS1581	1691	aCfaUfagcaUfaUfgagGfuGfaAfaAfcAfcUfgsGfsc		
D1582	S1582	600	UfuUfcAfcCfuCfaUfuUfgcuAfuUfl96	AS1582	1692	aAfcAfuAfgCfaUfaugAfgGfuGfaAfasAfcsc		
D1583	S1583	601	UfuUfcAfcCfuCfaUfuUfgcuAfuUfl96	AS1583	1693	aAfcAfuagCfaUfaugAfgGfuGfaAfasAfcsc		
D1584	S1584	602	UfuCfaCfcUfcAfuAfuUfgcuUfuUfl96	AS1584	1694	uAfaCfaUfaUfgAfcAfuauGfaGfgUfgAfasAfsa		
D1585	S1585	603	UfuCfaCfcUfcAfuAfuUfgcuUfuUfl96	AS1585	1695	uAfaCfaauGfcAfuauGfaGfgUfgAfasAfsa		
D1586	S1586	604	UfcAfcCfuCfaUfaUfgCfuauGfuUfl96	AS1586	1696	cUfaAfcAfuAfuUfgCfaUfaUfgAfgGfuGfasAfsa		
D1587	S1587	605	UfcAfcCfuCfaUfaUfgCfuauGfuUfl96	AS1587	1697	cUfaAfcAfuAfuUfgCfaUfaUfgAfgGfuGfasAfsa		

D1588	S1588	606	CfaCfcUfcAfuAfuGfcUfaugUfuAfgAfl96	AS1588	1698	uCuAfaCfaUfaGfcuAfuGfaGfgUfgsAfsa	
D1589	S1589	607	CfaCfcUfcAfuAfuGfcUfaUfgUfuAfgAfl96	AS1589	1699	uCuAfaCfaUfaGfcuAfuGfaGfgUfgsAfsa	
D1590	S1590	608	AfcCfuCfaUfaUfgCfuAfuUfgUfaGfaAfl96	AS1590	1700	uUcUfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1591	S1591	609	AfcCfuCfaUfaUfgCfuAfuUfgUfaGfaAfl96	AS1591	1701	uUcUfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1592	S1592	610	CfcUfcAfuAfuGfcUfaUfguuAfgAfgAfl96	AS1592	1702	cUfuCfuAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1593	S1593	611	CfcUfcAfuAfuGfcUfaUfgUfaAfgAfgAfl96	AS1593	1703	cUfuCfuAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1594	S1594	612	CfuCfaUfaUfgCfuAfuGfuuaGfaAfgUfl96	AS1594	1704	aCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1595	S1595	613	CfuCfaUfaUfgCfuAfuGfuUfaAfgAfgUfl96	AS1595	1705	aCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1596	S1596	614	UfcAfuAfuGfcUfaUfgUfuAfgAfgAfgUfl96	AS1596	1706	gAfcUfuCfuAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1597	S1597	615	UfcAfuAfuGfcUfaUfgUfuAfgAfgAfgUfl96	AS1597	1707	gAfcUfuCfuAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1598	S1598	616	CfaUfaUfgCfuAfuGfuUfaAfgAfgUfl96	AS1598	1708	gGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1599	S1599	617	CfaUfaUfgCfuAfuGfuUfaAfgAfgUfl96	AS1599	1709	gGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1600	S1600	618	AfuAfuGfcUfaUfgUfuAfgAfgAfgUfl96	AS1600	1710	uGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1601	S1601	619	AfuAfuGfcUfaUfgUfuAfgAfgAfgUfl96	AS1601	1711	uGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1602	S1602	620	UfaUfgCfuAfuGfuUfaGfaagUfcCfaGfl96	AS1602	1712	cUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1603	S1603	621	UfaUfgCfuAfuGfuUfaGfaAfgUfcCfaGfl96	AS1603	1713	cUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1604	S1604	622	AfuGfcUfaUfgUfaAfgAfgAfgUfl96	AS1604	1714	cCfuGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1605	S1605	623	AfuGfcUfaUfgUfaAfgAfgAfgUfl96	AS1605	1715	cCfuGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1606	S1606	624	UfgCfuAfuGfuUfaAfgAfgAfgUfl96	AS1606	1716	gCfcUfgGfaAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1607	S1607	625	UfgCfuAfuGfuUfaAfgAfgAfgUfl96	AS1607	1717	gCfcUfgGfaAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1608	S1608	626	GfcUfaUfgUfaAfgAfgAfgUfl96	AS1608	1718	uGfcCfuGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1609	S1609	627	GfcUfaUfgUfaAfgAfgAfgUfl96	AS1609	1719	uGfcCfuGfgAfcUfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1610	S1610	628	CfuAfuGfuUfaGfaAfgUfccAfgCfaGfl96	AS1610	1720	cUfgCfcUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1611	S1611	629	CfuAfuGfuUfaGfaAfgUfccAfgCfaGfl96	AS1611	1721	cUfgCfcUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1612	S1612	630	UfaUfgUfaAfgAfgUfccAfgCfaGfl96	AS1612	1722	uCfuGfcCfuUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1613	S1613	631	UfaUfgUfaAfgAfgUfccAfgCfaGfl96	AS1613	1723	uCfuGfcCfuUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	
D1614	S1614	632	AfuGfuUfaGfaAfgUfccAfgCfaGfl96	AS1614	1724	cUfcUfgCfcUfgGfaCfuUfcUfaAfaAfcAfuAfgcaUfaUfgAfgGfusGfsa	

D1615	S1615	633	AfuGfuUfaGfaAfgUfcCfaGfGfcfaGfaGfL96	AS1615	1725	cUfcUfgccUfgGfacuUfcUfaAfcAfuAfsfg		
D1616	S1616	634	UfgUfuAfgAfaGfuUfcCfaAfggAfgAfl96	AS1616	1726	uCfuCfuGfCfcUfgGfacUfcUfaAfcAfuAfsfg		
D1617	S1617	635	UfgUfuAfgAfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1617	1727	uCfuCfugcCfuGfgacUfcUfaAfcAfuAfsfg		
D1618	S1618	636	GfuUfaGfaAfgUfcCfaGfGcaGfaGfaCfl96	AS1618	1728	gUfcUfcUfgCfcUfggacUfcUfaAfcAfuAfsfg		
D1619	S1619	637	GfuUfaGfaAfgUfcCfaGfGcaGfaGfaCfl96	AS1619	1729	gUfcUfcUfgCfcUfggacUfcUfaAfcAfuAfsfg		
D1620	S1620	638	UfuAfgAfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1620	1730	uGfuCfuUfgCfcUfggacUfcUfaAfcAfuAfsfg		
D1621	S1621	639	UfuAfgAfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1621	1731	uGfuCfucuGfcCfugGfCfuUfaAfcAfuAfsfg		
D1622	S1622	640	UfaGfaAfgUfcCfaAfgGfCfaAfgAfl96	AS1622	1732	uUfgUfcUfcUfgCfcUfgGfCfuUfaAfcAfuAfsfg		
D1623	S1623	641	UfaGfaAfgUfcCfaAfgGfCfaAfgAfl96	AS1623	1733	uUfgUfcUfcUfgCfcUfgGfCfuUfaAfcAfuAfsfg		
D1624	S1624	642	AfgAfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1624	1734	aUfuGfuUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1625	S1625	643	AfgAfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1625	1735	aUfuGfucuUfcUfgGfCfuUfaAfcAfuAfsfg		
D1626	S1626	644	GfaAfgUfcCfaGfGcaGfaGfaCfl96	AS1626	1736	uAfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1627	S1627	645	GfaAfgUfcCfaGfGcaGfaGfaCfl96	AS1627	1737	uAfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1628	S1628	646	AfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1628	1738	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1629	S1629	647	AfaGfuUfcCfaAfgGfCfaAfgAfl96	AS1629	1739	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1630	S1630	648	AfgUfcCfaGfGcaGfaGfaCfl96	AS1630	1740	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1631	S1631	649	AfgUfcCfaGfGcaGfaGfaCfl96	AS1631	1741	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1632	S1632	650	GfuCfaAfgGfCfaAfgGfCfaAfgAfl96	AS1632	1742	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1633	S1633	651	GfuCfaAfgGfCfaAfgGfCfaAfgAfl96	AS1633	1743	uUfaUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1634	S1634	652	UfcCfaGfGcaGfaGfaCfl96	AS1634	1744	gUfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1635	S1635	653	UfcCfaGfGcaGfaGfaCfl96	AS1635	1745	gUfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1636	S1636	654	CfcAfgGfCfaAfgGfCfaAfgAfl96	AS1636	1746	uGfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1637	S1637	655	CfcAfgGfCfaAfgGfCfaAfgAfl96	AS1637	1747	uGfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1638	S1638	656	CfaGfGcaGfaGfCfaAfgGfCfaAfgAfl96	AS1638	1748	aUfgUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1639	S1639	657	CfaGfGcaGfaGfCfaAfgGfCfaAfgAfl96	AS1639	1749	aUfgUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1640	S1640	658	AfgGfCfaAfgGfCfaAfgGfCfaAfgAfl96	AS1640	1750	aAfuGfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		
D1641	S1641	659	AfgGfCfaAfgGfCfaAfgGfCfaAfgAfl96	AS1641	1751	aAfuGfuUfuUfgUfcUfcUfgGfCfuUfaAfcAfuAfsfg		

D1642	S1642	660	GfgCfaGfaGfaCfaAfaAfaAfaaCfaUfuCfl96	AS1642	1752	gAfaUfgUfuUfuAfuugUfcUfcUfgCfcsUfsg	
D1643	S1643	661	GfgCfaGfaGfaCfaAfaAfaAfaAfaCfaUfuCfl96	AS1643	1753	gAfaUfguuUfuAfuugUfcUfcUfgCfcsUfsg	
D1644	S1644	662	GfcAfgAfgAfaAfaAfaAfaAfaaCfaUfuCfl96	AS1644	1754	gGfaAfuGfuUfuUfaUfuGfuCfuCfuGfcsCfsu	
D1645	S1645	663	GfcAfgAfgAfaAfaAfaAfaAfaAfaUfuCfl96	AS1645	1755	gGfaAfguuUfuUfaUfuGfuCfuCfuGfcsCfsu	
D1646	S1646	664	CfaGfaGfaCfaAfaAfaAfaAfaCfaUfuCfl96	AS1646	1756	aGfgAfaUfgUfuUfuUfuUfgUfcUfcUfsgCfsc	
D1647	S1647	665	CfaGfaGfaCfaAfaAfaAfaAfaCfaUfuCfl96	AS1647	1757	aGfgAfaugUfuUfuUfuUfgUfcUfcUfsgCfsc	
D1648	S1648	666	AfgAfgAfaAfaAfaAfaAfaAfaUfuCfl96	AS1648	1758	cAfgGfaAfgUfuUfuUfaUfuGfuCfuCfusGfsc	
D1649	S1649	667	AfgAfgAfaAfaAfaAfaAfaAfaUfuCfl96	AS1649	1759	cAfgGfaauGfuUfuUfaUfuGfuCfuCfusGfsc	
D1650	S1650	668	GfaGfaCfaAfaAfaAfaAfaAfaUfuCfl96	AS1650	1760	aCfaGfgAfaUfgUfuUfuUfaUfuGfuCfusUfsg	
D1651	S1651	669	GfaGfaCfaAfaAfaAfaAfaAfaUfuCfl96	AS1651	1761	aCfaGfgaaUfgUfuUfuUfaUfuGfuCfusUfsg	
D1652	S1652	670	AfgAfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1652	1762	cAfcAfgGfaAfaUfguuUfaUfuGfuCfusCfsu	
D1653	S1653	671	AfgAfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1653	1763	cAfcAfggaAfuGfuUfuUfaUfuGfuCfusCfsu	
D1654	S1654	672	GfaCfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1654	1764	uCfaCfaGfgAfaUfguuUfaUfuGfuCfusUfsc	
D1655	S1655	673	GfaCfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1655	1765	uCfaCfaggAfaUfguuUfaUfuGfuCfusUfsc	
D1656	S1656	674	AfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1656	1766	uUfaAfaAfgGfaAfaUfuUfaUfuGfusCfsu	
D1657	S1657	675	AfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1657	1767	uUfaAfcagGfaAfaUfuUfaUfuGfusCfsu	
D1658	S1658	676	CfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1658	1768	uUfuCfaCfaAfgAfaUfuUfaUfuGfusUfsc	
D1659	S1659	677	CfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1659	1769	uUfuCfacaGfgAfaUfuUfaUfuGfusUfsc	
D1660	S1660	678	AfaUfaAfaAfaAfaAfaAfaUfuCfl96	AS1660	1770	cUfuUfaAfaAfgGfaUfuUfaUfuGfusGfsu	
D1661	S1661	679	AfaUfaAfaAfaAfaAfaAfaUfuCfl96	AS1661	1771	cUfuUfcacAfgGfaUfuUfaUfuGfusGfsu	
D1662	S1662	680	AfuAfaAfaAfaAfaAfaAfaUfuCfl96	AS1662	1772	cCfuUfuCfaCfaGfgaaUfgUfuUfaUfuGfusUfsg	
D1663	S1663	681	AfuAfaAfaAfaAfaAfaAfaUfuCfl96	AS1663	1773	cCfuUfucaCfaGfgaaUfgUfuUfaUfuGfusUfsg	
D1664	S1664	682	UfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1664	1774	gCfcUfuUfcAfaAfggaAfuGfuUfuUfasUfsc	
D1665	S1665	683	UfaAfaAfaAfaAfaAfaAfaUfuCfl96	AS1665	1775	gCfcUfuUfcAfaAfggaAfuGfuUfuUfasUfsc	
D1666	S1666	684	AfaAfaCfaUfuCfUfuGfgaaAfgGfcAfl96	AS1666	1776	uGfcCfuUfuCfaCfaggAfaUfgUfuUfusAfsu	
D1667	S1667	685	AfaAfaCfaUfuCfUfuGfgaaAfgGfcAfl96	AS1667	1777	uGfcCfuUfuCfaCfaggAfaUfgUfuUfusAfsu	
D1668	S1668	686	AfaAfaCfaUfuCfUfuGfgaaAfgGfcAfl96	AS1668	1778	gUfgCfcUfuUfuCfaCfaggAfaUfgUfuUfusAfsu	

D1669	S1669	687	AfaAfcAfuUfcCfuUfgUgUfaAfaAfgGfCfaCfl96	AS1669	1779	gUfgCfcuuUfcAfcagGfaAfuGfuUfusUfsa		
D1670	S1670	688	AfaCfaUfuCfcUfgUfGfUfgAfaagGfcAfcUfl96	AS1670	1780	aGfuGfcCfuUfuCfaacaGfgAfaUfgUfusUfsu		
D1671	S1671	689	AfaCfaUfuCfcUfgUfGfUfgAfaAfgGfGfcAfcUfl96	AS1671	1781	aGfuGfcuUfuCfacaGfgAfaUfgUfusUfsu		
D1672	S1672	690	AfaAfuUfcCfuUfgUfGfAfaAfaAfgGfCfaCfuUfl96	AS1672	1782	aAfgUfgCfcUfuUfcacaAfgGfaAfuGfusUfsu		
D1673	S1673	691	AfaAfuUfcCfuUfgUfGfAfaAfaAfgGfCfaCfuUfl96	AS1673	1783	aAfgUfgccUfuUfcacaAfgGfaAfuGfusUfsu		
D1674	S1674	692	CfaUfuCfcUfgUfGfAfaAfaAfgGfcAfcUfuUfl96	AS1674	1784	aAfaGfuGfCfcUfuUfacaCfaGfgAfaUfGfUfsu		
D1675	S1675	693	CfaUfuCfcUfgUfGfAfaAfaAfgGfcAfcUfuUfl96	AS1675	1785	aAfaGfugcCfuUfucaCfaGfgAfaUfGfUfsu		
D1676	S1676	694	AfuUfcCfuUfgUfGfAfaAfaAfgGfcAfcUfuUfl96	AS1676	1786	aAfaAfgUfGfCfcUfuucAfcAfgGfaAfaUfGfUfsu		
D1677	S1677	695	AfuUfcCfuUfgUfGfAfaAfaAfgGfcAfcUfuUfl96	AS1677	1787	aAfaAfgugCfcUfuucAfcAfgGfaAfaUfGfUfsu		
D1678	S1678	696	UfuCfcUfgUfGfAfaAfaAfgGfcacUfuUfuCfl96	AS1678	1788	gAfaAfaGfUfgCfcUfuucCfaCfaGfgAfaUfGfUfsu		
D1679	S1679	697	UfuCfcUfgUfGfAfaAfaAfgGfcAfcUfuUfuCfl96	AS1679	1789	gAfaAfaGfcCfuuuCfaCfaGfgAfaUfGfUfsu		
D1680	S1680	698	UfcCfuGfuGfaAfaAfgGfcAfcUfuUfuCfl96	AS1680	1790	uGfaAfaAfgUfgCfcUfuCfaAfcAfgGfAfaUfGfUfsu		
D1681	S1681	699	UfcCfuGfuGfaAfaAfgGfcAfcUfuUfuCfl96	AS1681	1791	uGfaAfaagUfgCfcUfuCfaAfcAfgGfAfaUfGfUfsu		
D1682	S1682	700	CfcUfgUfgAfaAfgGfcAfcUfuUfuCfl96	AS1682	1792	aUfgAfaAfaAfgUfgGfcUfuCfaCfaGfgAfaUfGfUfsu		
D1683	S1683	701	CfcUfgUfgAfaAfgGfcAfcUfuUfuCfl96	AS1683	1793	aUfgAfaaaGfuGfcUfuCfaCfaGfgAfaUfGfUfsu		
D1684	S1684	702	CfuGfuGfaAfaGfgCfaCfuUfuUfuCfl96	AS1684	1794	aAfuGfaAfaAfgUfgccUfuUfcAfcAfgGfAfaUfGfUfsu		
D1685	S1685	703	CfuGfuGfaAfaGfgCfaCfuUfuUfuCfl96	AS1685	1795	aAfuGfaaaAfgUfgccUfuUfcAfcAfgGfAfaUfGfUfsu		
D1686	S1686	704	UfgUfgAfaAfgGfcAfcUfuUfuCfl96	AS1686	1796	gAfaUfgAfaAfaGfugcCfuUfuCfaCfasGfsg		
D1687	S1687	705	UfgUfgAfaAfgGfcAfcUfuUfuCfl96	AS1687	1797	gAfaUfgaaAfaGfugcCfuUfuCfaCfasGfsg		
D1688	S1688	706	GfuGfaAfaGfgCfaCfuUfuUfuCfl96	AS1688	1798	gGfaAfuGfAfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1689	S1689	707	GfuGfaAfaGfgCfaCfuUfuUfuCfl96	AS1689	1799	gGfaAfuGfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1690	S1690	708	UfgAfaAfgGfcAfcUfuUfuCfl96	AS1690	1800	uGfgAfaUfgAfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1691	S1691	709	UfgAfaAfgGfcAfcUfuUfuCfl96	AS1691	1801	uGfgAfaugAfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1692	S1692	710	GfaAfaGfgCfaCfuUfuUfuCfl96	AS1692	1802	gUfgGfaAfuGfAfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1693	S1693	711	GfaAfaGfgCfaCfuUfuUfuCfl96	AS1693	1803	gUfgGfaauGfaAfaAfgUfgCfcUfuUfcAfcAfgGfAfaUfGfUfsu		
D1694	S1694	712	AfaAfgGfcAfcUfuUfuCfl96	AS1694	1804	aGfuGfgAfaAfaAfgUfgAfaaaGfuGfcCfuUfusCfAfaUfGfUfsu		
D1695	S1695	713	AfaAfgGfcAfcUfuUfuCfl96	AS1695	1805	aGfuGfgaaUfgAfaaaGfuGfcCfuUfusCfAfaUfGfUfsu		

D1723	S1723	741	UfuCfcAfcUfuUfaAfaCfuUfgAfuUfuUfl96	AS1723	1833	aAfaAfaaAfaGfuUaAfaGfuGfgAfasUfsg	
D1724	S1724	742	UfcCfaCfuUfuAfaCfuUfgauUfuUfuUfl96	AS1724	1834	aAfaAfaUfCfaAfguuAfaAfgUfgGfasAfsu	
D1725	S1725	743	UfcCfaCfuUfuAfaCfuUfgAfuUfuUfl96	AS1725	1835	aAfaAfaaCfaAfguuAfaAfgUfgGfasAfsu	
D1726	S1726	744	CfcAfcUfuUfaAfcUfuGfaUfuUfuUfl96	AS1726	1836	uAfaAfaAfaUfCfaAfgUfaAfaGfuGfgsAfsa	
D1727	S1727	745	CfcAfcUfuUfaAfcUfuGfaUfuUfuUfl96	AS1727	1837	uAfaAfaaaUfcAfaGfuUfaAfaGfuGfgsAfsa	
D1728	S1728	746	CfaCfuUfuAfaCfuUfgAfuUfuUfaAfl96	AS1728	1838	uUfaAfaAfaAfuCfaagUfuAfaAfgUfgsGfsa	
D1729	S1729	747	CfaCfuUfuAfaCfuUfgAfuUfuUfaAfl96	AS1729	1839	uUfaAfaaaAfuCfaagUfuAfaAfgUfgsGfsa	
D1730	S1730	748	AfcUfuUfaAfcUfgfaUfuUfuUfaAfl96	AS1730	1840	uUfaAfaAfaAfuUfcaaGfuUfaAfaGfusGfsg	
D1731	S1731	749	AfcUfuUfaAfcUfgfaUfuUfuUfaAfl96	AS1731	1841	uUfaAfaaaAfaUfcaaGfuUfaAfaGfusGfsg	
D1732	S1732	750	CfuUfaAfaCfuUfgAfuUfuUfaAfl96	AS1732	1842	aUfuUfaAfaAfaAfuAfucaAfgUfuAfaAfgsUfsg	
D1733	S1733	751	CfuUfaAfaCfuUfgAfuUfuUfaAfl96	AS1733	1843	aUfuUfaaaAfaAfucaAfgUfuAfaAfgsUfsg	
D1734	S1734	752	UfuUfaAfcUfuGfaUfuUfuUfaAfl96	AS1734	1844	aAfuUfuAfaAfaAfaucAfaGfuUfaAfasGfsu	
D1735	S1735	753	UfuUfaAfcUfuGfaUfuUfuUfaAfl96	AS1735	1845	aAfuUfuuaAfaAfaucAfaGfuUfaAfasGfsu	
D1736	S1736	754	UfuAfaCfuUfgAfuUfuUfaAfl96	AS1736	1846	gAfaUfuUfaAfaAfaaCfaAfgUfuAfasAfgs	
D1737	S1737	755	UfuAfaCfuUfgAfuUfuUfaAfl96	AS1737	1847	gAfaUfuuaAfaAfaaCfaAfgUfuAfasAfgs	
D1738	S1738	756	UfaAfcUfuGfaUfuUfuUfaAfl96	AS1738	1848	gGfaAfuUfuUfaAfaAfaaUfcAfaGfuUfasAfsa	
D1739	S1739	757	UfaAfcUfuGfaUfuUfuUfaAfl96	AS1739	1849	gGfaAfuuaAfaAfaaUfcAfaGfuUfasAfsa	
D1740	S1740	758	AfaCfuUfgAfuUfuUfaAfl96	AS1740	1850	gGfgAfaUfuUfaAfaaAfuCfaAfgUfusAfsa	
D1741	S1741	759	AfaCfuUfgAfuUfuUfaAfl96	AS1741	1851	gGfgAfauuUfaAfaaAfuCfaAfgUfusAfsa	
D1742	S1742	760	AfcUfuGfaUfuUfuUfaAfl96	AS1742	1852	aGfgGfaUfuUfaAfaaaAfaUfcAfaGfusUfsa	
D1743	S1743	761	AfcUfuGfaUfuUfuUfaAfl96	AS1743	1853	aGfgGfaaUfuUfaaAfaUfcAfaGfusUfsa	
D1744	S1744	762	CfuUfgAfuUfuUfaAfaaCfcUfuUfl96	AS1744	1854	aAfgGfgAfaUfuUfaaaAfaAfaCfaAfgsUfsu	
D1745	S1745	763	CfuUfgAfuUfuUfaAfaUfuUfcCfuUfl96	AS1745	1855	aAfgGfgaaUfuUfaaaAfaAfaCfaAfgsUfsu	
D1746	S1746	764	UfuGfaUfuUfuUfaAfaAfuUfaAfl96	AS1746	1856	uAfaGfgGfaAfuUfuuaAfaAfaUfcAfasGfsu	
D1747	S1747	765	UfuGfaUfuUfuUfaAfaAfuUfaAfl96	AS1747	1857	uAfaGfggaAfuUfuuaAfaAfaUfcAfasGfsu	
D1748	S1748	766	UfgAfuUfuUfaAfaUfuccCfuUfaUfl96	AS1748	1858	aUfaAfgGfgAfaUfuuaAfaAfaUfcAfasAfgs	
D1749	S1749	767	UfgAfuUfuUfaAfaUfCfcCfuUfaUfl96	AS1749	1859	aUfaAfgggAfaUfuuaAfaAfaUfcAfasAfgs	

D1750	S1750	768	GfaUfuUfuUfaAfaUfuUfccUfuAfuUfl96	AS1750	1860	aAfaUfaGfGfGfaAfaUuuAfaAfaUfcsAfsa		
D1751	S1751	769	GfaUfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1751	1861	aAfaUfaggGfaAfaUuuAfaAfaUfcsAfsa		
D1752	S1752	770	AfuUfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1752	1862	cAfaUfaAfGfGfAfaUuUfaAfaAfaAfaUfcsAfsa		
D1753	S1753	771	AfuUfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1753	1863	cAfaUfaagGfGfAfaUuUfaAfaAfaAfaUfcsAfsa		
D1754	S1754	772	UfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1754	1864	aCfaAfaUfaAfaGfGfAfaUuUfaAfaAfaAfaUfcsAfsa		
D1755	S1755	773	UfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1755	1865	aCfaAfaUfaGfGfAfaUuUfaAfaAfaAfaUfcsAfsa		
D1756	S1756	774	UfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1756	1866	gAfaAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1757	S1757	775	UfuUfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1757	1867	gAfaAfaUfaGfGfAfaUuUfaAfaAfaAfaUfcsAfsa		
D1758	S1758	776	UfuUfuAfaUfuUfcCfcUfuAfuUfl96	AS1758	1868	gGfaCfaAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1759	S1759	777	UfuUfuAfaUfuUfcCfcUfuAfuUfl96	AS1759	1869	gGfaCfaaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1760	S1760	778	UfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1760	1870	gGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1761	S1761	779	UfuUfaAfaUfuUfcCfcUfuAfuUfl96	AS1761	1871	gGfGfAfaaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1762	S1762	780	UfuAfaUfuUfcCfcUfuAfuUfl96	AS1762	1872	aGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1763	S1763	781	UfuAfaUfuUfcCfcUfuAfuUfl96	AS1763	1873	aGfGfAfaaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1764	S1764	782	UfaAfaUfuUfcCfcUfuAfuUfl96	AS1764	1874	aAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1765	S1765	783	UfaAfaUfuUfcCfcUfuAfuUfl96	AS1765	1875	aAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1766	S1766	784	AfaAfuUfcCfcUfuAfuUfl96	AS1766	1876	gAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1767	S1767	785	AfaAfuUfcCfcUfuAfuUfl96	AS1767	1877	gAfaGfGfaCfaAfaaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1768	S1768	786	AfaUfuUfcCfcUfuAfuUfl96	AS1768	1878	gGfaAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1769	S1769	787	AfaUfuUfcCfcUfuAfuUfl96	AS1769	1879	gGfaAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1770	S1770	788	AfuUfcCfcUfuAfuUfl96	AS1770	1880	uGfGfAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1771	S1771	789	AfuUfcCfcUfuAfuUfl96	AS1771	1881	uGfGfAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1772	S1772	790	UfuUfcCfcUfuAfuUfl96	AS1772	1882	uUfgGfaAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1773	S1773	791	UfuUfcCfcUfuAfuUfl96	AS1773	1883	uUfgGfaaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1774	S1774	792	UfcCfcUfuAfuUfl96	AS1774	1884	uUfuGfGfAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1775	S1775	793	UfcCfcUfuAfuUfl96	AS1775	1885	uUfuGfGfaaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		
D1776	S1776	794	CfcCfuUfaUfuUfl96	AS1776	1886	uUfuUfgGfAfaGfGfAfaUfaAfaGfGfaaUfuUfaAfaAfaAfaUfcsAfsa		

D1831	S1831	849	GfaAfuCfaAfaUfuUfuUfaCfaAfaGfaAfl96	AS1831	1941	uUfcUfuugUfaAfaaaUfuUfgAfuUfcsUfsc	
D1832	S1832	850	AfaUfcAfaAfaUfuUfuUfaAfaaaAfgAfaUfl96	AS1832	1942	aUfuCfuUfuUfgUfaAfaaaUfuUfuGfaUfusCfsu	
D1833	S1833	851	AfaUfcAfaAfaUfuUfuUfaAfaAfaAfgAfaUfl96	AS1833	1943	aUfuCfuuuGfuAfaaaUfuUfuGfaUfusCfsu	
D1834	S1834	852	AfuCfaAfaAfuUfuUfaCfaaaGfaAfaUcfl96	AS1834	1944	gAfuUfUfuUfgUfaaaAfuUfuUfgAfuUfsc	
D1835	S1835	853	AfuCfaAfaAfuUfuUfaCfaAfaGfaAfuCfl96	AS1835	1945	gAfuUfuuUfgUfaaaAfuUfuUfgAfuUfsc	
D1836	S1836	854	UfaAfaAfaUfuUfaAfaAfaagAfaUfcafl96	AS1836	1946	uGfaUfuCfuUfuUfguaaAfaUfuUfuGfasUfsc	
D1837	S1837	855	UfaAfaAfaUfuUfaAfaAfaGfaAfuCfl96	AS1837	1947	uGfaUfuuUfgUfaaaAfuUfuUfuGfasUfsc	
D1838	S1838	856	CfaAfaAfuUfuUfaAfaAfaAfaAfuCfl96	AS1838	1948	uUfgAfuUfCfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1839	S1839	857	CfaAfaAfuUfuUfaAfaAfaAfaAfuCfl96	AS1839	1949	uUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1840	S1840	858	AfaAfaUfuUfaAfaAfaAfaAfuCfl96	AS1840	1950	uUfuGfaUfuCfuUfguaAfaAfuUfuUfgsAfsa	
D1841	S1841	859	AfaAfaUfuUfaAfaAfaAfaAfuCfl96	AS1841	1951	uUfuGfaUfuCfuUfguaAfaAfuUfuUfgsAfsa	
D1842	S1842	860	AfaAfaUfuUfaAfaAfaAfaAfuCfl96	AS1842	1952	cUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1843	S1843	861	AfaAfaUfuUfaAfaAfaAfaAfuCfl96	AS1843	1953	cUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1844	S1844	862	AfaUfuUfaAfaAfaAfaAfuCfl96	AS1844	1954	cUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1845	S1845	863	AfaUfuUfaAfaAfaAfaAfuCfl96	AS1845	1955	cUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1846	S1846	864	AfuUfuUfaAfaAfaAfaAfuCfl96	AS1846	1956	uUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1847	S1847	865	AfuUfuUfaAfaAfaAfaAfuCfl96	AS1847	1957	uUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1848	S1848	866	UfuUfuUfaAfaAfaAfaAfuCfl96	AS1848	1958	uUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1849	S1849	867	UfuUfuUfaAfaAfaAfaAfuCfl96	AS1849	1959	uUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1850	S1850	868	UfuUfaCfaAfaAfaAfaAfuCfl96	AS1850	1960	aUfuCfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1851	S1851	869	UfuUfaCfaAfaAfaAfaAfuCfl96	AS1851	1961	aUfuCfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1852	S1852	870	UfuUfaCfaAfaAfaAfaAfuCfl96	AS1852	1962	aUfuCfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1853	S1853	871	UfuUfaCfaAfaAfaAfaAfuCfl96	AS1853	1963	aUfuCfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsu	
D1854	S1854	872	UfaCfaAfaAfaAfaAfaAfaAfuCfl96	AS1854	1964	gAfaUfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsa	
D1855	S1855	873	UfaCfaAfaAfaAfaAfaAfaAfuCfl96	AS1855	1965	gAfaUfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsa	
D1856	S1856	874	AfaAfaAfaAfaAfaAfaAfuCfl96	AS1856	1966	aGfaAfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsa	
D1857	S1857	875	AfaAfaAfaAfaAfaAfaAfuCfl96	AS1857	1967	aGfaAfuUfuUfgAfuUfuUfguaAfaAfuUfuUfgsAfsa	

D1858	S1858	876	CfaAfaGfaAfuCfaAfaGfaAfuGfaAfuCfuAfl96	AS1858	1968	uAfgAfaUfUfcUfuugAfuUfcUfuUfGsuUfsa	
D1859	S1859	877	CfaAfaGfaAfuCfaAfaGfaAfuGfaAfuCfuAfl96	AS1859	1969	uAfgAfaUfcUfuugAfuUfcUfuUfGsuUfsa	
D1860	S1860	878	AfaAfgAfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1860	1970	cUfaGfaAfuUfcCfuuuGfaUfuCfuUfusGfsu	
D1861	S1861	879	AfaAfgAfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1861	1971	cUfaGfaaUfcCfuuuGfaUfuCfuUfusGfsu	
D1862	S1862	880	AfaGfaAfuCfaAfaGfaAfuUfcUfaGfl96	AS1862	1972	uCfaAfgAfaUfcCfuUfGfaUfuUfcUfusUfsg	
D1863	S1863	881	AfaGfaAfuCfaAfaGfaAfuUfcUfaGfl96	AS1863	1973	uCfaAfgaaUfcCfuUfGfaUfuUfcUfusUfsg	
D1864	S1864	882	AfgAfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1864	1974	uUfcUfaGfaAfuUfcuUfuGfaUfuCfusUfsu	
D1865	S1865	883	AfgAfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1865	1975	uUfcUfagaAfuUfcuUfuGfaUfuCfusUfsu	
D1866	S1866	884	GfaAfuCfaAfaGfaAfuUfcUfaGfl96	AS1866	1976	uUfuCfuAfgAfaUfuccUfuUfgAfuUfcsUfsu	
D1867	S1867	885	GfaAfuCfaAfaGfaAfuUfcUfaGfl96	AS1867	1977	uUfuCfuagAfaUfuccUfuUfgAfuUfcsUfsu	
D1868	S1868	886	AfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1868	1978	cUfuUfcUfAfgAfaUfuUfcUfuUfcsUfsu	
D1869	S1869	887	AfaUfcAfaAfgGfaAfuUfcUfaGfl96	AS1869	1979	cUfuUfcuaGfaAfuUfcUfuUfcsUfsu	
D1870	S1870	888	AfuCfaAfaGfaAfuUfcUfaGfl96	AS1870	1980	aCfuUfuCfuAfgAfaUfuUfgAfuUfsc	
D1871	S1871	889	AfuCfaAfaGfaAfuUfcUfaGfl96	AS1871	1981	aCfuUfucuAfgAfaUfuUfgAfuUfsc	
D1872	S1872	890	UfcAfaAfgGfaAfuUfcUfaGfl96	AS1872	1982	uAfcUfuUfcUfaGfaaUfcCfuUfuGfasUfsu	
D1873	S1873	891	UfcAfaAfgGfaAfuUfcUfaGfl96	AS1873	1983	uAfcUfuucUfaGfaaUfcCfuUfuGfasUfsu	
D1874	S1874	892	CfaAfaGfaAfuUfcUfaGfl96	AS1874	1984	aUfaCfuUfcUfuAfgaaUfuCfuUfGsuUfsc	
D1875	S1875	893	CfaAfaGfaAfuUfcUfaGfl96	AS1875	1985	aUfaCfuuuCfuAfgaaUfuCfuUfGsuUfsc	
D1876	S1876	894	AfaAfgGfaAfuUfcUfaGfl96	AS1876	1986	gAfuAfcUfuUfcUfagaAfuUfcCfuUfusGfsa	
D1877	S1877	895	AfaAfgGfaAfuUfcUfaGfl96	AS1877	1987	gAfuAfcuuUfcUfagaAfuUfcCfuUfusGfsa	
D1878	S1878	896	AfaGfgAfaUfuCfuAfgAfaUfuUfL96	AS1878	1988	aGfaUfaCfuUfuCfuagAfaUfuCfuUfusUfsg	
D1879	S1879	897	AfaGfgAfaUfuCfuAfgAfaUfuUfL96	AS1879	1989	aGfaUfacuUfuCfuagAfaUfuCfuUfusUfsg	
D1880	S1880	898	AfgGfaAfuUfcUfaGfaAfgaAfuCfuGfl96	AS1880	1990	cAfgAfuAfcUfuUfcuaGfaAfuUfcCfusUfsu	
D1881	S1881	899	AfgGfaAfuUfcUfaGfaAfgaAfuCfuGfl96	AS1881	1991	cAfgAfuacUfuUfcuaGfaAfuUfcCfusUfsu	
D1882	S1882	900	GfgAfaUfuCfuAfgAfaAfgaUfuUfGfl96	AS1882	1992	cCfaGfaUfaAfcUfuUfucuAfgAfaUfuCfusUfsu	
D1883	S1883	901	GfgAfaUfuCfuAfgAfaAfgaUfuUfGfl96	AS1883	1993	cCfaGfaaUfuUfucuAfgAfaUfuCfusUfsu	
D1884	S1884	902	GfaAfuUfcUfaGfaAfgaAfuUfGfl96	AS1884	1994	cCfaAfgAfuAfcUfuUfucuUfaGfaAfuUfcsUfsu	

D1885	S1885	903	GfaAfuUfaGfaAfaGfaUfaUfcUfGfGfL96	AS1885	1995	cfcAfgauAfcUfuucUfaGfaAfuUfcsCfsu		
D1886	S1886	904	AfaUfuCfuAfgAfAfAfaUfaUfcUfGfCfL96	AS1886	1996	gCfcCfaGfaUfaCfuuuCfuAfgAfaUfcsCfsc		
D1887	S1887	905	AfaUfuCfuAfgAfAfAfaUfaUfcUfGfGfCfL96	AS1887	1997	gCfcCfagaUfaCfuuuCfuAfgAfaUfcsCfsc		
D1888	S1888	906	AfuUfcUfaGfaAfaAfcUfaUfcUfGfGfCfAfl96	AS1888	1998	uGfcCfcAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1889	S1889	907	AfuUfcUfaGfaAfaAfcUfaUfcUfGfGfCfAfl96	AS1889	1999	uGfcCfcagAfaUfcUfaGfaAfuUfcsUfsc		
D1890	S1890	908	UfuCfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1890	2000	cUfgCfcCfAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1891	S1891	909	UfuCfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1891	2001	cUfgCfccagAfaUfcUfaGfaAfuUfcsUfsc		
D1892	S1892	910	UfuCfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1892	2002	uCfuGfcCfcAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1893	S1893	911	UfuCfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1893	2003	uCfuGfccagAfaUfcUfaGfaAfuUfcsUfsc		
D1894	S1894	912	CfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1894	2004	uUfcUfgCfcCfAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1895	S1895	913	CfuAfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1895	2005	uUfcUfgcccCfaGfaUfcUfaGfaAfuUfcsUfsc		
D1896	S1896	914	UfaGfaAfaGfaUfaUfcUfGfGfCfAfl96	AS1896	2006	gUfuCfuGfcCfcAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1897	S1897	915	UfaGfaAfaGfaUfaUfcUfGfGfCfAfl96	AS1897	2007	gUfuCfugcCfcAfgAfaUfcUfaGfaAfuUfcsUfsc		
D1898	S1898	916	AfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1898	2008	cGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1899	S1899	917	AfgAfaAfcUfaUfcUfGfGfCfAfl96	AS1899	2009	cGfuUfcUfcCfcCfagaUfaCfuUfcUfcsUfsc		
D1900	S1900	918	GfaAfaGfaUfaUfcUfGfGfCfAfl96	AS1900	2010	gCfGfuCfuUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1901	S1901	919	GfaAfaGfaUfaUfcUfGfGfCfAfl96	AS1901	2011	gCfGfuCfuUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1902	S1902	920	AfaAfgUfaUfcUfGfGfCfAfl96	AS1902	2012	aGfcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1903	S1903	921	AfaAfgUfaUfcUfGfGfCfAfl96	AS1903	2013	aGfcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1904	S1904	922	AfaAfgUfaUfcUfGfGfCfAfl96	AS1904	2014	uAfgCfGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1905	S1905	923	AfaGfaAfaGfaUfaUfcUfGfGfCfAfl96	AS1905	2015	uAfgCfGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1906	S1906	924	AfgUfaUfcUfGfGfCfAfl96	AS1906	2016	cUfaGfcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1907	S1907	925	AfgUfaUfcUfGfGfCfAfl96	AS1907	2017	cUfaGfcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1908	S1908	926	GfuAfuCfuGfGfCfAfl96	AS1908	2018	cCfuAfgCfGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1909	S1909	927	GfuAfuCfuGfGfCfAfl96	AS1909	2019	cCfuAfgcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1910	S1910	928	UfaUfcUfGfGfCfAfl96	AS1910	2020	uCfuUfaGfcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		
D1911	S1911	929	UfaUfcUfGfGfCfAfl96	AS1911	2021	uCfuUfagcGfuUfcUfGfCfcCfagaUfaCfuUfcUfcsUfsc		

D1912	S1912	930	AfuCfuGfgGfcAfgAfaCfcuaGfgGfaGfl96	AS1912	2022	cUfcCfuAfgCfcUfuGfcCfcAfgAfuAfsC	
D1913	S1913	931	AfuCfuGfgGfcAfgAfaCfcUfaGfgGfaGfl96	AS1913	2023	cUfcCfuagCfcUfuccUgfcCfcAfgAfuAfsC	
D1914	S1914	932	UfcUfgGfgCfaGfaAfaCfcuaGfgAfgAfl96	AS1914	2024	uCfuCfcUfaAfgGfcGfuUcUfgCfcCfaGfasUfsa	
D1915	S1915	933	UfcUfgGfgCfaGfaAfaCfcUfaGfgAfgAfl96	AS1915	2025	uCfuCfcuaGfcGfuUcUfgCfcCfaGfasUfsa	
D1916	S1916	934	CfuGfgGfcAfgAfaCfcUfaGfgGfaGfl96	AS1916	2026	cUfcUfcUfaAfgCfcuaCfuGfcCfcAfgAfsu	
D1917	S1917	935	CfuGfgGfcAfgAfaCfcUfaGfgGfaGfl96	AS1917	2027	cUfcUfccuAfgCfcuaCfuGfcCfcAfgAfsu	
D1918	S1918	936	UfgGfgCfaGfaAfcCfcUfaGfgAfgAfl96	AS1918	2028	uCfuCfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1919	S1919	937	UfgGfgCfaGfaAfcGfcUfaGfgAfgAfl96	AS1919	2029	uCfuCfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1920	S1920	938	GfgGfcAfgAfaCfcUfaGfgGfaGfl96	AS1920	2030	aUfcUfcUfaAfgGfcUfcUfgCfcCfasGfsa	
D1921	S1921	939	GfgGfcAfgAfaCfcUfaGfgGfaGfl96	AS1921	2031	aUfcUfcuUfaAfgGfcUfcUfgCfcCfasGfsa	
D1922	S1922	940	GfgCfaGfaAfcGfcUfaGfgAfgAfl96	AS1922	2032	gAfuCfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1923	S1923	941	GfgCfaGfaAfcGfcUfaGfgAfgAfl96	AS1923	2033	gAfuCfcuUfaGfgUfcUfgCfcCfasGfsa	
D1924	S1924	942	GfcAfaCfcUfaGfgGfaGfl96	AS1924	2034	gGfaUfcUfcUfaGfgUfcUfgCfcCfasGfsa	
D1925	S1925	943	GfcAfaCfcUfaGfgGfaGfl96	AS1925	2035	gGfaUfcuUfaGfgUfcUfgCfcCfasGfsa	
D1926	S1926	944	CfaGfaAfcGfcUfaGfgAfgAfl96	AS1926	2036	uGfgAfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1927	S1927	945	CfaGfaAfcGfcUfaGfgAfgAfl96	AS1927	2037	uGfgAfuCfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1928	S1928	946	AfgAfaCfcUfaGfgGfaGfl96	AS1928	2038	uUfgGfaUfcUfaGfgUfcUfgCfcCfasGfsa	
D1929	S1929	947	AfgAfaCfcUfaGfgGfaGfl96	AS1929	2039	uUfgGfaUfcUfccuAfgCfcUfcUfgCfcCfasGfsa	
D1930	S1930	948	GfaAfcGfcUfaGfgAfgAfl96	AS1930	2040	uUfuGfgAfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1931	S1931	949	GfaAfcGfcUfaGfgAfgAfl96	AS1931	2041	uUfuGfgauCfcUfaGfgUfcUfgCfcCfasGfsa	
D1932	S1932	950	AfaCfcUfaGfgAfgAfl96	AS1932	2042	aUfuUfgGfaUfcUfaGfgUfcUfgCfcCfasGfsa	
D1933	S1933	951	AfaCfcUfaGfgAfgAfl96	AS1933	2043	aUfuUfggaUfcUfaGfgUfcUfgCfcCfasGfsa	
D1934	S1934	952	AfcGfcUfaGfgAfgAfl96	AS1934	2044	aAfuUfuGfgAfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1935	S1935	953	AfcGfcUfaGfgAfgAfl96	AS1935	2045	aAfuUfuggAfuCfcUfaGfgUfcUfgCfcCfasGfsa	
D1936	S1936	954	CfgCfuAfgGfaGfgAfl96	AS1936	2046	aAfaUfuUfgGfaUfcUfaGfgUfcUfgCfcCfasGfsa	
D1937	S1937	955	CfgCfuAfgGfaGfgAfl96	AS1937	2047	aAfaUfuugGfaUfcUfaGfgUfcUfgCfcCfasGfsa	
D1938	S1938	956	GfcUfaGfgAfgAfl96	AS1938	2048	gAfaAfuUfuUfgGfaUfcUfaGfgUfcUfgCfcCfasGfsa	

D1939	S1939	957	GfcUfaGfgAfgAfGfaFufcCfaAfAfAfUfuUfuCfl96	AS1939	2049	gAfaAfaUuuGfgAfufuUfuCfcUfaGfcsGfsu	
D1940	S1940	958	CfuAfgGfaGfaGfaUfucCfaaUfuUfucfl96	AS1940	2050	gGfaAfaUfuUfGfgfaucUfcUfcCfuAfgsCfsg	
D1941	S1941	959	CfuAfgGfaGfaGfaUfucCfaAfAfUfuUfucfl96	AS1941	2051	gGfaAfaUfuUfGfgfaucUfcUfcCfuAfgsCfsg	
D1942	S1942	960	UfaGfgAfgAfGfaUfucCfaaUfuUfucfl96	AS1942	2052	uGfgAfaUfuUfGfgauCfuCfuCfuUfasGfsc	
D1943	S1943	961	UfaGfgAfgAfGfaUfucCfaAfAfUfuUfucfl96	AS1943	2053	uGfgAfaUfuUfGfgauCfuCfuCfuUfasGfsc	
D1944	S1944	962	AfgGfaGfaUfucCfaAfAfUfuUfucfl96	AS1944	2054	aUfgGfaAfAfUfuUfGfgaUfcUfcUfcCfusAfsG	
D1945	S1945	963	AfgGfaGfaUfucCfaAfAfUfuUfucfl96	AS1945	2055	aUfgGfaaUfuUfGfgaUfcUfcUfcCfusAfsG	
D1946	S1946	964	GfgAfgAfGfaUfucCfaAfAfUfuUfucfl96	AS1946	2056	aAfuGfgAfAfUfuUfuggAfuCfuCfuCfcsUfsa	
D1947	S1947	965	GfgAfgAfGfaUfucCfaAfAfUfuUfucfl96	AS1947	2057	aAfuGfgaaAfuUfuggAfuCfuCfuCfcsUfsa	
D1948	S1948	966	GfaGfaGfaUfucCfaAfAfUfuUfucfl96	AS1948	2058	cAfaUfgGfaAfAfUfuUfgGfaUfcUfcUfcsCfsu	
D1949	S1949	967	GfaGfaGfaUfucCfaAfAfUfuUfucfl96	AS1949	2059	cAfaUfGfgaAfaUfuUfgGfaUfcUfcUfcsCfsu	
D1950	S1950	968	AfgAfgAfGfaUfucCfaAfAfUfuUfucfl96	AS1950	2060	aCfaAfuGfgAfaAfaUfuUfgAfuCfuCfusCfsc	
D1951	S1951	969	AfgAfgAfGfaUfucCfaAfAfUfuUfucfl96	AS1951	2061	aCfaAfuGfgAfaAfaUfuUfgAfuCfuCfusCfsc	
D1952	S1952	970	GfaGfaUfucCfaAfAfUfuUfucfl96	AS1952	2062	gAfaAfaUfGfgAfaaUfuUfgGfaUfcUfcsUfsc	
D1953	S1953	971	GfaGfaUfucCfaAfAfUfuUfucfl96	AS1953	2063	gAfaAfaUfGfgAfaaUfuUfgGfaUfcUfcsUfsc	
D1954	S1954	972	AfgAfuCfaAfAfUfuUfucCfaUfucfl96	AS1954	2064	aGfaCfaAfUfGfgAfaaUfuUfgAfuCfusCfsu	
D1955	S1955	973	AfgAfuCfaAfAfUfuUfucCfaUfucfl96	AS1955	2065	aGfaCfaaUfGfgAfaaUfuUfgAfuCfusCfsu	
D1956	S1956	974	GfaUfucCfaAfAfUfuUfucCfaUfucfl96	AS1956	2066	aAfgAfaUfGfgAfaaUfuUfgGfaUfcsUfsc	
D1957	S1957	975	GfaUfucCfaAfAfUfuUfucCfaUfucfl96	AS1957	2067	aAfgAfaUfGfgAfaaUfuUfgGfaUfcsUfsc	
D1958	S1958	976	AfuCfaAfAfUfuUfucCfaUfucfl96	AS1958	2068	cAfaGfaCfaAfAfUfGfgaAfuUfuUfgAfuCfusCfsu	
D1959	S1959	977	AfuCfaAfAfUfuUfucCfaUfucfl96	AS1959	2069	cAfaGfaCfaAfAfUfGfgaAfuUfuUfgAfuCfusCfsu	
D1960	S1960	978	UfucCfaAfAfUfuUfucCfaUfucfl96	AS1960	2070	gCfaAfgAfaUfGfgaAfaUfuUfgGfasUfsc	
D1961	S1961	979	UfucCfaAfAfUfuUfucCfaUfucfl96	AS1961	2071	gCfaAfgaCfaUfGfgaAfaUfuUfgGfasUfsc	
D1962	S1962	980	CfaAfaUfuUfucCfaUfucfl96	AS1962	2072	uGfaCfaGfaCfaAfUfGfgaAfaUfuUfgGfasUfsc	
D1963	S1963	981	CfaAfaUfuUfucCfaUfucfl96	AS1963	2073	uGfaCfaGfaCfaAfUfGfgaAfaUfuUfgGfasUfsc	
D1964	S1964	982	CfaAfaUfuUfucCfaUfucfl96	AS1964	2074	uUfgCfaAfGfaCfaUfGfgaAfaUfuUfgGfasUfsc	
D1965	S1965	983	CfaAfaUfuUfucCfaUfucfl96	AS1965	2075	uUfgCfaaGfaCfaUfGfgaAfaUfuUfgGfasUfsc	

D1966	S1966	984	AfaAfuUfuCfcAfuUfgUfcuuGfcAfaGfl96	AS1966	2076	cUfuGfcAfaGfaCfaauGfgAfaAfuUfusGfsg	
D1967	S1967	985	AfaAfuUfuCfcAfuUfgUfcUfgCfaAfaGfl96	AS1967	2077	cUfuGfcaaGfaCfaauGfgAfaAfuUfusGfsg	
D1968	S1968	986	AfaUfuUfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1968	2078	gCfuUfgCfaAfaGfaUfgGfaAfaUfusUfsg	
D1969	S1969	987	AfaUfuUfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1969	2079	gCfuUfgcaAfgAfaGfaUfgGfaAfaUfusUfsg	
D1970	S1970	988	AfuUfuCfcAfuUfgUfcUfgCfaAfaGfl96	AS1970	2080	uGfcUfuGfcAfaGfaAfaUfgGfaAfaUfusUfsg	
D1971	S1971	989	AfuUfuCfcAfuUfgUfcUfgCfaAfaGfl96	AS1971	2081	uGfcUfuGfcAfaGfaAfaUfgGfaAfaUfusUfsg	
D1972	S1972	990	UfuUfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1972	2082	uUfgCfuUfgCfaAfgacAfaUfgGfaAfaUfusUfsg	
D1973	S1973	991	UfuUfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1973	2083	uUfgCfuugCfaAfgacAfaUfgGfaAfaUfusUfsg	
D1974	S1974	992	UfuCfcAfuUfgUfcUfuUfgCfaAfaGfl96	AS1974	2084	uUfuGfcUfuUfgCfaAfaGfaAfaUfgGfaAfaUfusUfsg	
D1975	S1975	993	UfuCfcAfuUfgUfcUfuUfgCfaAfaGfl96	AS1975	2085	uUfuGfcuuGfcAfaGfaAfaUfgGfaAfaUfusUfsg	
D1976	S1976	994	UfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1976	2086	cUfuUfgCfuUfgCfaagAfaUfgGfaAfaUfusUfsg	
D1977	S1977	995	UfcCfaUfuGfcUfuUfgCfaAfaGfl96	AS1977	2087	cUfuUfgcuUfgCfaagAfaUfgGfaAfaUfusUfsg	
D1978	S1978	996	CfcAfuUfgUfcUfuUfgCfaAfaGfl96	AS1978	2088	gCfuUfuGfcUfuUfgCfaaGfaCfaAfaUfgGfaAfaUfusUfsg	
D1979	S1979	997	CfcAfuUfgUfcUfuUfgCfaAfaGfl96	AS1979	2089	gCfuUfuugCfuUfgCfaaGfaCfaAfaUfgGfaAfaUfusUfsg	
D1980	S1980	998	CfaUfuGfcUfuUfgCfaAfaGfl96	AS1980	2090	uGfcUfuUfgCfuUfgcaAfgAfaUfgGfaAfaUfusUfsg	
D1981	S1981	999	CfaUfuGfcUfuUfgCfaAfaGfl96	AS1981	2091	uGfcUfuugCfuUfgcaAfgAfaUfgGfaAfaUfusUfsg	
D1982	S1982	1000	AfuUfgUfcUfuGfcAfaGfaAfaGfl96	AS1982	2092	gUfgCfuUfuUfgCfuUfgcaAfaGfaCfaAfaUfusUfsg	
D1983	S1983	1001	AfuUfgUfcUfuGfcAfaGfaAfaGfl96	AS1983	2093	gUfgCfuuuGfcUfuGcaAfaGfaCfaAfaUfusUfsg	
D1984	S1984	1002	UfuGfcUfuUfgCfaAfaGfaAfaGfl96	AS1984	2094	cGfuGfcUfuUfgCfuugCfaAfgAfaAfaUfusUfsg	
D1985	S1985	1003	UfuGfcUfuUfgCfaAfaGfaAfaGfl96	AS1985	2095	cGfuGfcuuUfgCfuugCfaAfgAfaAfaUfusUfsg	
D1986	S1986	1004	UfgUfcUfuGfcAfaGfaAfaGfl96	AS1986	2096	aCfgUfgCfuUfuGfcuuGfcAfaGfaCfaAfaUfusUfsg	
D1987	S1987	1005	UfgUfcUfuGfcAfaGfaAfaGfl96	AS1987	2097	aCfuUfgcuUfuGfcuuGfcAfaGfaCfaAfaUfusUfsg	
D1988	S1988	1006	GfuCfuUfgCfaAfaGfaAfaGfl96	AS1988	2098	uAfcGfuGfcUfuUfgcuUfgCfaAfgAfaAfaUfusUfsg	
D1989	S1989	1007	GfuCfuUfgCfaAfaGfaAfaGfl96	AS1989	2099	uAfcGfuugCfuUfgcuUfgCfaAfgAfaAfaUfusUfsg	
D1990	S1990	1008	UfcUfuGfcAfaGfaAfaGfl96	AS1990	2100	aUfaCfuUfgCfuUfuGfcUfuGfaAfaGfaAfaUfusUfsg	
D1991	S1991	1009	UfcUfuGfcAfaGfaAfaGfl96	AS1991	2101	aUfaCfuugCfuUfuGfcUfuGfaAfaGfaAfaUfusUfsg	
D1992	S1992	1010	CfuUfgCfaAfaGfaAfaGfl96	AS1992	2102	aAfuAfcUfgCfuUfuGfcUfuGfaAfaGfaAfaUfusUfsg	

D1993	S1993	1011	CfuUfgCfaAfgCfaAfaGfcAfcGfuAfuUfl96	AS1993	2103	aAfaAfcguGfcUfuugCfuUfgCfaAfgsAfc		
D1994	S1994	1012	UfuGfcAfaGfcAfaAfaAfgCfaGfuAfuUfl96	AS1994	2104	uAfaUfaCfGfUfgCfuuuGfcUfuGfcAfasGfsa		
D1995	S1995	1013	UfuGfcAfaGfcAfaAfaAfgCfaGfuAfuUfl96	AS1995	2105	uAfaUfacgUfgCfuuuGfcUfuGfcAfasGfsa		
D1996	S1996	1014	UfgCfaAfgCfaAfaAfaAfgCfaGfuAfuUfl96	AS1996	2106	uUfaAfaAfcGfuGfcuuUfgCfuUfgCfasAfg		
D1997	S1997	1015	UfgCfaAfgCfaAfaAfaAfgCfaGfuAfuUfl96	AS1997	2107	uUfaAfaAfcGfuGfcuuUfgCfuUfgCfasAfg		
D1998	S1998	1016	GfcAfaGfcAfaAfaAfaAfgCfaGfuAfuUfl96	AS1998	2108	uUfaAfaAfcGfuUfgcuUfuGfcUfuGfcsAfsa		
D1999	S1999	1017	GfcAfaGfcAfaAfaAfaAfgCfaGfuAfuUfl96	AS1999	2109	uUfaAfaAfcGfuUfgcuUfuGfcUfuGfcsAfsa		
D2000	S2000	1018	CfaAfgCfaAfaGfcAfaGfuUfaAfaUfl96	AS2000	2110	aUfuUfaAfaAfcGfuGfcUfuUfgCfuUfgsCfsa		
D2001	S2001	1019	CfaAfgCfaAfaGfcAfaGfuUfaAfaUfl96	AS2001	2111	aUfuUfaAfaAfcGfuGfcUfuUfgCfuUfgsCfsa		
D2002	S2002	1020	AfaGfcAfaAfgCfaGfuUfaAfaUfl96	AS2002	2112	uAfuUfaAfaAfcGfuGfcUfuUfgCfuUfusGfsc		
D2003	S2003	1021	AfaGfcAfaAfgCfaGfuUfaAfaUfl96	AS2003	2113	uAfuUfaAfaAfcGfuGfcUfuUfgCfuUfusGfsc		
D2004	S2004	1022	AfgCfaAfaGfcAfcGfuAfaAfaUfl96	AS2004	2114	aUfaUfuUfaAfaAfcguGfcUfuUfgCfuUfsg		
D2005	S2005	1023	AfgCfaAfaGfcAfcGfuAfaAfaUfl96	AS2005	2115	aUfaUfuAfaAfcguGfcUfuUfgCfuUfsg		
D2006	S2006	1024	GfcAfaAfgCfaGfuUfaAfaUfl96	AS2006	2116	cAfuAfuUfaAfaUfaccgUfgCfuUfuGfcsUfsu		
D2007	S2007	1025	GfcAfaAfgCfaGfuUfaAfaUfl96	AS2007	2117	cAfuAfuUfaAfaUfaccgUfgCfuUfuGfcsUfsu		
D2008	S2008	1026	CfaAfaGfcAfcGfuUfaAfaUfl96	AS2008	2118	uCfaUfaUfuUfaAfaAfuacGfuGfcUfuUfgsCfsu		
D2009	S2009	1027	CfaAfaGfcAfcGfuUfaAfaUfl96	AS2009	2119	uCfaUfaAfaAfuacGfuGfcUfuUfgsCfsu		
D2010	S2010	1028	AfaAfgCfaGfuUfaAfaUfl96	AS2010	2120	aUfcAfaUfuUfaAfaAfcgUfgCfuUfusGfsc		
D2011	S2011	1029	AfaAfgCfaGfuUfaAfaUfl96	AS2011	2121	aUfcAfaUfuUfaAfaAfcgUfgCfuUfusGfsc		
D2012	S2012	1030	AfaGfcAfcGfuAfuUfaAfaUfl96	AS2012	2122	gAfuCfaUfaAfuUfaAfaAfcGfuGfcUfusUfsg		
D2013	S2013	1031	AfaGfcAfcGfuAfuUfaAfaUfl96	AS2013	2123	gAfuCfaUfuUfaAfaAfcGfuGfcUfusUfsg		
D2014	S2014	1032	AfgCfaCfuUfaUfaAfaAfaUfl96	AS2014	2124	aGfaUfcAfaUfaUfaAfaAfcgUfgCfuUfusUfsg		
D2015	S2015	1033	AfgCfaCfuUfaUfaAfaAfaUfl96	AS2015	2125	aGfaUfcAfaUfaUfaAfaAfcgUfgCfuUfusUfsg		
D2016	S2016	1034	GfcAfcGfuAfuUfaAfaAfaUfl96	AS2016	2126	cAfgAfuCfaUfaUfaAfaAfcGfuGfcsUfsu		
D2017	S2017	1035	GfcAfcGfuAfuUfaAfaAfaUfl96	AS2017	2127	cAfgAfucaUfaUfaAfaAfcGfuGfcsUfsu		
D2018	S2018	1036	CfaCfuUfaUfaAfaAfaUfl96	AS2018	2128	gCfaGfaUfcAfaAfaAfaUfaAfcgUfgsCfsu		
D2019	S2019	1037	CfaCfuUfaUfaAfaAfaUfl96	AS2019	2129	gCfaGfaucAfaAfaAfaUfaAfcgUfgsCfsu		

D2020	S2020	1038	AfcGfuAfuUfaAfaUfaUfgauCfuGfcAfl96	AS2020	2130	uGfcAfgAfUfCfaUfaUuUfaAfuAfcGfusGfsc	
D2021	S2021	1039	AfcGfuAfuUfaAfaUfaUfgAuUfCfuGfcAfl96	AS2021	2131	uGfcAfgauCfaUfaUuUfaAfuAfcGfusGfsc	
D2022	S2022	1040	CfgUfaUfuAfaUfaUfgAuGfauUfgCfaGfl96	AS2022	2132	cUfgCfaGfAfUfCfaUfaUuUfaAfaCfugsUfsg	
D2023	S2023	1041	CfgUfaUfuAfaUfaUfgAuGfauUfCfufgCfaGfl96	AS2023	2133	cUfgCfagaUfCfaUfaUuUfaAfaCfugsUfsg	
D2024	S2024	1042	GfuAfuUfaAfaUfaUfgAfUfCfuGfcAfl96	AS2024	2134	gCfuGfcAfGfAfuCfaUuUfaAfuAfcGfisu	
D2025	S2025	1043	GfuAfuUfaAfaUfaUfgAfUfCfuGfcAfl96	AS2025	2135	gCfuGfcagAfuCfaUuUfaAfuAfcGfisu	
D2026	S2026	1044	UfaUfuAfaUfaUfgAuUfCfuGfcAfl96	AS2026	2136	gGfcUfgCfAfGfaUfcauAfuUfaUfasCfsg	
D2027	S2027	1045	UfaUfuAfaUfaUfgAuUfCfuGfcAfl96	AS2027	2137	gGfcUfgcaGfaUfcauAfuUfaUfasCfsg	
D2028	S2028	1046	AfuUfaAfaUfaUfgAfUfCfuGfcAfl96	AS2028	2138	uGfgCfuGfCfAfgAfucaUfaUfuUfaAfuAfc	
D2029	S2029	1047	AfuUfaAfaUfaUfgAfUfCfuGfcAfl96	AS2029	2139	uGfgCfugcAfgAfucaUfaUfuUfaAfuAfc	
D2030	S2030	1048	UfuAfaUfaUfaUfgAfUfCfuGfcAfl96	AS2030	2140	aUfgGfcUfGfCfaGfaucaUfaUfuUfaAfuAfc	
D2031	S2031	1049	UfuAfaUfaUfaUfgAfUfCfuGfcAfl96	AS2031	2141	aUfgCfugcCfaGfaucaUfaUfuUfaAfuAfc	
D2032	S2032	1050	UfaAfaUfaUfgAfUfCfuGfcAfl96	AS2032	2142	aAfuGfGcUfGfCfaGfaucaUfaUfuUfaAfuAfc	
D2033	S2033	1051	UfaAfaUfaUfgAfUfCfuGfcAfl96	AS2033	2143	aAfuGfGcuGfcAfgauCfaUfaUfuUfaAfuAfc	
D2034	S2034	1052	AfaAfuAfuUfgAfUfCfuGfcAfl96	AS2034	2144	uAfaUfgGfCfUfgCfagaUfCfaUfaUfuUfaAfuAfc	
D2035	S2035	1053	AfaAfuAfuUfgAfUfCfuGfcAfl96	AS2035	2145	uAfaUfgggUfgCfagaUfCfaUfaUfuUfaAfuAfc	
D2036	S2036	1054	AfaUfaUfgAfUfCfuGfcAfl96	AS2036	2146	uUfaAfuGfGfCfuGfcagAfuCfaUfaUfuUfaAfuAfc	
D2037	S2037	1055	AfaUfaUfgAfUfCfuGfcAfl96	AS2037	2147	uUfaAfuGgCfuGfcagAfuCfaUfaUfuUfaAfuAfc	
D2038	S2038	1056	AfuAfuGfaUfCfuGfcAfl96	AS2038	2148	uUfuAfaUfGfGfcUfgcaGfaUfCfaUfaUfuUfaAfuAfc	
D2039	S2039	1057	AfuAfuGfaUfCfuGfcAfl96	AS2039	2149	uUfuAfaugGfcUfgcaGfaUfCfaUfaUfuUfaAfuAfc	
D2040	S2040	1058	UfaUfgAfuCfuGfCfAfgCfcaUfaAfaAfl96	AS2040	2150	uUfuUfaAfuUfgCfugcAfgAfuCfaUfaUfuUfaAfuAfc	
D2041	S2041	1059	UfaUfgAfuCfuGfCfAfgCfcaUfaAfaAfl96	AS2041	2151	uUfuUfaaUfgCfugcAfgAfuCfaUfaUfuUfaAfuAfc	
D2042	S2042	1060	AfuGfaUfCfuGfCfAfgCfcaUfaAfaAfl96	AS2042	2152	uUfuUfaAfaUfGfGfcUfgCfagCfaGfaUfCfaUfaUfuUfaAfuAfc	
D2043	S2043	1061	AfuGfaUfCfuGfCfAfgCfcaUfaAfaAfl96	AS2043	2153	uUfuUfaaUfgCfugcCfaGfaUfCfaUfaUfuUfaAfuAfc	
D2044	S2044	1062	UfgAfuCfuGfCfAfgCfcaUfaAfaAfl96	AS2044	2154	cUfuUfuUfaAfaUfGfGfcUfgcUfgAfuCfaUfaUfuUfaAfuAfc	
D2045	S2045	1063	UfgAfuCfuGfCfAfgCfcaUfaAfaAfl96	AS2045	2155	cUfuUfuuaUfgCfugcGfcaUfCfaUfaUfuUfaAfuAfc	
D2046	S2046	1064	GfaUfCfuGfCfAfgCfcaUfaAfaAfl96	AS2046	2156	uCfuUfuUfaAfaUfGgCfugCfcaGfaUfCfaUfaUfuUfaAfuAfc	

D2047	S2047	1065	GfaUfcUfgCfaGfcCfaUfaUfaAfaAfaAfaAfl96	AS2047	2157	uCfuUfuuuAfaUfggcUfgCfaGfaUfcsAfsu		
D2048	S2048	1066	AfuCfuGfcAfgCfcAfuUfaaaAfaGfaCfl96	AS2048	2158	gUfcUfuUfuUfaAfaUfuggCfuGfcAfgAfusCfsa		
D2049	S2049	1067	AfuCfuGfcAfgCfcAfuUfaAfaAfaGfaCfl96	AS2049	2159	gUfcUfuuuUfaAfaUfuggCfuGfcAfgAfusCfsa		
D2050	S2050	1068	UfcUfgCfaGfcCfaUfaUfaAfaAfaAfaAfl96	AS2050	2160	uGfuCfuUfuUfuUfaAfaUfgCfaGfasUfsc		
D2051	S2051	1069	UfcUfgCfaGfcCfaUfaUfaAfaAfaAfaAfl96	AS2051	2161	uGfuCfuuuUfuUfaAfaUfgCfaGfasUfsc		
D2052	S2052	1070	CfuGfcAfgCfcAfuUfaAfaAfaAfaAfl96	AS2052	2162	gUfgUfcUfuUfuUfaaUfgCfuGfcAfgsAfsu		
D2053	S2053	1071	CfuGfcAfgCfcAfuUfaAfaAfaAfaAfl96	AS2053	2163	gUfgUfcuuUfuUfaaUfgCfuGfcAfgsAfsu		
D2054	S2054	1072	UfgCfaGfcCfaUfaAfaAfaAfaAfl96	AS2054	2164	uGfuGfuCfuUfuUfaaUfgGfcUfgCfasGfsa		
D2055	S2055	1073	UfgCfaGfcCfaUfaAfaAfaAfaAfl96	AS2055	2165	uGfuGfucUfuUfuuaUfgGfcUfgCfasGfsa		
D2056	S2056	1074	GfcAfgCfcAfuUfaAfaAfaAfaAfl96	AS2056	2166	aUfgUfgCfuUfuUfaaUfgCfuGfcUfgCfasGfsa		
D2057	S2057	1075	GfcAfgCfcAfuUfaAfaAfaAfaAfl96	AS2057	2167	aUfgUfgucUfuUfuuaUfgCfuGfcUfgCfasGfsa		
D2058	S2058	1076	CfaGfcCfaUfaAfaAfaAfaAfl96	AS2058	2168	aAfuGfuUfcUfuUfuuuAfaUfgGfcUfgsCfsa		
D2059	S2059	1077	CfaGfcCfaUfaAfaAfaAfaAfl96	AS2059	2169	aAfuGfuguCfuUfuuuAfaUfgGfcUfgsCfsa		
D2060	S2060	1078	AfgCfcAfuUfaAfaAfaAfaAfl96	AS2060	2170	gAfaUfgUfgUfcUfuuuUfaAfuGfgCfusGfsc		
D2061	S2061	1079	AfgCfcAfuUfaAfaAfaAfaAfl96	AS2061	2171	gAfaUfgucUfuUfuuuUfaAfuGfgCfusGfsc		
D2062	S2062	1080	GfcCfaUfaAfaAfaAfaAfl96	AS2062	2172	aGfaAfuGfuUfgUfcuuUfaAfuUfgGfcsUfsg		
D2063	S2063	1081	GfcCfaUfaAfaAfaAfaAfl96	AS2063	2173	aGfaAfuguGfuCfuuuUfaAfuUfgGfcsUfsg		
D2064	S2064	1082	CfcAfuUfaAfaAfaAfaAfl96	AS2064	2174	cAfgAfaUfgUfgUfcuuUfaAfuGfcsCfsu		
D2065	S2065	1083	CfcAfuUfaAfaAfaAfaAfl96	AS2065	2175	cAfgAfaugUfgUfcuuUfaAfuGfcsCfsu		
D2066	S2066	1084	CfaUfaAfaAfaAfaAfl96	AS2066	2176	aCfaGfaAfuGfuGfucUfuUfaAfuUfgsGfsc		
D2067	S2067	1085	CfaUfaAfaAfaAfaAfl96	AS2067	2177	aCfaGfaaUfgUfcuuUfaAfuUfgsGfsc		
D2068	S2068	1086	AfuUfaAfaAfaAfaAfl96	AS2068	2178	uAfcAfgAfaUfgUfgucUfuUfaAfuUfgsGfsc		
D2069	S2069	1087	AfuUfaAfaAfaAfaAfl96	AS2069	2179	uAfcAfgaaUfgUfgucUfuUfaAfuUfgsGfsc		
D2070	S2070	1088	UfuAfaAfaAfaAfaAfl96	AS2070	2180	uUfaCfaGfaAfaUfgUfcuuUfaAfuUfasUfsg		
D2071	S2071	1089	UfuAfaAfaAfaAfaAfl96	AS2071	2181	uUfaCfagaAfuGfuguCfuUfaAfuUfasUfsg		
D2072	S2072	1090	UfaAfaAfaAfaAfaAfl96	AS2072	2182	uUfaAfaAfaAfaAfl96		
D2073	S2073	1091	UfaAfaAfaAfaAfaAfl96	AS2073	2183	uUfaAfcagAfaUfgUfcuuUfaAfuUfasAfsu		

D2074	S2074	1092	AfaAfaAfgAfcAfaUfcUgUfaAfaAflL96	AS2074	2184	uUfuUfaCfaAfaGfaAfaUfgUfaUfuUfsa		
D2075	S2075	1093	AfaAfaAfgAfcAfaUfcUgUfaAfaAflL96	AS2075	2185	uUfuUfacaGfaAfaUfgUfaUfuUfsa		
D2076	S2076	1094	AfaAfaGfaCfaCfaUfcUfgUfaAfaAflL96	AS2076	2186	uUfuUfuAfcAfaAfaUfgUfcUfuUfsa		
D2077	S2077	1095	AfaAfaGfaCfaCfaUfcUfgUfaAfaAflL96	AS2077	2187	uUfuUfuacAfaAfaUfgUfcUfuUfsa		
D2078	S2078	1096	AfaAfgAfcAfaUfcUfgUfaAfaAflL96	AS2078	2188	uUfuUfuUfaCfaAfaUfgUfaUfuUfsa		
D2079	S2079	1097	AfaAfgAfcAfaUfcUfgUfaAfaAflL96	AS2079	2189	uUfuUfuuaCfaAfaUfgUfaUfuUfsa		
D2080	S2080	1098	AfaGfaCfaUfcUfgUfaAfaAflL96	AS2080	2190	uUfuUfuUfaAfcAfaUfgUfcUfuUfsa		
D2081	S2081	1099	AfaGfaCfaUfcUfgUfaAfaAflL96	AS2081	2191	uUfuUfuuuAfcAfaUfgUfcUfuUfsa		
D2082	S2082	1100	AfgAfcAfaUfcUfgUfaAfaAflL96	AS2082	2192	uUfuUfuUfaCfaAfaUfgUfaUfuUfsa		
D2083	S2083	1101	AfgAfcAfaUfcUfgUfaAfaAflL96	AS2083	2193	uUfuUfuuuUfaCfaAfaUfgUfaUfuUfsa		
D2084	S2084	1102	GfaCfaCfaUfcUfgUfaAfaAflL96	AS2084	2194	uUfuUfuUfuUfaCfaAfaUfgUfcUfuUfsa		
D2085	S2085	1103	GfaCfaCfaUfcUfgUfaAfaAflL96	AS2085	2195	uUfuUfuuuUfaCfaAfaUfgUfcUfuUfsa		
D2086	S2086	1104	AfcAfaUfcUfgUfaAfaAflL96	AS2086	2196	uUfuUfuUfuUfaCfaAfaUfgUfaUfuUfsa		
D2087	S2087	1105	AfcAfaUfcUfgUfaAfaAflL96	AS2087	2197	uUfuUfuuuUfaCfaAfaUfgUfaUfuUfsa		
D2088	S2088	1106	CfaCfaUfcUfgUfaAfaAflL96	AS2088	2198	uUfuUfuUfuUfaCfaAfaUfgUfcUfuUfsa		
D2089	S2089	1107	CfaCfaUfcUfgUfaAfaAflL96	AS2089	2199	uUfuUfuuuUfaCfaAfaUfgUfcUfuUfsa		
D2090	S2090	1108	AfcAfaUfcUfgUfaAfaAflL96	AS2090	2200	uUfuUfuUfuUfaCfaAfaUfgUfaUfuUfsa		
D2091	S2091	1109	AfcAfaUfcUfgUfaAfaAflL96	AS2091	2201	uUfuUfuuuUfaCfaAfaUfgUfaUfuUfsa		

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 GalNAc₃ ligand.

Example 4: *In vitro* screening of RNAi AgentsCell 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
5 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
10 temperature for 15 minutes. 80 µl of complete growth media without antibiotic containing ~2 x 10⁴ 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.

15

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
20 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
25 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
30 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 μ l 10X Buffer, 0.4 μ l 25X dNTPs, 1 μ l Random primers, 0.5 μ l Reverse Transcriptase, 0.5 μ l RNase inhibitor and 1.6 μ l of H₂O per reaction were added into 5 μ l 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.

10 Real time PCR

2 μ l of cDNA were added to a master mix containing 0.5 μ l GAPDH TaqMan Probe (Applied Biosystems Cat #4326317E (human) Cat # 4308313 (rodent)), 0.5 μ l TTR TaqMan probe (Applied Biosystems cat # HS00174914 _m1 (human) cat # Rn00562124_m1 (rat)) and 5 μ l 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 $\Delta\Delta C_t$ method and normalized to assays performed with cells transfected with 10nM AD-1955, or mock transfected cells. IC₅₀s 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. IC₅₀s were calculated for each individual transfection as well as in combination, where a single IC₅₀ 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.

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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 GalNAc₃ 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 GGATGGGATTTTCATGTAACCAAGA (SEQ ID NO: 2204) and the region or complementarity of RNAi agents AD-45163, AD-51544, and AD-51545 is TTCATGTAACCAAGAGTATTCCAT (SEQ ID NO: 2205).

Protocol for assessment of IC₅₀ in Hep3B cells

The IC₅₀ 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% CO₂ 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 IC₅₀ 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 IC₅₀

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
5 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% CO₂ incubator for 24 hours prior to lysis and analysis of TTR and GAPDH
10 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.

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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)	aUfgGfaAfuAfcUfcUfuGfgUfuAfcAfusGfisa (SEQ ID NO: 2212)	0.04101	0.00820	0.0115
AD-51544	AfuGfuAfaCfcAfaGfaGfuAfuUfcCfaUfL96 (SEQ ID NO: 2207)	aUfgGf A fAfuAfcUfcuuGfgUfuAfcAfusGfisa (SEQ ID NO: 2213)	0.00346	0.00374	0.0014
AD-51545	AfuGfuAfa A fCfcAfaGfaGfuAfuUfcCfaUfL96 (SEQ ID NO: 2208)	aUfgGfaAfuAfcUfcuuGfguuAfcAfusGfisa (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 G fAfuUfuCfa A UfgUfaAfcCfa A fAfgAFL96 (SEQ ID NO: 2210)	uCfu u gGfuUfaCfa u gAfaAfu u ccCfasUfsc (SEQ ID NO: 2216)	0.00317	0.00263	0.0017
AD-51547	UfgGfgAfuUfuCfa A UfgUfaacCfaAfgAFL96 (SEQ ID NO: 2211)	uCfuUfgG U fUfaCfa 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 GalNA₃ 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

5 Parent RNAi agents with alternating chemical modifications and a GalNAc₃ ligand provided an IC₅₀ 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 IC₅₀ values in Hep3B cells that
10 were 5-8 fold better than the corresponding parent agent.

Results: Free Uptake IC₅₀s 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
15 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
20 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.

25

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,
5 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.

10 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
15 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
20 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
25 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
30 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

5 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.

10 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
15 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 ($\mu\text{g/mL}$)	9.6	11.7	10.9	11.7	12.1
Plasma AUC (h* $\mu\text{g/mL}$)	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	
	25		25		25		25	
siRNA Dose (mg/kg)								
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} ($\mu\text{g/mL}$)	96.5 ^a	11.7	108 ^a	10.9	128 ^a	10.9	123 ^a	12.1
$AUC_{0-\text{last}}$ ($\text{h} \cdot \mu\text{g/mL}$)	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, $AUC_{0-\infty}$, CL and V_{ss}) 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-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	
	25		25		25		25	
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} ($\mu\text{g/g}$)	67.9	126	37.0	80.5	35.3	117	73.8	174
AUC_{0-last} ($\text{h}\cdot\mu\text{g/g}$)	632	1092	324	763	984	2131	1429	4583
MRT_{0-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 cynomolgous 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 TTRIn 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 μ M 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), IL1 β , IL-1ra, IL-6, IL-8, IP-10, MCP-1, MIP-1 α , MIP-1 β , TNF α . (Results not shown).

10

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 subcutaneous 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.

20

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).

25

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
5 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.

10

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.

15

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.

15

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 cynomolgous 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

25

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.

5 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
10 days after the day of administration of the last maintenance dose for the 5 and 2.5 mg/kg
dose levels.

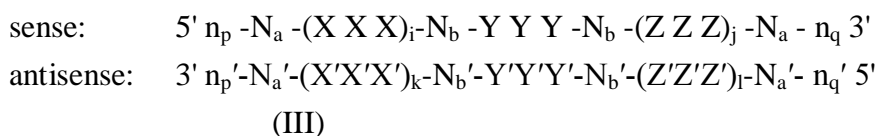
Equivalents:

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

5 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 sequence that is complementary to nucleotides 504 to 526 of the transthyretin (TTR) gene (SEQ ID NO:1), wherein the sense strand is 21 nucleotides in length and the antisense strand is 23 nucleotides in length, wherein said double stranded RNAi agent is represented by formula (III):



10

wherein:

$j = 1$; and i , k , and l are 0;

p' is 2; p , q , and q' are 0;

each N_a and $N_{a'}$ independently represents an oligonucleotide sequence comprising 2-10 nucleotides which are modified nucleotides;

15

each N_b and $N_{b'}$ independently represents an oligonucleotide sequence comprising 0-7 nucleotides which are modified nucleotides;

$n_{p'}$ represents an overhang nucleotide;

YYY, ZZZ, and Y'Y'Y', each independently represent one motif of three identical modifications on three consecutive nucleotides, wherein the Y nucleotides contain a 2'-fluoro modification, the Y' nucleotides contain a 2'-O-methyl modification, and the Z nucleotides contain a 2'-O-methyl modification; and

20

wherein the sense strand is conjugated to at least one ligand, wherein the ligand is one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

25

2. The RNAi agent of claim 1, wherein the YYY motif occurs at or near the cleavage site of the sense strand; or wherein the Y'Y'Y' motif occurs at the 11, 12 and 13 positions of the antisense strand from the 5'-end.

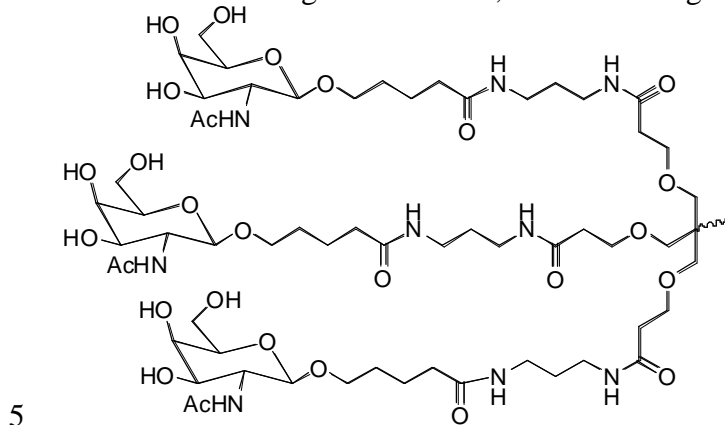
30

3. The RNAi agent of claim 1 or claim 2, wherein the modifications on the N_a , $N_{a'}$, N_b , and $N_{b'}$ nucleotides are each independently 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.

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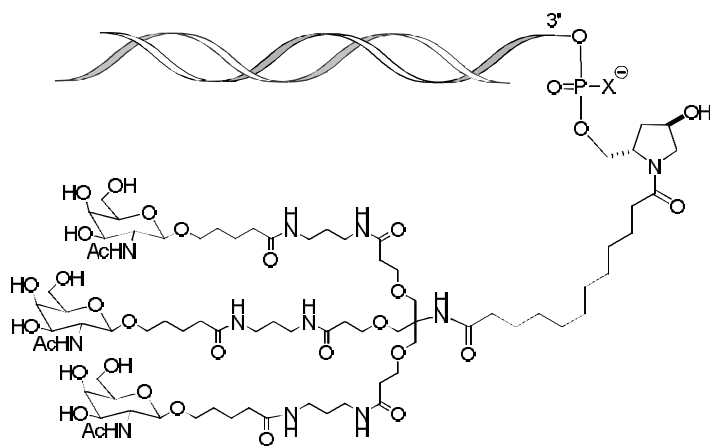
4. The RNAi agent of claim 3, wherein the modifications on the N_a , N_a' , N_b , and N_b' nucleotides are 2'-O-methyl, 2'-fluoro or both.

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



6. The RNAi agent of claim of any one of claims 1 to 5, wherein the ligand is attached to the 3' end of the sense strand.

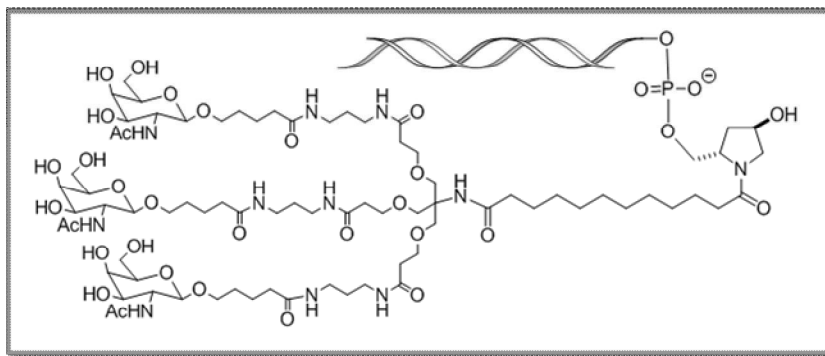
10 7. The RNAi agent of claim 6, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



wherein X is O or S.

15

8. The RNAi agent of claim 7, wherein the RNAi agent is conjugated to the ligand as shown in the following schematic



9. The RNAi agent of any one of claims 1 to 8 further comprising at least one phosphorothioate or methylphosphonate internucleotide linkage.
- 5
10. The RNAi agent of claim 9, wherein the phosphorothioate or methylphosphonate internucleotide linkage is at the 3'-terminal of one strand.
11. The RNAi agent of claim 10, wherein said strand is the antisense strand or the
- 10 sense strand.
12. The RNAi agent of any one of claims 1 to 11, wherein the base pair at the 1 position of the 5'-end of the antisense strand of the duplex is an AU base pair.
- 15
13. The RNAi agent of any one of claims 1 to 11, wherein the p' overhang nucleotides are complementary to the target mRNA or wherein the p' overhang nucleotides are non-complementary to the target mRNA.
14. The RNAi agent of any one of claims 1 to 11, wherein at least one np' is linked
- 20 to a neighboring nucleotide via a phosphorothioate linkage.
15. The RNAi agent of claim 14, wherein all np' are linked to neighboring nucleotides via phosphorothioate linkages.
- 25
16. An isolated cell containing an RNAi agent of any one of claims 1-15.
17. A pharmaceutical composition comprising an RNAi agent of any one of claims 1-15.

18. The pharmaceutical composition of claim 17, wherein the RNAi agent is administered in an unbuffered solution.
19. The pharmaceutical composition of claim 18, wherein said unbuffered solution is saline or water.
20. The pharmaceutical composition of claim 17, wherein said RNAi agent is administered with a buffer solution.
21. The pharmaceutical composition of claim 20, wherein said buffer solution comprises acetate, citrate, prolamine, carbonate, phosphate or any combination thereof.
22. The pharmaceutical composition of claim 21, wherein said buffer solution is phosphate buffered saline (PBS).
23. A method of inhibiting expression of a transthyretin (TTR) in an isolated cell comprising contacting said isolated cell with an RNAi agent of any one of claims 1-15 in an amount effective to inhibit expression of said TTR in said isolated cell, thereby inhibiting expression of said transthyretin (TTR) in said isolated cell.
24. Use of an RNAi agent of any one of claims 1-15, for the manufacture of a medicament for treating a TTR-associated disease in a subject.
25. A kit for performing the method of claim 23, comprising
- a) said RNAi agent, and
 - b) instructions for use.
26. A kit for the use of claim 24, said kit comprising
- a) said RNAi agent, and
 - b) instructions for said use.
27. The double stranded RNAi agent of claim 1, wherein the sense strand comprises the nucleotide sequence 5' – UGGGAUUUCAUGUAACCAAGA – 3' (SEQ ID NO:2211).

35

28. The double stranded RNAi agent of claim 1, wherein the sense strand comprises the nucleotide sequence 5'- UfgGfgAfuUfuCfAfUfgUfaacCfaAfgAfL96-3' (SEQ ID NO:2) and the antisense strand comprises the nucleotide sequence 5'-
uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc-3' (SEQ ID NO:3),
5 wherein a, g, c and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C and U; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.
29. The use of claim 24, wherein said medicament is suitable for administration to a
10 human.
30. The method of claim 24, wherein said subject carries a TTR gene mutation that is associated with the development of a TTR-associated disease.
- 15 31. The use of claim 24, 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.
20
32. The use of claim 24, wherein said TTR-associated-disease is TTR-associated amyloidosis.
33. The use of claim 24, wherein said medicament is suitable for subcutaneous
25 administration.
34. A double stranded RNAi agent, comprising a sense strand and an antisense strand, wherein the sense strand comprises the nucleotide sequence 5'-
UfgGfgAfuUfuCfAfUfgUfaacCfaAfgAfL96-3' (SEQ ID NO:2) and the antisense strand
30 comprises the nucleotide sequence 5'-uCfuUfgGfUfUfaCfaugAfaAfuCfcCfasUfsc-3' (SEQ ID NO:3),
wherein a, g, c, and u are 2'-O-methyl (2'-OMe) A, G, C, and U; Af, Gf, Cf, and Uf are 2'-fluoro A, G, C, and U; s is a phosphorothioate linkage; and L96 is a GalNAc3 ligand.

FIG. 1

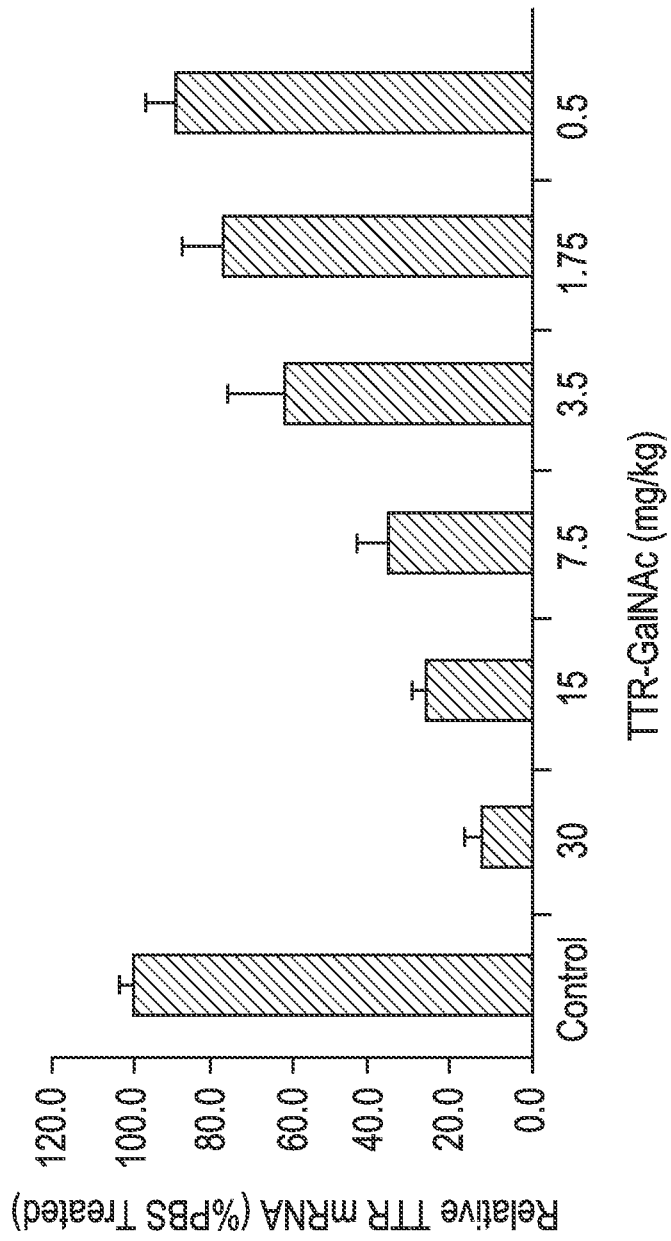


FIG. 2

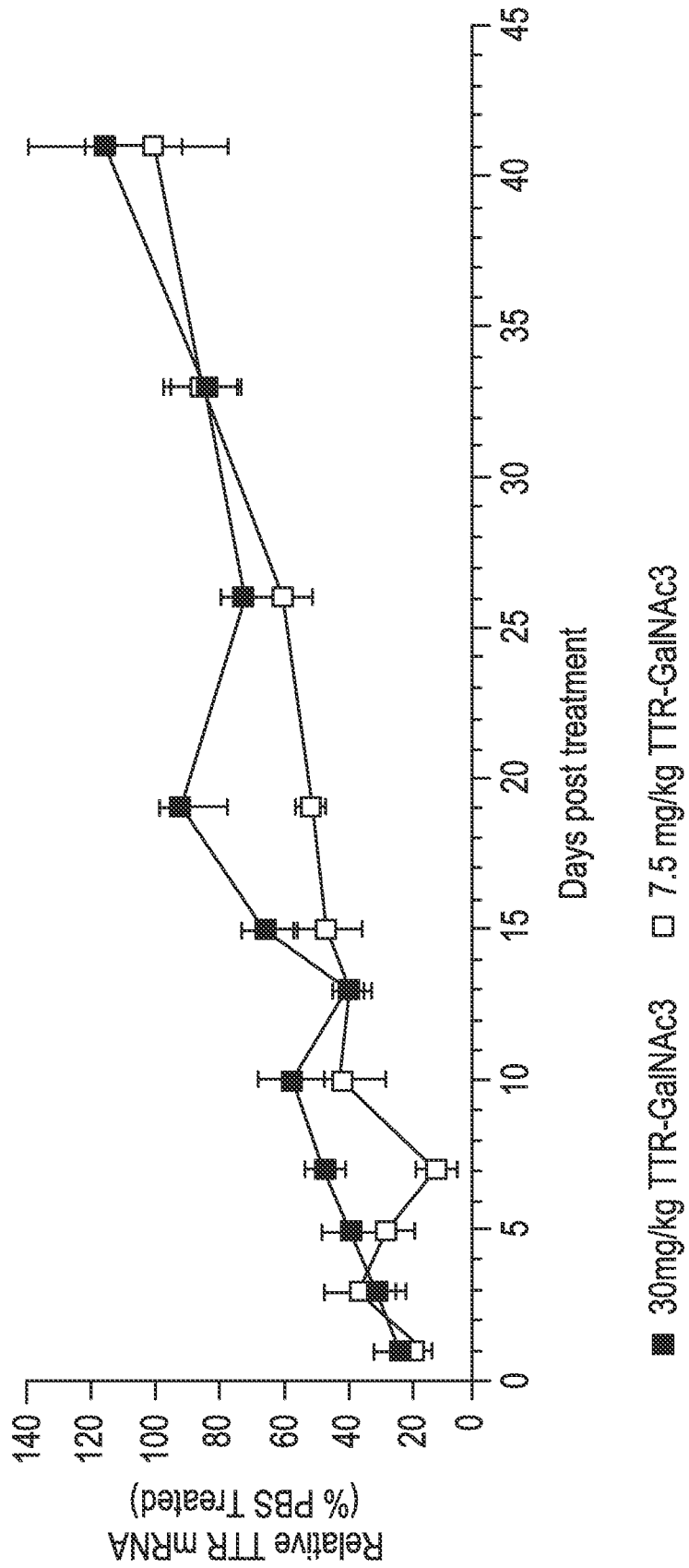


FIG. 3

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

GI: 189583

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```

1   cagaagtcca  ctcattcttg  gcaggatggc  ttctcatcgt  ctgctcctcc  tctgccttgc
61  tggactggta  tttgtgtctg  aggctggccc  tacgggcacc  ggtgaatcca  agtgtcctct
121 gatggtcaaa  gttctagatg  ctgtccgagg  cagtcctgcc  atcaatgtgg  ccgtgcatgt
181 gttcagaag  gctgctgatg  acacctggga  gccatttgcc  tctgggaaaa  ccagtgagtc
241 tggagagctg  catgggctca  cactgagga  ggaatttgta  gaaggatat  acaaagtgga
301 aatagacacc  aaatcttact  ggaaggcact  tggcatctcc  ccattccatg  agcatgacaga
361 ggtggtattc  acagccaacg  actccggccc  ccgccgtac  accattgccg  ccctgctgag
421 cccctactcc  tattcacca  cggctgtcgt  caccaatccc  aaggaatgag  ggacttctcc
481 tccagtggac  ctgaaggacg  agggatggga  tttcatgtaa  ccaagagtat  tccattttta
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601 ttcctgtgaa  aggc

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FIG. 4

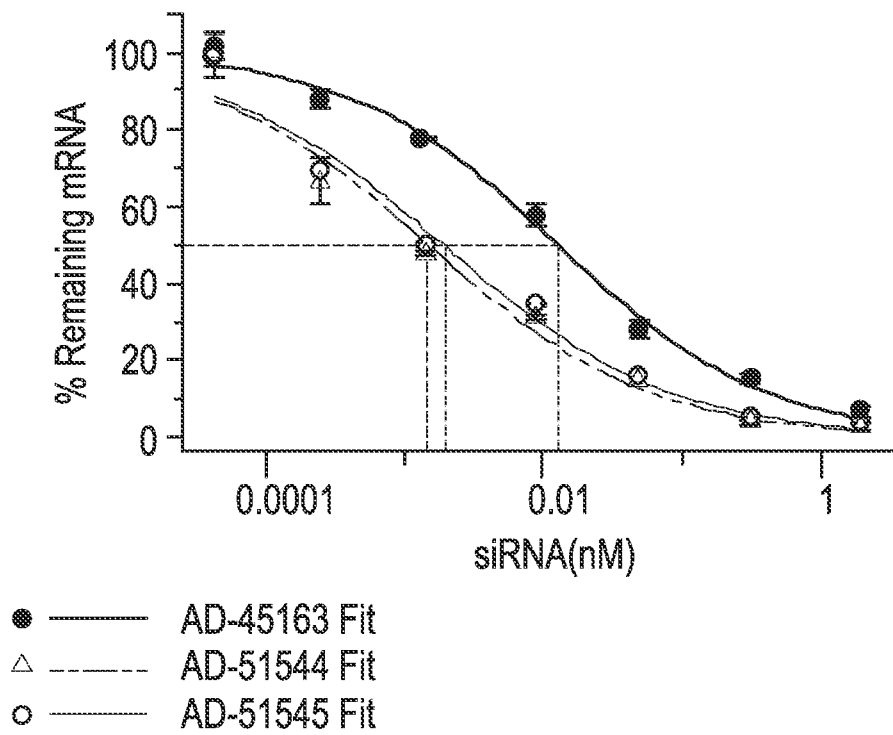
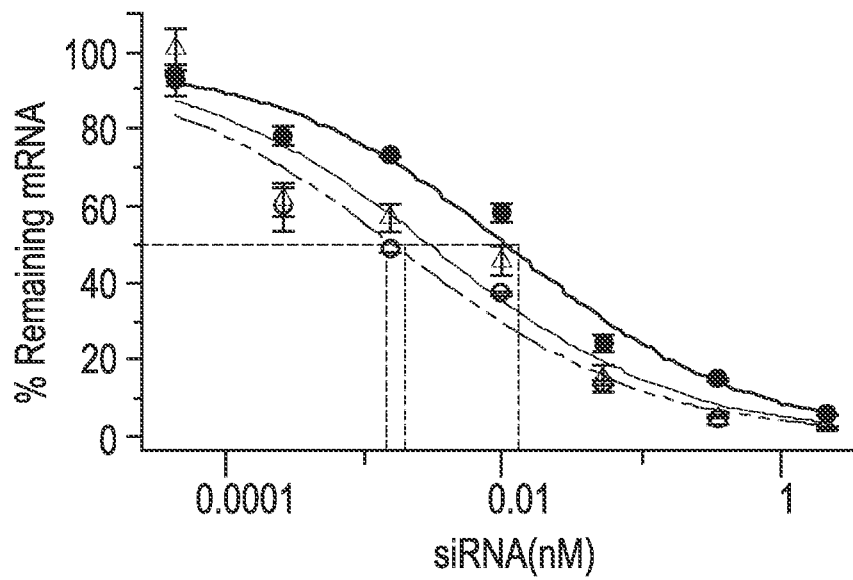
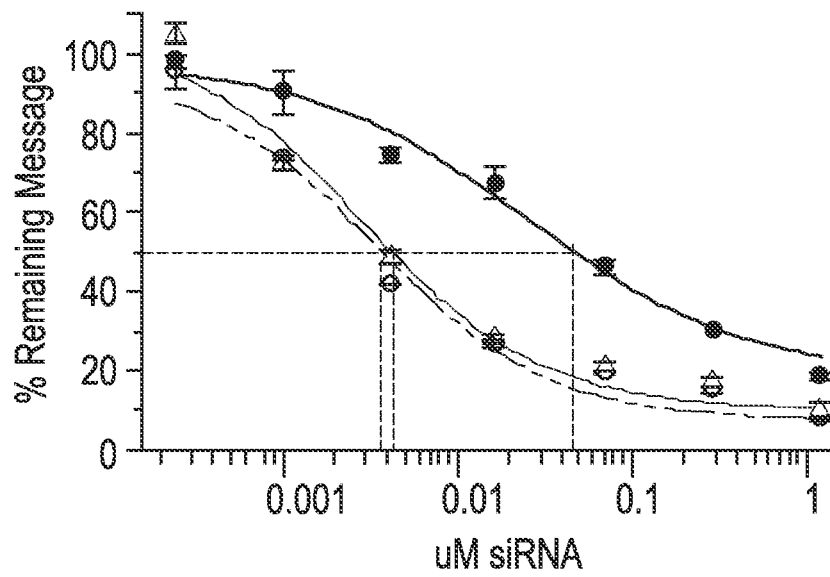


FIG. 5



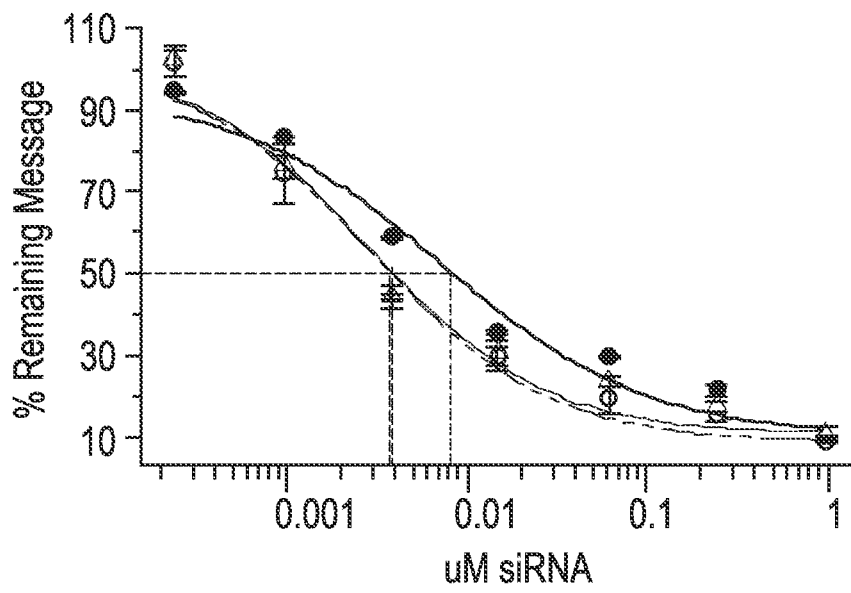
- — AD-45165 Fit
- △ - - AD-51546 Fit
- — AD-51547 Fit

FIG. 6



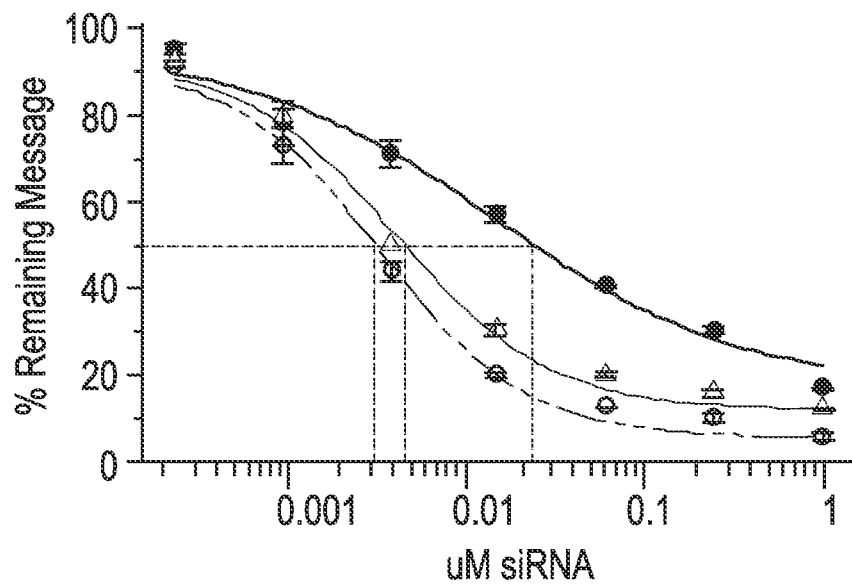
- ——— 45163, 4hr Fit
- △ - - - - 51544, 4hr Fit
- ——— 51545, 4hr Fit

FIG. 7



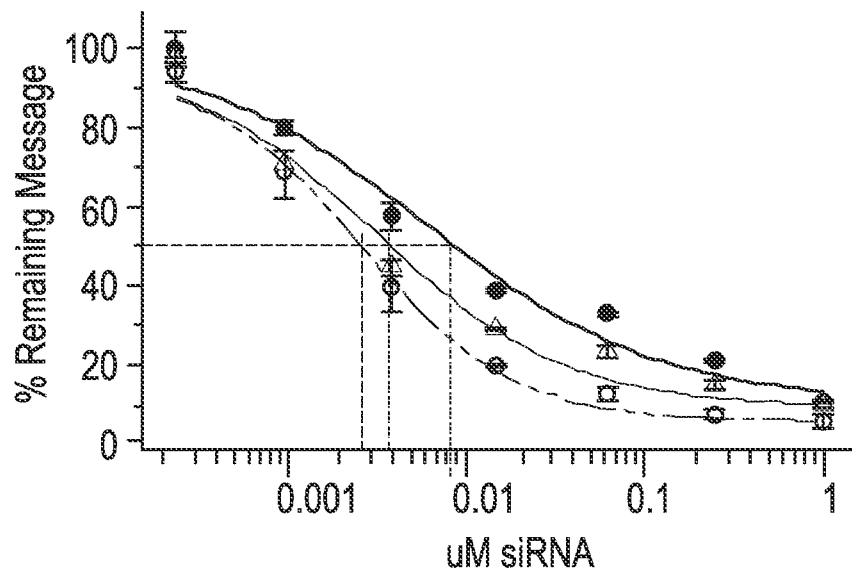
- ——— 45163, 24hr Fit
- △ - - - - 51544, 24hr Fit
- ——— 51545, 24hr Fit

FIG. 8



- ——— 45165, 4 hr Fit
- △ - - - - 51546, 4 hr Fit
- ——— 51547, 4 hr Fit

FIG. 9

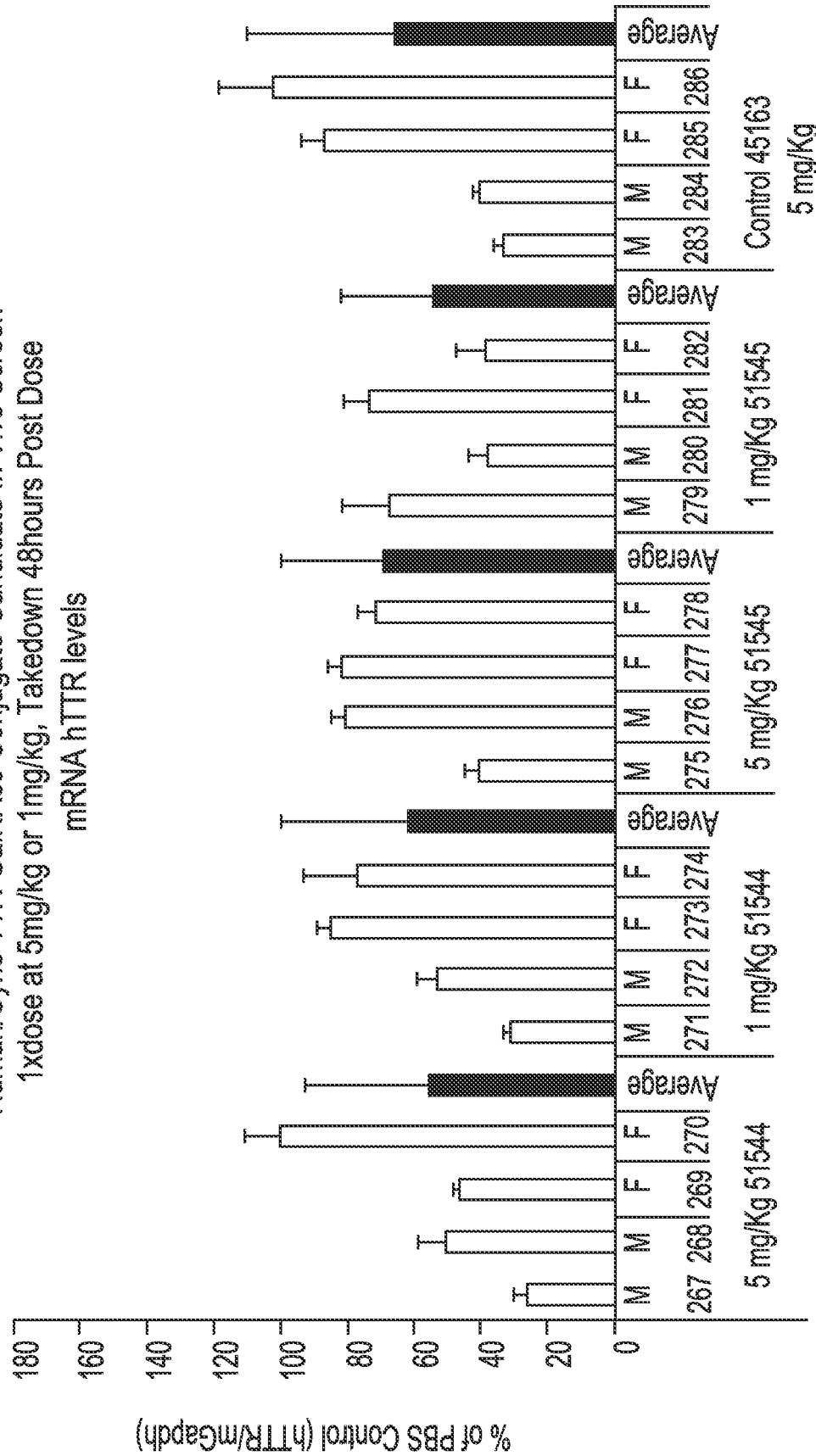


- ——— 45165, 24 hr Fit
- △ - - - - 51546, 24 hr Fit
- ——— 51547, 24 hr Fit

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FIG. 10A

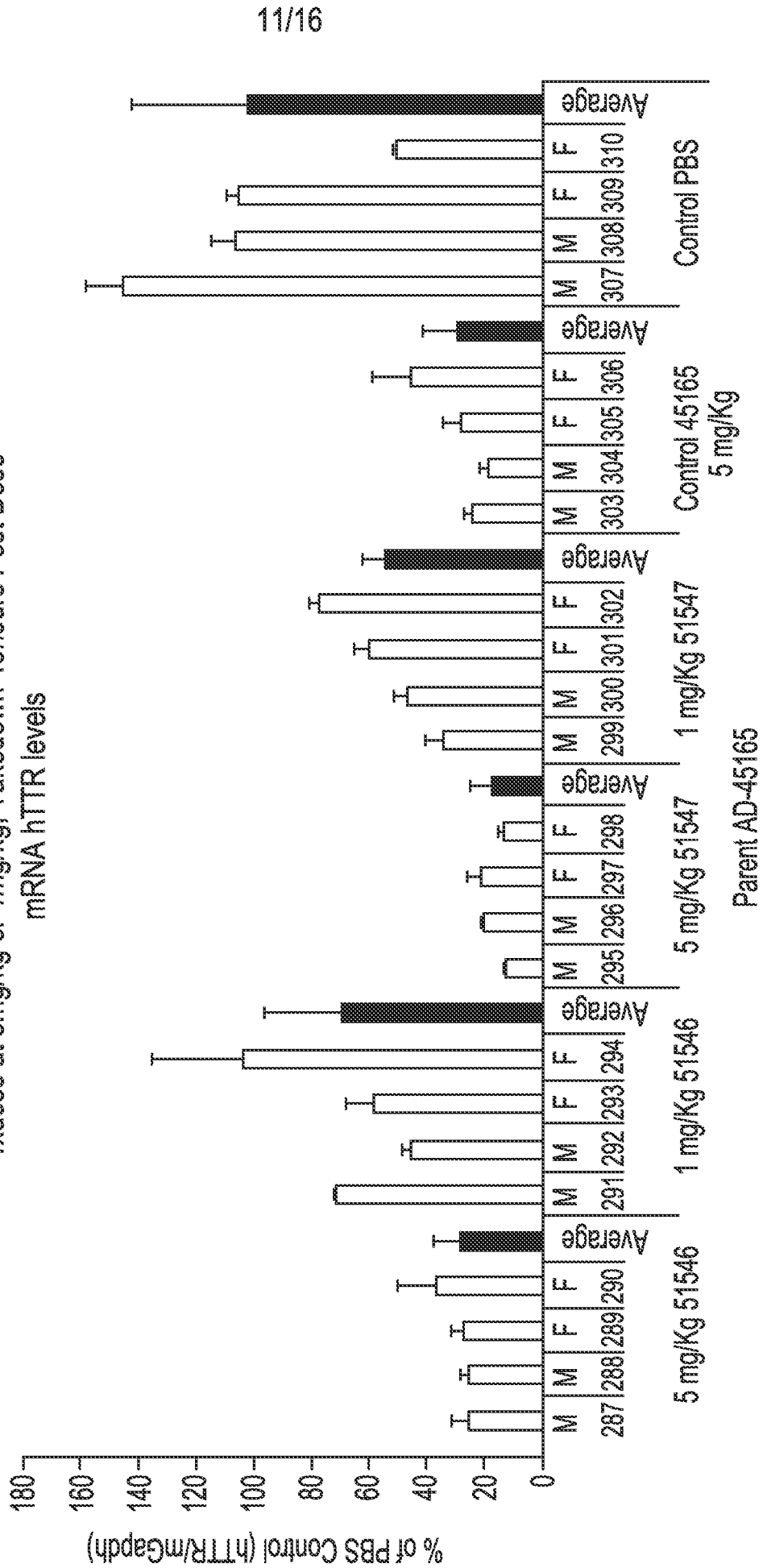
Human/Cyno TTR-GalNAc3 Conjugate Candidate In Vivo Screen
 1xdose at 5mg/kg or 1mg/kg, Takedown 48hours Post Dose
 mRNA hTTR levels



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



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FIG. 11

Relative TTR Protein - Single s.c. dose, 48h

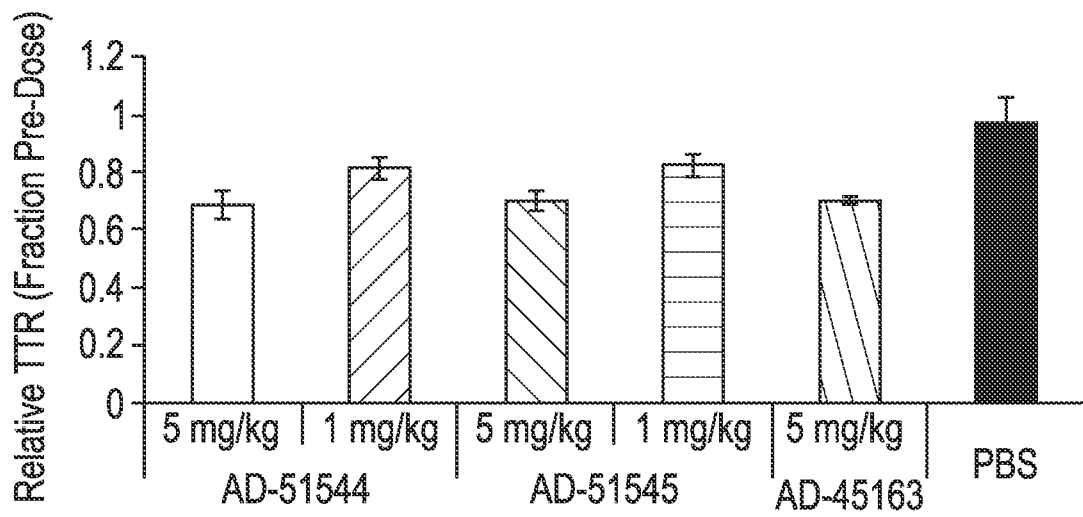


FIG. 12

Relative TTR Protein - Single s.c. dose, 48h

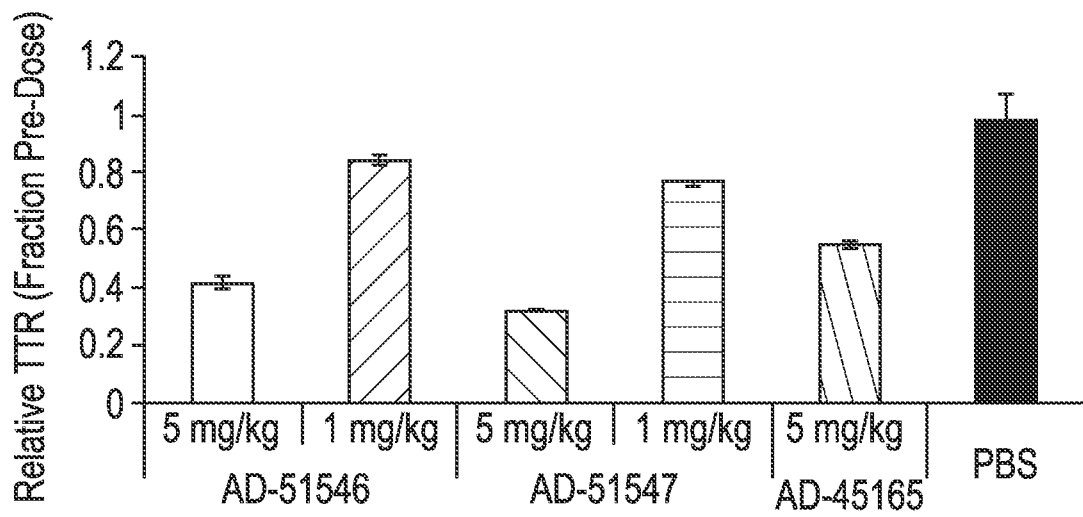
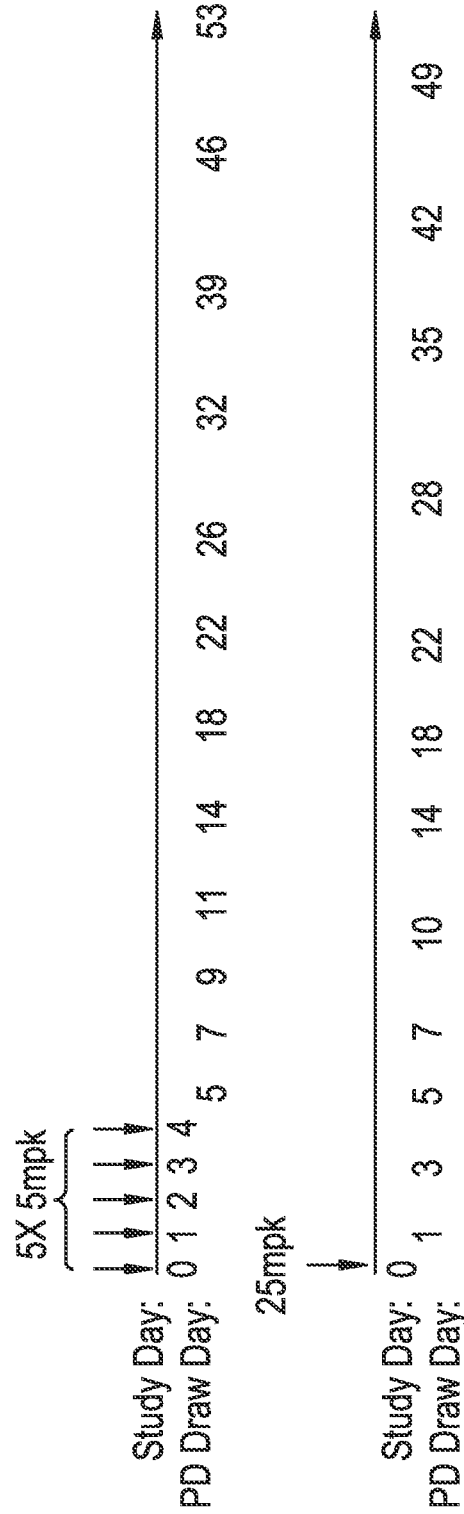


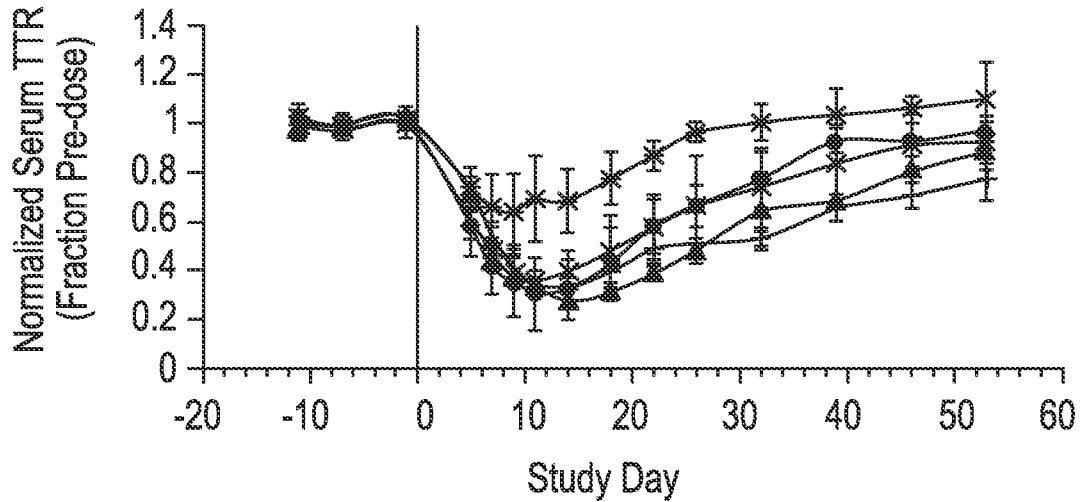
FIG. 13



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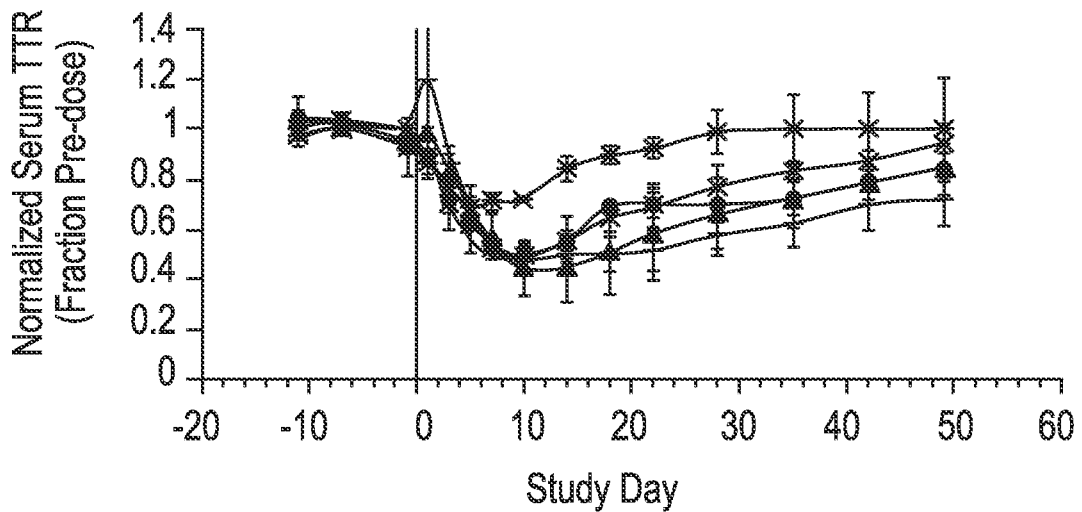
FIG. 14

5X 5mg/kg



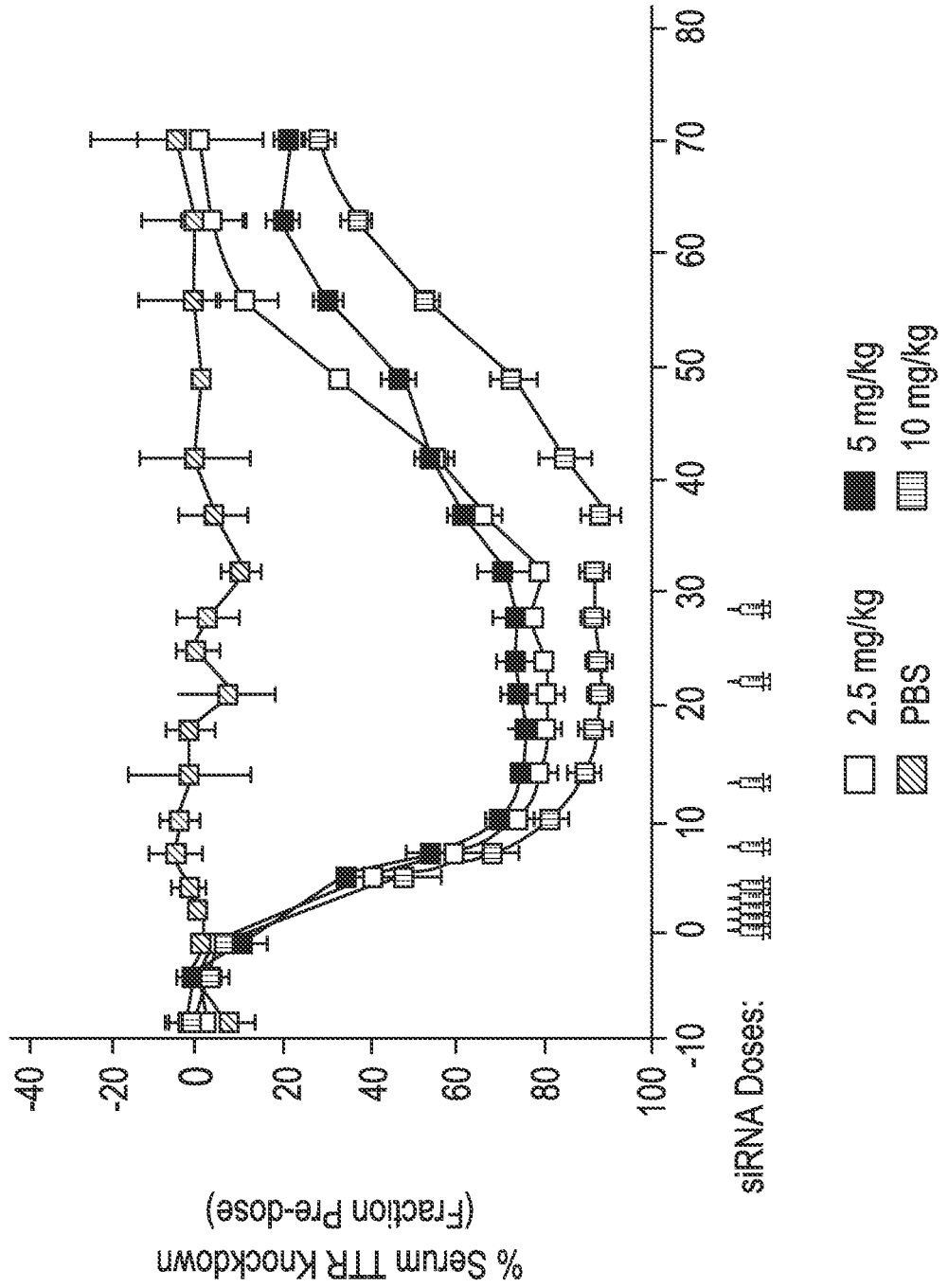
- ▲ 5x5 mk 45163 × 5x5 mpk 51544 * 5x5 mpk 51545
- 5x5 mpk 51546 + 5x5 mpk 51547

25mg/kg



- ▲ 25mpk 45163 × 25mpk 51544 * 25mpk 51545
- 25mpk 51546 + 25mpk 51547

FIG. 15



1351630_1.txt
SEQUENCE LISTING

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<120> RNAI AGENTS, COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING
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<130> 121301-00120

<140> New Application

<141> Concurrently Herewith

<150> 61/680,098

<151> 2012-08-06

<150> 61/615,618

<151> 2012-03-26

<150> 61/561,710

<151> 2011-11-18

<160> 2217

<170> PatentIn version 3.5

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<212> DNA

<213> Homo sapiens

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gatggtcaaa gttctagatg ctgtccgagg cagtcctgcc atcaatgtgg ccgtgcatgt      180
gttcagaaag gctgctgatg acacctggga gccatttgcc tctgggaaaa ccagtgagtc      240
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tccagtggac ctgaaggacg agggatggga tttcatgtaa ccaagagtat tccattttta      540
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 1 5 10 15

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23

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 <210> 643
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 <210> 644
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<210> 646
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<210> 649
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<210> 657
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<210> 658
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<210> 659
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<210> 660
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<210> 776
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<210> 777
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<210> 779
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<210> 780
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oligonucleotide

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oligonucleotide

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oligonucleotide

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<210> 869
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<210> 870
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<210> 881
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<210> 884
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 oligonucleotide

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<210> 902
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 oligonucleotide

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<210> 903
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 oligonucleotide

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<210> 904
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<210> 905
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oligonucleotide

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<210> 991
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<210> 992
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<210> 993
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<210> 996
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<210> 998
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 <210> 1004
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 <400> 1004
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 <210> 1005
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<213> Artificial Sequence

<220>
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<400> 1005
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<210> 1006
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<210> 1007
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<210> 1008
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<210> 1009
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<210> 1010
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<210> 1011
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<210> 1012
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<210> 1013
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<210> 1014
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<210> 1015
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<210> 1016
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<210> 1017
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<400> 1017
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<210> 1019
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<210> 1020
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<210> 1021
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<210> 1060
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<210> 1072
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<210> 1073
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<213> Artificial Sequence

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<210> 1074
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<210> 1075
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<210> 1076
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<210> 1077
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<210> 1079
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<210> 1080
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<210> 1081
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<210> 1082
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<210> 1083
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<210> 1084
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<210> 1085
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<210> 1086
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<210> 1087
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<220>
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<210> 1088
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<220>
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<210> 1089
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<220>
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<210> 1090
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<220>
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<210> 1091
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<210> 1092
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<210> 1093
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<220>
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<210> 1094
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<210> 1095
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<210> 1096
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<210> 1097
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 oligonucleotide

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<210> 1098
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 oligonucleotide

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<210> 1099
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<220>
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 oligonucleotide

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<210> 1100
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<220>
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 oligonucleotide

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<220>
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<210> 1103
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 <212> RNA
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<220>
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 oligonucleotide

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<210> 1104
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<220>
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 oligonucleotide

<400> 1104
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<210> 1105
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<220>
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oligonucleotide

<400> 1105
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<210> 1106
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<220>
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oligonucleotide

<400> 1106
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oligonucleotide

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<220>
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<210> 1110
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<220>
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<400> 1110
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<210> 1111
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<220>
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<210> 1112
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<220>
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<210> 1113
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<220>
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<400> 1113
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<210> 1114
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<220>
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<210> 1115

<211> 23
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 oligonucleotide

 <400> 1115
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 <210> 1116
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 oligonucleotide

 <400> 1116
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 <210> 1117
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 oligonucleotide

 <400> 1117
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 <210> 1118
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 <210> 1119
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 oligonucleotide

 <400> 1119
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 <210> 1120
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<220>
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 oligonucleotide

 <400> 1120
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 <210> 1121
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 <220>
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 oligonucleotide

 <400> 1121
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 <210> 1122
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 <220>
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 <210> 1123
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 oligonucleotide

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 <210> 1124
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<220>
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<220>
<223> Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide

<400> 1132
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<210> 1133
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<220>
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<210> 1134
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<400> 1134
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<210> 1135

<211> 23
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 oligonucleotide

 <400> 1135
 ucuugguuac augaaaauccc auc 23

<210> 1136
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 oligonucleotide

 <400> 1136
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<210> 1137
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 oligonucleotide

 <400> 1137
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<210> 1138
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 oligonucleotide

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 oligonucleotide

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<210> 1139
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 oligonucleotide

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 oligonucleotide

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<210> 1140
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<210> 1141
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23

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<210> 1704
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 <210> 1918
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 <210> 1921
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 <210> 1922
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 <210> 1925
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 <210> 1926
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<210> 1928
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<210> 1931
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<210> 1932
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<210> 1933
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<210> 1934
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<210> 1935
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<210> 1936
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<210> 1937
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<210> 1938
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 <210> 1939
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cagaaugugu cuuuuuuaug gcu 23

<210> 2176
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2176
acagaaugug uuuuuuaau ggc 23

<210> 2177
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2177
acagaaugug uuuuuuaau ggc 23

<210> 2178
<211> 23
<212> RNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2178
uacagaaugu gucuuuuuaa ugg 23

<210> 2179
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2179
uacagaaugu gucuuuuuaa ugg 23

<210> 2180
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2180
uuacagaaug ugucuuuuua aug 23

<210> 2181
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2181
uuacagaaug ugucuuuuua aug 23

<210> 2182
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2182
uuuacagaaugugucuuuuu aau 23

<210> 2183
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2183
 uuuacagaau gugucuuuuu aau 23

<210> 2184
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<220>
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 oligonucleotide

<400> 2184
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<210> 2185
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 <212> RNA
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<220>
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 oligonucleotide

<400> 2185
 uuuuacagaa ugugucuuuu uaa 23

<210> 2186
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 <212> RNA
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<220>
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 oligonucleotide

<400> 2186
 uuuuuacaga augugucuuu uua 23

<210> 2187
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<220>
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 oligonucleotide

<400> 2187
 uuuuuacaga augugucuuu uua 23

<210> 2188
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<220>
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 oligonucleotide

<400> 2188
 uuuuuuacag aaugugucuu uuu 23

<210> 2189
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 <212> RNA
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 oligonucleotide

 <400> 2189
 uuuuuuacag aaugugucu uuu 23

 <210> 2190
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
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 oligonucleotide

 <400> 2190
 uuuuuuuaca gaaugugucu uuu 23

 <210> 2191
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 <212> RNA
 <213> Artificial Sequence

 <220>
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 oligonucleotide

 <400> 2191
 uuuuuuuaca gaaugugucu uuu 23

 <210> 2192
 <211> 23
 <212> RNA
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 <220>
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 oligonucleotide

 <400> 2192
 uuuuuuuuac agaauguguc uuu 23

 <210> 2193
 <211> 23
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 <213> Artificial Sequence

 <220>
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 oligonucleotide

 <400> 2193
 uuuuuuuuac agaauguguc uuu 23

 <210> 2194
 <211> 23

<212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2194
 uuuuuuuuuu cagaaugugu cuu 23

 <210> 2195
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2195
 uuuuuuuuuu cagaaugugu cuu 23

 <210> 2196
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2196
 uuuuuuuuuu acagaaugug ucu 23

 <210> 2197
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2197
 uuuuuuuuuu acagaaugug ucu 23

 <210> 2198
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2198
 uuuuuuuuuu uacagaaugu guc 23

 <210> 2199
 <211> 23
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

 <400> 2199
 uuuuuuuuuu uacagaaugu guc 23

<210> 2200
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

 <400> 2200
 uuuuuuuuuu uuacagaaug ugu 23

<210> 2201
 <211> 23
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

 <400> 2201
 uuuuuuuuuu uuacagaaug ugu 23

<210> 2202
 <211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

 <220>
 <223> Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide

 <400> 2202
 cuuacgcuga guacuucgat t 21

<210> 2203
 <211> 20
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic oligonucleotide

 <220>
 <223> Description of Combined DNA/RNA Molecule: Synthetic oligonucleotide

 <400> 2203
 ucgaagucuc agcguaagtt 20

<210> 2204
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2204
 ggatgggatt tcatgtaacc aaga 24

 <210> 2205
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2205
 ttcattgtaac caagagtatt ccat 24

 <210> 2206
 <211> 21
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2206
 auguaaccaa gaguauucca u 21

 <210> 2207
 <211> 21
 <212> RNA
 <213> Artificial Sequence

 <220>
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 oligonucleotide

 <400> 2207
 auguaaccaa gaguauucca u 21

 <210> 2208
 <211> 21
 <212> RNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: Synthetic
 oligonucleotide

 <400> 2208
 auguaaccaa gaguauucca u 21

 <210> 2209
 <211> 21
 <212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2209
 ugggauuuca uguaaccaag a 21

<210> 2210
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 <212> RNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2210
 ugggauuuca uguaaccaag a 21

<210> 2211
 <211> 21
 <212> RNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2211
 ugggauuuca uguaaccaag a 21

<210> 2212
 <211> 23
 <212> RNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2212
 auggauacu cuugguaca uga 23

<210> 2213
 <211> 23
 <212> RNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2213
 auggauacu cuugguaca uga 23

<210> 2214
 <211> 23
 <212> RNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2214
auggaauacu cugguuaca uga 23

<210> 2215
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2215
ucugguuac augaaauccc auc 23

<210> 2216
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2216
ucugguuac augaaauccc auc 23

<210> 2217
<211> 23
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2217
ucugguuac augaaauccc auc 23