COMPOSITIONS FOR MODULATING C9ORF72 EXPRESSION

Sequence Listing

The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0269WOSEQ_ST25.txt created April 15, 2016, which is 104 Kb in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

Provided are compositions and methods for modulating expression of C9ORF72 mRNA and protein in cells and animals. Such compositions and methods are useful to treat, prevent, ameliorate, or slow progression of neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, and olivopontocerebellar degeneration (OPCD).

Background

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized clinically by progressive paralysis leading to death from respiratory failure, typically within two to three years of symptom onset (Rowland and Shneider, N. Engl. J. Med., 2001, 344, 1688-1700). ALS is the third most common neurodegenerative disease in the Western world (Hirtz et al., Neurology, 2007, 68, 326-337), and there are currently no effective therapies. Approximately 10% of cases are familial in nature, whereas the bulk of patients diagnosed with the disease are classified as sporadic as they appear to occur randomly throughout the population (Chio et al., Neurology, 2008, 70, 533-537). There is growing recognition, based on clinical, genetic, and epidemiological data, that ALS and frontotemporal dementia (FTD) represent an overlapping continuum of disease, characterized pathologically by the presence of TDP-43 positive inclusions throughout the central nervous system (Lillo and Hodges, J. Clin. Neurosci., 2009, 16, 1131-1135; Neumann et al., Science, 2006, 314, 130-133).

To date, a number of genes have been discovered as causative for classical familial ALS, for example, SOD1, TARDBP, FUS, OPTN, and VCP (Johnson et al., Neuron, 2010, 68, 857-864; Kwiatkowski et al., Science, 2009, 323, 1205-1208; Maruyama et al., Nature, 2010, 465, 223-226;

Rosen et al., Nature, 1993, 362, 59-62; Sreedharan et al., Science, 2008, 319, 1668-1672; Vance et al., Brain, 2009, 129, 868-876). Recently, linkage analysis of kindreds involving multiple cases of ALS, FTD, and ALS-FTD had suggested that there was an important locus for the disease on the short arm of chromosome 9 (Boxer et al., J. Neurol. Neurosurg. Psychiatry, 2011, 82, 196-203; Morita et al., Neurology, 2006, 66, 839-844; Pearson et al. J. Nerol., 2011, 258, 647-655; Vance et al., Brain, 2006, 129, 868-876). The mutation in the C9ORF72 gene is the most common genetic cause of ALS and FTD. The ALS-FTD causing mutation is a large hexanucleotide (GGGGCC) repeat expansion in the first intron of the C9ORF72 gene (Renton et al., Neuron, 2011, 72, 257-268; DeJesus-Hernandez et al., Neuron, 2011, 72, 245-256). A founder haplotype, covering the C9ORF72 gene, is present in the majority of cases linked to this region (Renton et al., Neuron, 2011, 72, 257-268). This locus on chromosome 9p21 accounts for nearly half of familial ALS and nearly one-quarter of all ALS cases in a cohort of 405 Finnish patients (Laaksovirta et al, Lancet Neurol., 2010, 9, 978-985).

A founder haplotype, covering the C9ORF72 gene, is present in the majority of cases linked to this region.

There are currently no effective therapies to treat such neurodegenerative diseases. Therefore, it is an object to provide compositions and methods for the treatment of such neurodegenerative diseases.

Summary

Certain embodiments provide methods, compounds, and compositions for inhibiting expression of C9ORF72 mRNA and protein in cells, tissues, and animals. Certain embodiments provide methods, compounds, and compositions for reducing C9ORF72 mRNA and protein levels in cells, tissues, and animals. Certain embodiments provide antisense compounds targeted to a C9ORF72 nucleic acid. In certain embodiments, the antisense compounds are modified oligonucleotides. In certain embodiments, the modified oligonucleotides are single-stranded.

In certain embodiments, C9ORF72 associated Repeat Associated Non-ATG Translation (RAN translation) products are reduced. In certain embodiments, the C9ORF72 associated RAN translation products are poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine). In certain embodiments, certain C9ORF72 mRNA variants are preferentially reduced. In certain embodiments, the C9ORF72 mRNA variants preferentially reduced are variants processed from a pre-mRNA containing intron 1. In certain embodiments, intron 1 contains a hexanucleotide repeat

expansion. In certain embodiments, the C9ORF72 mRNA variant preferentially reduced is a C9ORF72 pathogenic associated mRNA variant. In certain embodiments, the C9ORF72 pathogenic associated mRNA variant is NM_001256054.1 (SEQ ID NO: 1). In certain embodiments, the hexanucleotide repeat expansion is associated with a C9ORF72 associated disease. In certain embodiments, the hexanucleotide repeat expansion is associated with a C9ORF72 hexanucleotide repeat expansion associated disease. In certain embodiments, the hexanucleotide repeat expansion comprises at least 30 GGGGCC repeats, more than 30 GGGGCC repeats, more than 100 GGGGCC repeats, more than 500 GGGGCC repeats, or more than 1000 GGGGCC repeats. In certain embodiments, the hexanucleotide repeat expansion is associated with nuclear foci. In certain embodiments, C9ORF72 associated RAN translation products are associated with nuclear foci. In certain embodiments, the C9ORF72 associated RAN translation products are are poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine). In certain embodiments, the compositions and methods described herein are useful for reducing C9ORF72 mRNA levels, C9ORF72 protein levels, C9ORF72 RAN translation products, and nuclear foci. In certain embodiments, the compositions and methods described herein are useful for selectively reducing C9ORF72 pathogenic associated mRNA variants. Such reduction can occur in a time-dependent manner or in a dose-dependent manner.

Also provided are methods useful for preventing, treating, ameliorating, and slowing progression of diseases associated with C9ORF72. In certain embodiments, such C9ORF72 associated diseases are neurodegenerative diseases. In certain embodiments, the neurodegenerative disease is amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, and olivopontocerebellar degeneration (OPCD).

Such diseases can have one or more risk factors, causes, or outcomes in common. Certain risk factors and causes for development of a neurodegenerative disease, and, in particular, ALS and FTD, include genetic predisposition and older age.

In certain embodiments, methods of treatment include administering a C9ORF72 antisense compound to an individual in need thereof. In certain embodiments, the antisense compound is a single-stranded modified oligonucleotide. In certain embodiments, the single-stranded modified oligonucleotide is complementary to a C9ORF72 nucleic acid.

Detailed Description

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed. Herein, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Additionally, as used herein, the use of "and" means "and/or" unless stated otherwise. Furthermore, the use of the term "including" as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit, unless specifically stated otherwise.

Compounds of the invention include variations of the disclosed compounds in which one or more hydrogen, carbon, nitrogen, oxygen, or sulfur atoms is replaced with a stable isotope of the same element.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this disclosure, including, but not limited to, patents, patent applications, published patent applications, articles, books, treatises, and GENBANK Accession Numbers and associated sequence information obtainable through databases such as National Center for Biotechnology Information (NCBI) and other data referred to throughout in the disclosure herein are hereby expressly incorporated by reference for the portions of the document discussed herein, as well as in their entirety.

Definitions

Unless specific definitions are provided, the nomenclature utilized in connection with, and the procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical synthesis, and chemical analysis.

Unless otherwise indicated, the following terms have the following meanings:

"2'-O-methoxyethyl" (also 2'-MOE and 2'-OCH_2CH_2-OCH_3 and MOE) refers to an O-methoxy-ethyl modification of the 2' position of a furanose ring. A 2'-O-methoxyethyl modified sugar is a modified sugar.

"2'-MOE nucleoside" (also 2'-O-methoxyethyl nucleoside) means a nucleoside comprising a MOE modified sugar moiety.

"2'-substituted nucleoside" means a nucleoside comprising a substituent at the 2'-position of the furanose ring other than H or OH. In certain embodiments, 2'-substituted nucleosides include nucleosides with bicyclic sugar modifications.

"5-methylcytosine" means a cytosine modified with a methyl group attached to the 5' position. A 5-methylcytosine is a modified nucleobase.

"Administered concomitantly" refers to the co-administration of two pharmaceutical agents in any manner in which the pharmacological effects of both are manifest in the patient at the same time. Concomitant administration does not require that both pharmaceutical agents be administered in a single pharmaceutical composition, in the same dosage form, or by the same route of administration. The effects of both pharmaceutical agents need not manifest themselves at the same time. The effects need only be overlapping for a period of time and need not be coextensive.

"Administering" means providing a pharmaceutical agent to an animal, and includes, but is not limited to administering by a medical professional and self-administering.

"Amelioration" refers to a lessening, slowing, stopping, or reversing of at least one indicator of the severity of a condition or disease. The severity of indicators may be determined by subjective or objective measures, which are known to those skilled in the art.

"Animal" refers to a human or non-human animal, including, but not limited to, mice, rats, rabbits, dogs, cats, pigs, and non-human primates, including, but not limited to, monkeys and chimpanzees.

"Antibody" refers to a molecule characterized by reacting specifically with an antigen in some way, where the antibody and the antigen are each defined in terms of the other. Antibody may refer to a complete antibody molecule or any fragment or region thereof, such as the heavy chain, the light chain, Fab region, and Fc region.

"Antisense activity" means any detectable or measurable activity attributable to the hybridization of an antisense compound to its target nucleic acid. In certain embodiments, antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid.

"Antisense compound" means an oligomeric compound that is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding. Examples of antisense compounds include single-stranded and double-stranded compounds, such as, antisense oligonucleotides, siRNAs, shRNAs, ssRNAs, and occupancy-based compounds.

"Antisense inhibition" means reduction of target nucleic acid levels in the presence of an antisense compound complementary to a target nucleic acid compared to target nucleic acid levels or in the absence of the antisense compound.

"Antisense mechanisms" are all those mechanisms involving hybridization of a compound with a target nucleic acid, wherein the outcome or effect of the hybridization is either target degradation or target occupancy with concomitant stalling of the cellular machinery involving, for example, transcription or splicing.

"Antisense oligonucleotide" means a single-stranded oligonucleotide having a nucleobase sequence that permits hybridization to a corresponding segment of a target nucleic acid.

"Base complementarity" refers to the capacity for the precise base pairing of nucleobases of an antisense oligonucleotide with corresponding nucleobases in a target nucleic acid (i.e., hybridization), and is mediated by Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen binding between corresponding nucleobases.

"Bicyclic sugar" means a furanose ring modified by the bridging of two atoms. A bicyclic sugar is a modified sugar.

"Bicyclic nucleoside" (also BNA) means a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring.

"C9ORF72 antisense transcript" means transcripts produced from the non-coding strand (also antisense strand and template strand) of the C9ORF72 gene. The C9ORF72 antisense transcript differs from the canonically transcribed "C9ORF72 sense transcript", which is produced from the coding strand (also sense strand) of the C9ORF72 gene. In certain embodiments, a C9ORF72 antisense transcript is SEQ ID NO: 18.

"C9ORF72 antisense transcript specific inhibitor" refers to any agent capable of specifically inhibiting the expression of C9ORF72 antisense transcript and/or its expression products at the molecular level. As used herein, "specific" means reducing or inhibiting expression of C9ORF72 antisense transcript without reducing non-target transcript to an appreciable degree (e.g., a C9ORF72 antisense transcript specific inhibitor reduces expression of C9ORF72 antisense transcript, but does not reduce expression of C9ORF72 sense transcript to an appreciable degree). C9ORF72 specific antisense transcript inhibitors include antisense compounds, siRNAs, aptamers, antibodies, peptides, small molecules, and other agents capable of inhibiting the expression of

C9ORF72 antisense transcript and/or its expression products, such as C9ORF72 antisense transcript associated RAN translation products.

"C9ORF72 associated disease" means any disease associated with any C9ORF72 nucleic acid or expression product thereof. Such diseases may include a neurodegenerative disease. Such neurodegenerative diseases may include ALS and FTD. In certain embodiments, the C9ORF72 associated disease is caused by (or is associated with) a hexanucleotide repeat expansion. In certain embodiments, the hexanucleotide repeat expansion may comprise GGGGCC, GGGGGG, GGGGGC, or GGGGCG repeated at least 30 times, more than 30 times, more than 100 times, more than 500 times, or more than 1000 times.

"C9ORF72 associated RAN translation products" means aberrant peptide or di-peptide polymers translated through RAN translation (i.e., repeat-associated, and non-ATG-dependent translation). In certain embodiments, the C9ORF72 associated RAN translation products are any of poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine).

"C9ORF72 nucleic acid" means any nucleic acid encoding C9ORF72. For example, in certain embodiments, a C9ORF72 nucleic acid includes a DNA sequence encoding C9ORF72, an RNA sequence transcribed from DNA encoding C9ORF72 including genomic DNA comprising introns and exons (i.e., pre-mRNA), and an mRNA sequence encoding C9ORF72. "C9ORF72 mRNA" means an mRNA encoding a C9ORF72 protein.

"C9ORF72 pathogenic associated mRNA variant" means the C9ORF72 mRNA variant processed from a C9ORF72 pre-mRNA variant containing the hexanucleotide repeat. A C9ORF72 pre-mRNA contains the hexanucleotide repeat when transcription of the pre-mRNA begins in the region from the start site of exon 1A to the start site of exon 1B, e.g., nucleotides 1107 to 1520 of the genomic sequence (SEQ ID NO: 2, the complement of GENBANK Accession No. NT_008413.18 truncated from nucleosides 27535000 to 27565000). In certain embodiments, the level of a C9ORF72 pathogenic associated mRNA variant is measured to determine the level of a C9ORF72 pre-mRNA containing the hexanucleotide repeat in a sample.

"C9ORF72 specific inhibitor" refers to any agent capable of specifically inhibiting the expression of C9ORF72 mRNA and/or C9ORF72 protein at the molecular level. For example, C9ORF72 specific inhibitors include nucleic acids (including antisense compounds), siRNAs, aptamers, antibodies, peptides, small molecules, and other agents capable of inhibiting the expression of C9ORF72 mRNA and/or C9ORF72 protein. Similarly, in certain embodiments, C9ORF72 specific inhibitors may affect other molecular processes in an animal.

"Cap structure" or "terminal cap moiety" means chemical modifications, which have been incorporated at either terminus of an antisense compound.

"cEt" or "constrained ethyl" means a bicyclic nucleoside having a sugar moiety comprising a bridge connecting the 4'-carbon and the 2'-carbon, wherein the bridge has the formula: 4'-$CH(CH_3)$-O-2'.

"Constrained ethyl nucleoside" (also cEt nucleoside) means a nucleoside comprising a bicyclic sugar moiety comprising a 4'-$CH(CH_3)$-O-2' bridge.

"Chemically distinct region" refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleosides is chemically distinct from a region having nucleosides without 2'-O-methoxyethyl modifications.

"Chimeric antisense compound" means an antisense compound that has at least two chemically distinct regions, each position having a plurality of subunits.

"Co-administration" means administration of two or more pharmaceutical agents to an individual. The two or more pharmaceutical agents may be in a single pharmaceutical composition, or may be in separate pharmaceutical compositions. Each of the two or more pharmaceutical agents may be administered through the same or different routes of administration. Co-administration encompasses parallel or sequential administration.

"Complementarity" means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

"Comprise," "comprises," and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

"Contiguous nucleobases" means nucleobases immediately adjacent to each other.

"Designing" or"designed to" refer to the process of designing an oligomeric compound that specifically hybridizes with a selected nucleic acid molecule.

"Diluent" means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, in drugs that are injected, the diluent may be a liquid, e.g. saline solution.

"Dose" means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time period. In certain embodiments, a dose may be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where

subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections may be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period of time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week, or month.

"Effective amount" in the context of modulating an activity or of treating or preventing a condition means the administration of that amount of pharmaceutical agent to a subject in need of such modulation, treatment, or prophylaxis, either in a single dose or as part of a series, that is effective for modulation of that effect, or for treatment or prophylaxis or improvement of that condition. The effective amount may vary among individuals depending on the health and physical condition of the individual to be treated, the taxonomic group of the individuals to be treated, the formulation of the composition, assessment of the individual's medical condition, and other relevant factors.

"Efficacy" means the ability to produce a desired effect.

"Expression" includes all the functions by which a gene's coded information is converted into structures present and operating in a cell. Such structures include, but are not limited to the products of transcription and translation.

"Focus" or "foci" means a nuclear or cytoplasmic body comprising a C9ORF72 transcript. In certain embodiments, a focus comprises at least one C9ORF72 transcript. In certain embodiments, C9ORF72 foci comprise transcripts comprising any of the following hexanucleotide repeats: GGGGCC, GGGGGG, GGGGGC, and/or GGGGCG.

"Fully complementary" or "100% complementary" means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. In certain embodiments, a first nucleic acid is an antisense compound and a target nucleic acid is a second nucleic acid.

"Gapmer" means a chimeric antisense compound in which an internal region having a plurality of nucleosides that support RNase H cleavage is positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region may be referred to as a "gap" and the external regions may be referred to as the "wings."

"Hexanucleotide repeat expansion" means a series of six bases (for example, GGGGCC, GGGGGG, GGGGCG, or GGGGGC) repeated at least twice. In certain embodiments, the hexanucleotide repeat expansion may be located in intron 1 of a C9ORF72 nucleic acid. In certain

embodiments, a pathogenic hexanucleotide repeat expansion includes at least 30, more than 30, more than 100, more than 500, or more than 1000 repeats of GGGGCC, GGGGGG, GGGGCG, or GGGGGC in a C9ORF72 nucleic acid and is associated with disease. In certain embodiments, the repeats are consecutive. In certain embodiments, the repeats are interrupted by 1 or more nucleobases. In certain embodiments, a wild-type hexanucleotide repeat expansion includes 23 or fewer repeats of GGGGCC, GGGGGG, GGGGCG, or GGGGGC in a C9ORF72 nucleic acid. In certain embodiments, the repeats are consecutive. In certain embodiments, the repeats are interrupted by 1 or more nucleobases.

"Hybridization" means the annealing of complementary nucleic acid molecules. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense compound and a target nucleic acid. In certain embodiments, complementary nucleic acid molecules include, but are not limited to, an antisense oligonucleotide and a nucleic acid target.

"Identifying an animal having a C9ORF72 associated disease" means identifying an animal having been diagnosed with a C9ORF72 associated disease or predisposed to develop a C9ORF72 associated disease. Individuals predisposed to develop a C9ORF72 associated disease include those having one or more risk factors for developing a C9ORF72 associated disease, including, having a personal or family history or genetic predisposition of one or more C9ORF72 associated diseases. Such identification may be accomplished by any method including evaluating an individual's medical history and standard clinical tests or assessments, such as genetic testing.

"Immediately adjacent" means there are no intervening elements between the immediately adjacent elements.

"Individual" means a human or non-human animal selected for treatment or therapy.

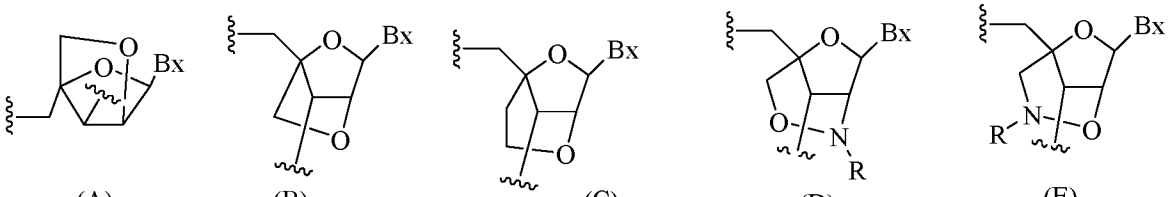
"Inhibiting C9ORF72" means reducing the level or expression of a C9ORF72 mRNA and/or protein. In certain embodiments, C9ORF72 mRNA and/or protein levels are inhibited in the presence of an antisense compound targeting C9ORF72, including an antisense oligonucleotide targeting C9ORF72, as compared to expression of C9ORF72 mRNA and/or protein levels in the absence of a C9ORF72 antisense compound, such as an antisense oligonucleotide.

"Inhibiting the expression or activity" refers to a reduction or blockade of the expression or activity and does not necessarily indicate a total elimination of expression or activity.

"Internucleoside linkage" refers to the chemical bond between nucleosides.

"Linked nucleosides" means adjacent nucleosides linked together by an internucleoside linkage.

"Locked nucleic acid" or " LNA" or "LNA nucleosides" means nucleic acid monomers having a bridge connecting two carbon atoms between the 4' and 2'position of the nucleoside sugar unit, thereby forming a bicyclic sugar. Examples of such bicyclic sugar include, but are not limited to A) α-L-Methyleneoxy (4'-CH_2-O-2') LNA , (B) β-D-Methyleneoxy (4'-CH_2-O-2') LNA , (C) Ethyleneoxy (4'-$(CH_2)_2$-O-2') LNA , (D) Aminooxy (4'-CH_2-O-N(R)-2') LNA and (E) Oxyamino (4'-CH_2-N(R)-O-2') LNA, as depicted below.



(A) (B) (C) (D) (E)

As used herein, LNA compounds include, but are not limited to, compounds having at least one bridge between the 4' and the 2' position of the sugar wherein each of the bridges independently comprises 1 or from 2 to 4 linked groups independently selected from -$[C(R_1)(R_2)]_n$-, -$C(R_1)$=$C(R_2)$-, -$C(R_1)$=N-, -C(=NR_1)-, -C(=O)-, -C(=S)-, -O-, -$Si(R_1)_2$-, -$S(=O)_x$- and -$N(R_1)$-; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R_1 and R_2 is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, a heterocycle radical, a substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl (C(=O)-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl (S(=O)-J_1); and each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl (C(=O)-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

Examples of 4'- 2' bridging groups encompassed within the definition of LNA include, but are not limited to one of formulae: -$[C(R_1)(R_2)]_n$-, -$[C(R_1)(R_2)]_n$-O-, -$C(R_1R_2)$-$N(R_1)$-O- or –$C(R_1R_2)$-O-$N(R_1)$-. Furthermore, other bridging groups encompassed with the definition of LNA are 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-$N(R_1)$-2' and 4'-CH_2-$N(R_1)$-O-2'- bridges, wherein each R_1 and R_2 is, independently, H, a protecting group or C_1-C_{12} alkyl.

Also included within the definition of LNA according to the invention are LNAs in which the 2'-hydroxyl group of the ribosyl sugar ring is connected to the 4' carbon atom of the sugar ring,

thereby forming a methyleneoxy (4'-CH_2-O-2') bridge to form the bicyclic sugar moiety. The bridge can also be a methylene (-CH_2-) group connecting the 2' oxygen atom and the 4' carbon atom, for which the term methyleneoxy (4'-CH_2-O-2') LNA is used. Furthermore; in the case of the bicylic sugar moiety having an ethylene bridging group in this position, the term ethyleneoxy (4'-CH_2CH_2-O-2') LNA is used. α -L- methyleneoxy (4'-CH_2-O-2'), an isomer of methyleneoxy (4'-CH_2-O-2') LNA is also encompassed within the definition of LNA, as used herein.

"Mismatch" or "non-complementary nucleobase" refers to the case when a nucleobase of a first nucleic acid is not capable of pairing with the corresponding nucleobase of a second or target nucleic acid.

"Modified internucleoside linkage" refers to a substitution or any change from a naturally occurring internucleoside bond (*i.e.*, a phosphodiester internucleoside bond).

"Modified nucleobase" means any nucleobase other than adenine, cytosine, guanine, thymidine, or uracil. An "unmodified nucleobase" means the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U).

"Modified nucleoside" means a nucleoside having, independently, a modified sugar moiety and/or modified nucleobase.

"Modified nucleotide" means a nucleotide having, independently, a modified sugar moiety, modified internucleoside linkage, and/or modified nucleobase.

"Modified oligonucleotide" means an oligonucleotide comprising at least one modified internucleoside linkage, modified sugar, and/or modified nucleobase.

"Modified sugar" means substitution and/or any change from a natural sugar moiety.

"Monomer" means a single unit of an oligomer. Monomers include, but are not limited to, nucleosides and nucleotides, whether naturally occurring or modified.

"Motif" means the pattern of unmodified and modified nucleoside in an antisense compound.

"Natural sugar moiety" means a sugar moiety found in DNA (2'-H) or RNA (2'-OH).

"Naturally occurring internucleoside linkage" means a 3' to 5' phosphodiester linkage.

"Non-complementary nucleobase" refers to a pair of nucleobases that do not form hydrogen bonds with one another or otherwise support hybridization.

"Nucleic acid" refers to molecules composed of monomeric nucleotides. A nucleic acid includes, but is not limited to, ribonucleic acids (RNA), deoxyribonucleic acids (DNA), single-stranded nucleic acids, double-stranded nucleic acids, small interfering ribonucleic acids (siRNA), and microRNAs (miRNA).

"Nucleobase" means a heterocyclic moiety capable of pairing with a base of another nucleic acid.

"Nucleobase complementarity" refers to a nucleobase that is capable of base pairing with another nucleobase. For example, in DNA, adenine (A) is complementary to thymine (T). For example, in RNA, adenine (A) is complementary to uracil (U). In certain embodiments, complementary nucleobase refers to a nucleobase of an antisense compound that is capable of base pairing with a nucleobase of its target nucleic acid. For example, if a nucleobase at a certain position of an antisense compound is capable of hydrogen bonding with a nucleobase at a certain position of a target nucleic acid, then the position of hydrogen bonding between the oligonucleotide and the target nucleic acid is considered to be complementary at that nucleobase pair.

"Nucleobase sequence" means the order of contiguous nucleobases independent of any sugar, linkage, and/or nucleobase modification.

"Nucleoside" means a nucleobase linked to a sugar.

"Nucleoside mimetic" includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo, or tricyclo sugar mimetics, *e.g.*, non furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by -N(H)-C(=O)-O- or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system. "Mimetic" refers to groups that are substituted for a sugar, a nucleobase, and/or internucleoside linkage. Generally, a mimetic is used in place of the sugar or sugar-internucleoside linkage combination, and the nucleobase is maintained for hybridization to a selected target.

"Nucleotide" means a nucleoside having a phosphate group covalently linked to the sugar portion of the nucleoside.

"Off-target effect" refers to an unwanted or deleterious biological effect assocaited with modulation of RNA or protein expression of a gene other than the intended target nucleic acid.

"Oligomeric compound" or "oligomer" means a polymer of linked monomeric subunits which is capable of hybridizing to at least a region of a nucleic acid molecule.

"Oligonucleotide" means a polymer of linked nucleosides each of which can be modified or unmodified, independent one from another.

"Parenteral administration" means administration through injection (e.g., bolus injection) or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g., intrathecal or intracerebroventricular administration.

"Peptide" means a molecule formed by linking at least two amino acids by amide bonds. Without limitation, as used herein, peptide refers to polypeptides and proteins.

"Pharmaceutical agent" means a substance that provides a therapeutic benefit when administered to an individual. For example, in certain embodiments, an antisense oligonucleotide targeted to C9ORF72 is a pharmaceutical agent.

"Pharmaceutically acceptable derivative" encompasses pharmaceutically acceptable salts, conjugates, prodrugs or isomers of the compounds described herein.

"Pharmaceutically acceptable salts" means physiologically and pharmaceutically acceptable salts of antisense compounds, *i.e.*, salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

"Phosphorothioate linkage" means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom. A phosphorothioate linkage is a modified internucleoside linkage.

"Portion" means a defined number of contiguous (*i.e.*, linked) nucleobases of a nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of a target nucleic acid. In certain embodiments, a portion is a defined number of contiguous nucleobases of an antisense compound.

"Prevent" or "preventing" refers to delaying or forestalling the onset or development of a disease, disorder, or condition for a period of time from minutes to days, weeks to months, or indefinitely.

"Prodrug" means a therapeutic agent that is prepared in an inactive form that is converted to an active form within the body or cells thereof by the action of endogenous enzymes or other chemicals or conditions.

"Prophylactically effective amount" refers to an amount of a pharmaceutical agent that provides a prophylactic or preventative benefit to an animal.

"Region" is defined as a portion of the target nucleic acid having at least one identifiable structure, function, or characteristic.

"Ribonucleotide" means a nucleotide having a hydroxy at the 2' position of the sugar portion of the nucleotide. Ribonucleotides may be modified with any of a variety of substituents.

"Salts" mean a physiologically and pharmaceutically acceptable salts of antisense compounds, i.e., salts that retain the desired biological activity of the parent oligonucleotide and do not impart undesired toxicological effects thereto.

"Segments" are defined as smaller or sub-portions of regions within a target nucleic acid.

"Shortened" or "truncated" versions of antisense oligonucleotides taught herein have one, two or more nucleosides deleted.

"Side effects" means physiological responses attributable to a treatment other than desired effects. In certain embodiments, side effects include, without limitation, injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, and myopathies.

"Single-stranded oligonucleotide" means an oligonucleotide which is not hybridized to a complementary strand. A "single-stranded modified oligonucleotide" means a modified oligonucleotide which is not hybridized to a complementary strand.

"Sites," as used herein, are defined as unique nucleobase positions within a target nucleic acid.

"Slows progression" means decrease in the development of the disease.

"Specifically hybridizable" refers to an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays and therapeutic treatments.

"Stringent hybridization conditions" or "stringent conditions" refer to conditions under which an oligomeric compound will hybridize to its target sequence, but to a minimal number of other sequences.

"Subject" means a human or non-human animal selected for treatment or therapy.

"Target" refers to a protein, the modulation of which is desired.

"Target gene" refers to a gene encoding a target.

"Targeting" or "targeted" means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

"Target nucleic acid," "target RNA," and "target RNA transcript" and "nucleic acid target" all mean a nucleic acid capable of being targeted by antisense compounds.

"Target region" means a portion of a target nucleic acid to which one or more antisense compounds is targeted.

"Target segment" means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. "5' target site" refers to the 5'-most nucleotide of a target segment. "3' target site" refers to the 3'-most nucleotide of a target segment.

"Therapeutically effective amount" means an amount of a pharmaceutical agent that provides a therapeutic benefit to an individual.

"Treat" or "treating" or "treatment" means administering a composition to effect an alteration or improvement of a disease or condition.

"Unmodified nucleobases" means the purine bases adenine (A) and guanine (G), and the pyrimidine bases (T), cytosine (C), and uracil (U).

"Unmodified nucleotide" means a nucleotide composed of naturally occuring nucleobases, sugar moieties, and internucleoside linkages. In certain embodiments, an unmodified nucleotide is an RNA nucleotide (*i.e.* β-D-ribonucleosides) or a DNA nucleotide (*i.e.* β-D-deoxyribonucleoside).

"Wing segment" means a plurality of nucleosides modified to impart to an oligonucleotide properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by in vivo nucleases.

Certain Embodiments

Certain embodiments provide compositions and methods for reducing total C9ORF72 mRNA and protein expression.

Certain embodiments provide compositions and methods for reducing C9ORF72 pathogenic associated mRNA variants.

Certain embodiments provide methods for the treatment, prevention, amelioration, or slowing progression of diseases associated with C9ORF72 in an individual in need thereof. Also contemplated are methods for the preparation of a medicament for the treatment, prevention, or amelioration of a disease associated with C9ORF72. C9ORF72 associated diseases include

neurodegenerative diseases. In certain embodiments, the neurodegenerative disease may be ALS or FTD. In certain embodiments, the neurodegenerative disease may be familial or sporadic.

The present disclosure provided the following non-limiting numbered embodiments:

Embodiment 1. A compound comprising a modified oligonucleotide consisting of 12 to 30 linked nucleosides and having a nucleobase sequence comprising at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, or at least 20 consecutive nucleobases of any of the nucleobase sequences of SEQ ID NOs: 22-55.

Embodiment 2. The compound of embodiment 1, wherein the nucleobase sequence of the modified oligonucleotide is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to SEQ ID NO: 1 or SEQ ID NO: 2.

Embodiment 3. The compound of embodiments 1 and 2, wherein the modified oligonucleotide is a single-stranded modified oligonucleotide.

Embodiment 4. The compound of any of embodiments 1-3, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

Embodiment 5. The compound of embodiment 4, wherein the at least one modified internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 6. The compound of embodiments 4 and 5, wherein the modified oligonucleotide comprises at least one phosphodiester linkage.

Embodiment 7. The compound of embodiment 5, wherein each modified internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 8. The compound of any of embodiments 1-7, wherein at least one nucleoside comprises a modified nucleobase.

Embodiment 9. The compound of embodiment 8, wherein the modified nucleobase is a 5-methylcytosine.

Embodiment 10. The compound of any of embodiments 1-9, wherein at least one nucleoside of the modified oligonucleotide comprises a modified sugar.

Embodiment 11. The compound of embodiment 10, wherein each nucleoside of the modified oligonucleotide comprises a modified sugar.

Embodiment 12. The compound of embodiments 10 or 11, wherein the at least one modified sugar is a bicyclic sugar.

Embodiment 13. The compound of embodiment 12, wherein the bicyclic sugar comprises a chemical bridge between the 4' and 2' positions of the sugar, wherein the chemical bridge is selected from: 4'-CH(R)-O-2' and 4'-$(CH_2)_2$-O-2', wherein R is independently H, C_1-C_6 alkyl, and C_1-C_6 alkoxy.

Embodiment 14. The compound of embodiment 13, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is methyl.

Embodiment 15. The compound of embodiment 13, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is H.

Embodiment 16. The compound of embodiment 13, wherein the chemical bridge is 4'-CH(R)-O-2' and wherein R is $–CH_2$-O-CH_3.

Embodiment 17. The compound of embodiments 10 or 11, wherein the at least one modified sugar comprises a 2'-O-methoxyethyl group.

Embodiment 18. The compound of any of embodiments 1-10 or 12-16, wherein the modified oligonucleotide is a gapmer.

Embodiment 19. The compound of embodiment 18, wherein the gapmer is any of a 3-8-7 MOE gapmer, a 3-10-7 MOE gapmer, a 4-8-6 MOE gapmer, a 4-10-6 MOE gapmer, a 6-10-4 MOE gapmer, a 6-8-4 MOE gapmer, a 7-8-3 MOE gapmer, or a 7-10-3 MOE gapmer.

Embodiment 20. The compound of claim 5, wherein the modified oligonucleotide comprises internucleoside linkages in any of the following patterns: soossssssssssooooss, sooossssssssssooooss, sooooossssssssssoss, soooooossssssssssss, sooooossssssssooss, sooossssssssssooooss, soooooossssssssssoss, soossssssssssooooss, sooooossssssssssoss, sosssssssssooooss, or soooooossssssssss, wherein,

s = a phosphorothioate linkage, and

o = a phosphodiester linkage.

Embodiment 19. A composition comprising the compound of any preceding embodiment or salt thereof and at least one of a pharmaceutically acceptable carrier or diluent.

Embodiment 20. The composition of embodiment 19, further comprising a C9ORF72 antisense transcript specific inhibitor.

Embodiment 21. The composition of embodiment 20, wherein the C9ORF72 antisense transcript specific inhibitor is an antisense compound.

Embodiment 22. The composition of embodiment 21, wherein the antisense compound is a modified oligonucleotide.

Embodiment 23. The composition of embodiment 22, wherein the modified oligonucleotide is single-stranded.

Embodiment 24. The composition of embodiments 22 or 23, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% complementary to a C9ORF72 antisense transcript.

Embodiment 25. The composition of embodiment 24, wherein the C9ORF72 antisense transcript has the nucleobase sequence of SEQ ID NO: 18.

Embodiment 26. A method comprising administering to an animal the compound or composition of any preceding embodiment.

Embodiment 27. The method of embodiment 26, wherein the animal is a human.

Embodiment 28. The method of embodiments 26 and 27, wherein administering the compound prevents, treats, ameliorates, or slows progression of a C9ORF72 associated disease.

Embodiment 29. The method of embodiment 28, wherein the C9ORF72 associated disease is caused by a hexanucleotide repeat expansion.

Embodiment 30. The method of embodiment 28, wherein the C9ORF72 associated disease is amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, and olivopontocerebellar degeneration (OPCD).

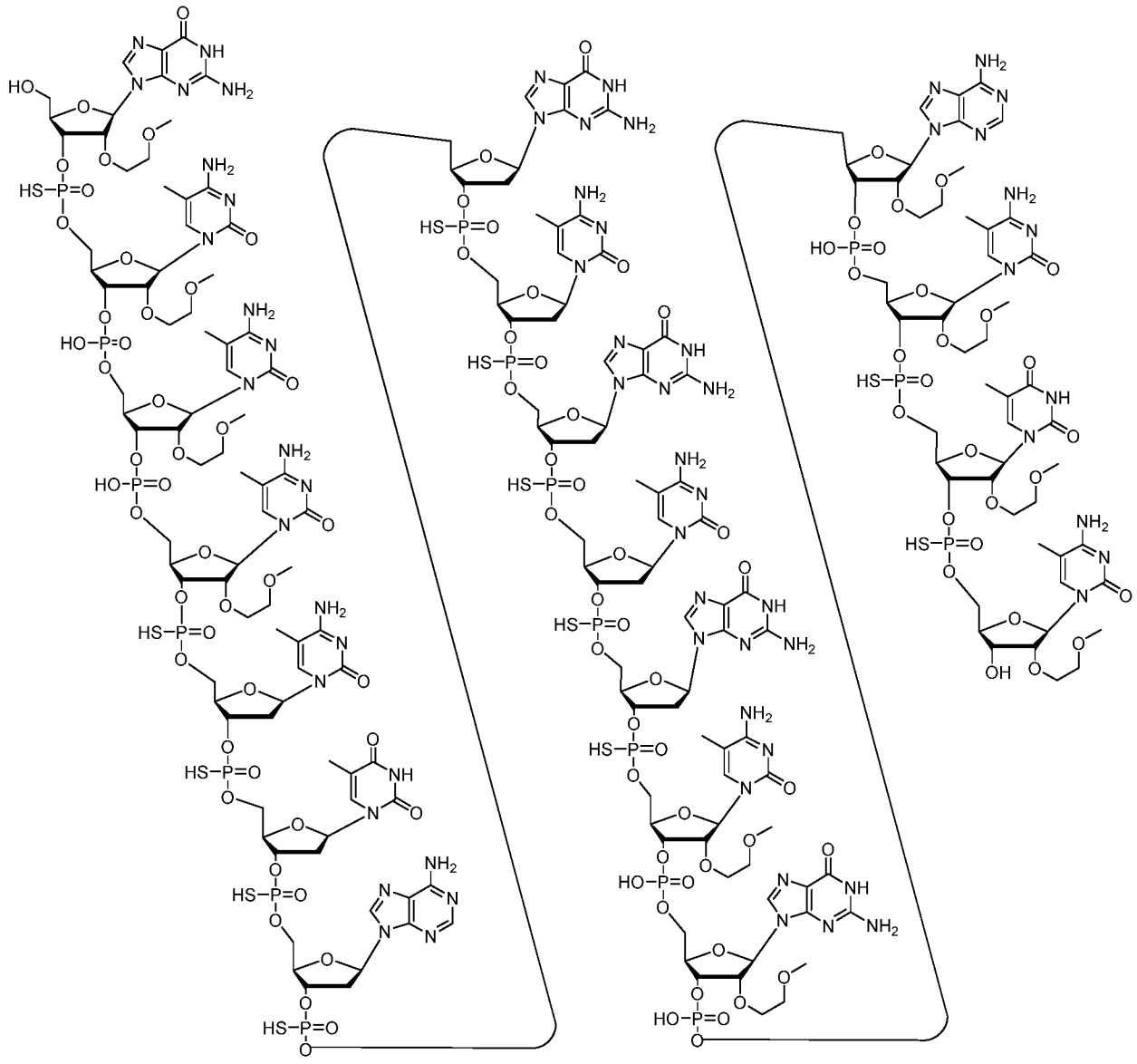
Embodiment 31. The method of embodiments 26-30, wherein the administering reduces nuclear foci.

Embodiment 32. The method of embodiments 26-31, wherein the administering reduces expression of C9ORF72 associated RAN translation products.

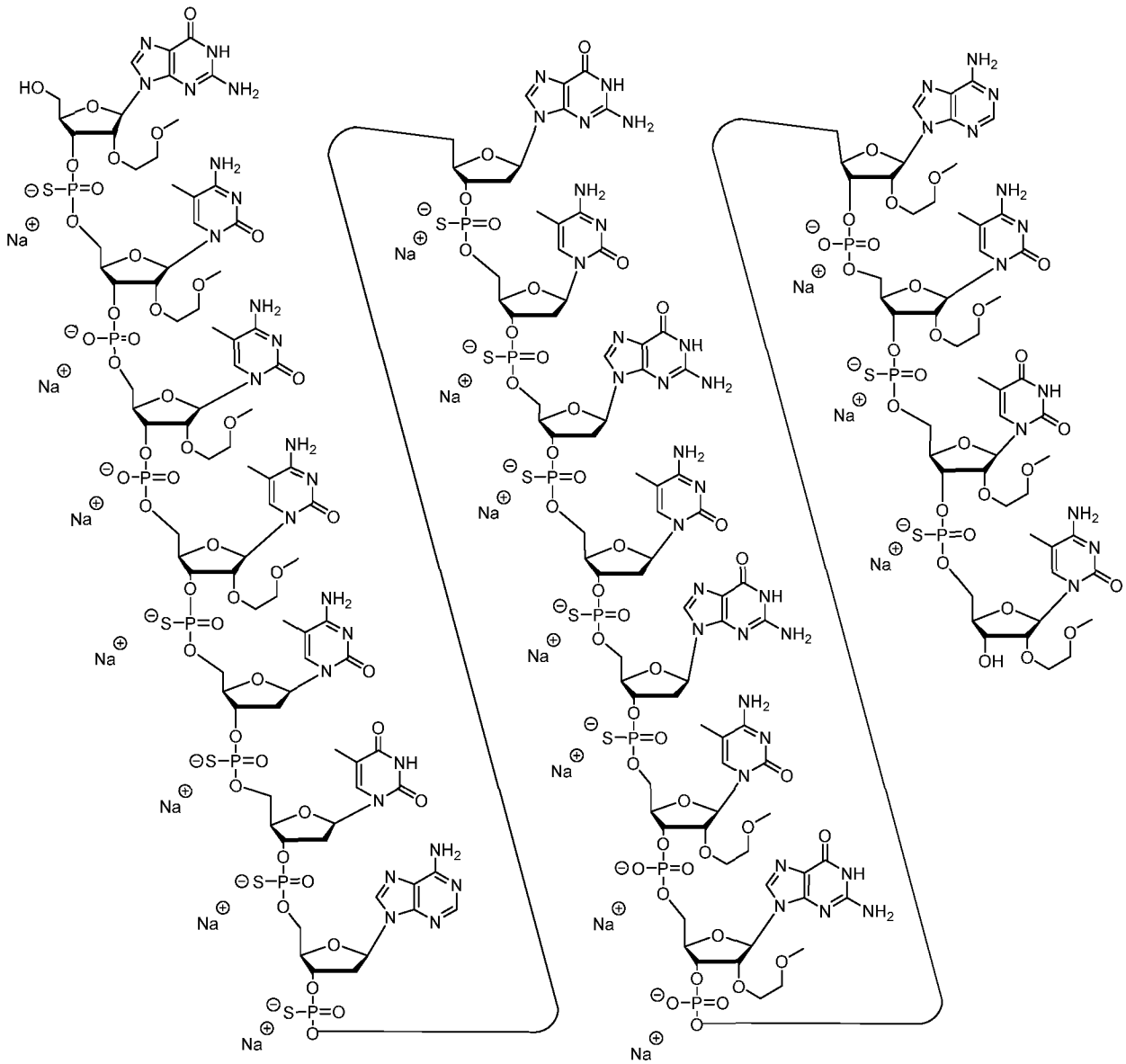
Embodiment 33. The method of embodiment 32, wherein the C9ORF72 associated RAN translation products are any of poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine).

Embodiment 34. Use of the compound or composition of any of embodiments 1-33 for the manufacture of a medicament for treating a neurodegenerative disorder.

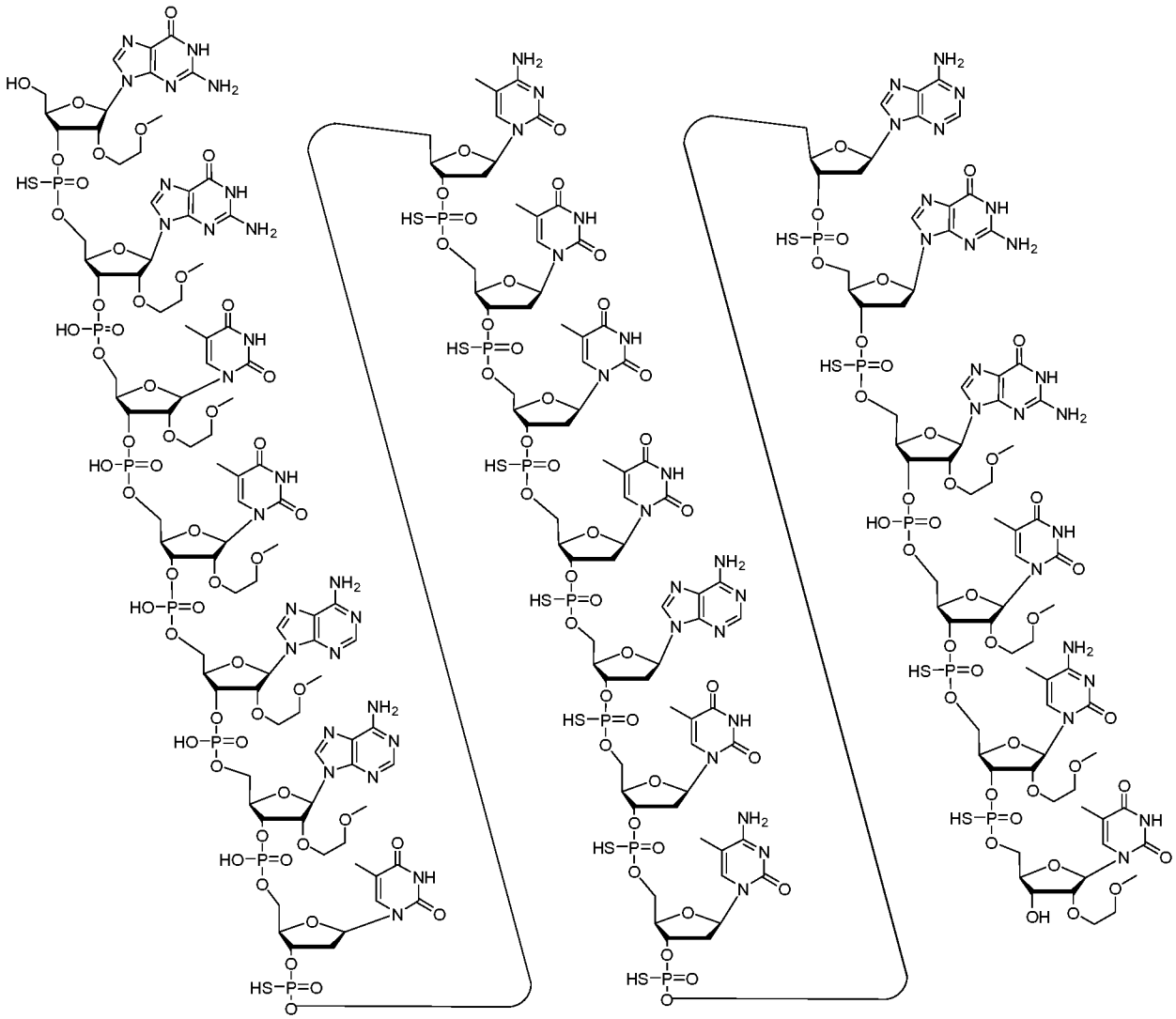
Embodiment 35. A compound consisting of a modified oligonucleotide according to the following formula, or a salt thereof:



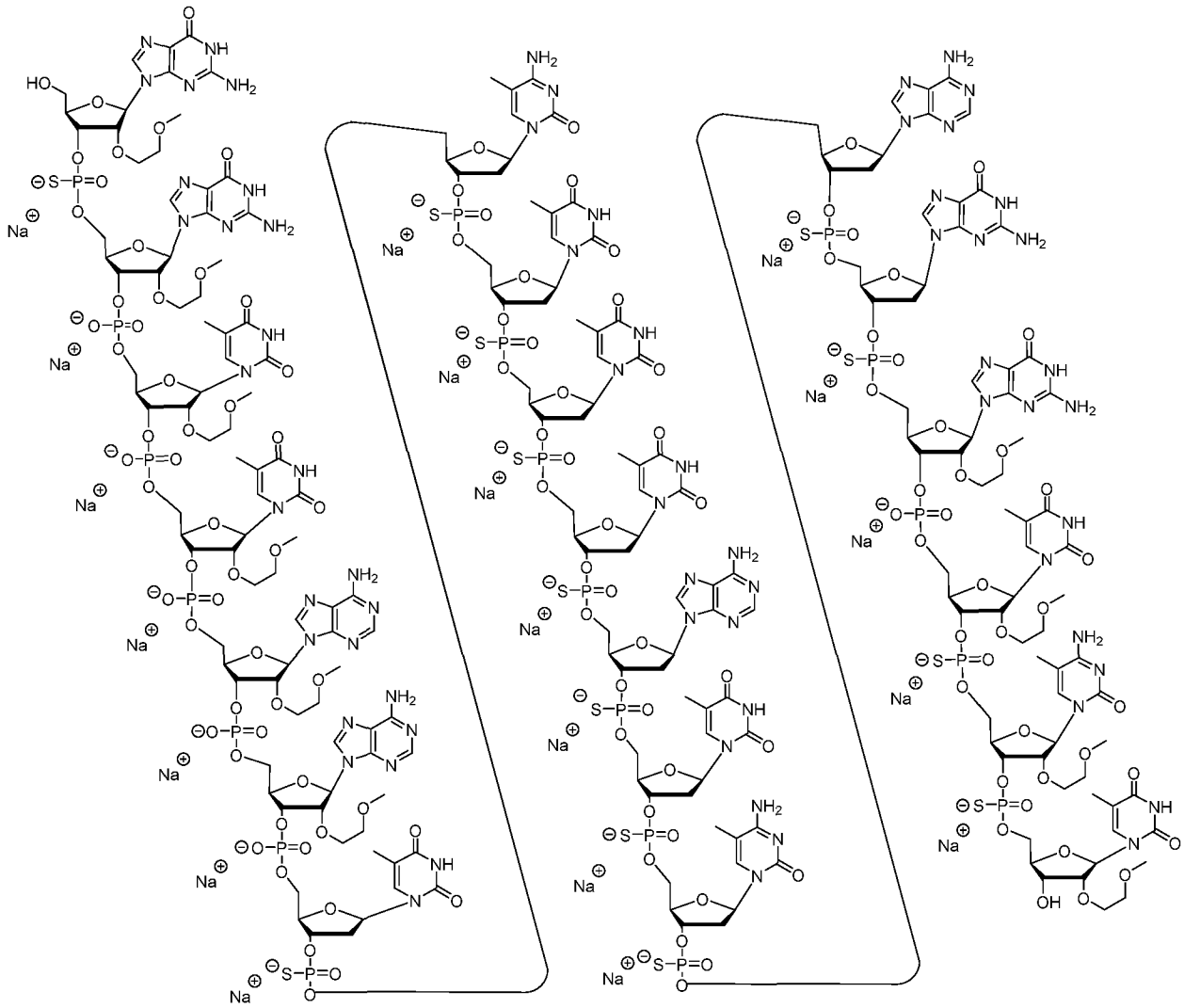
Embodiment 36. A composition consisting of the sodium salt of a modified oligonucleotide according to the following formula:



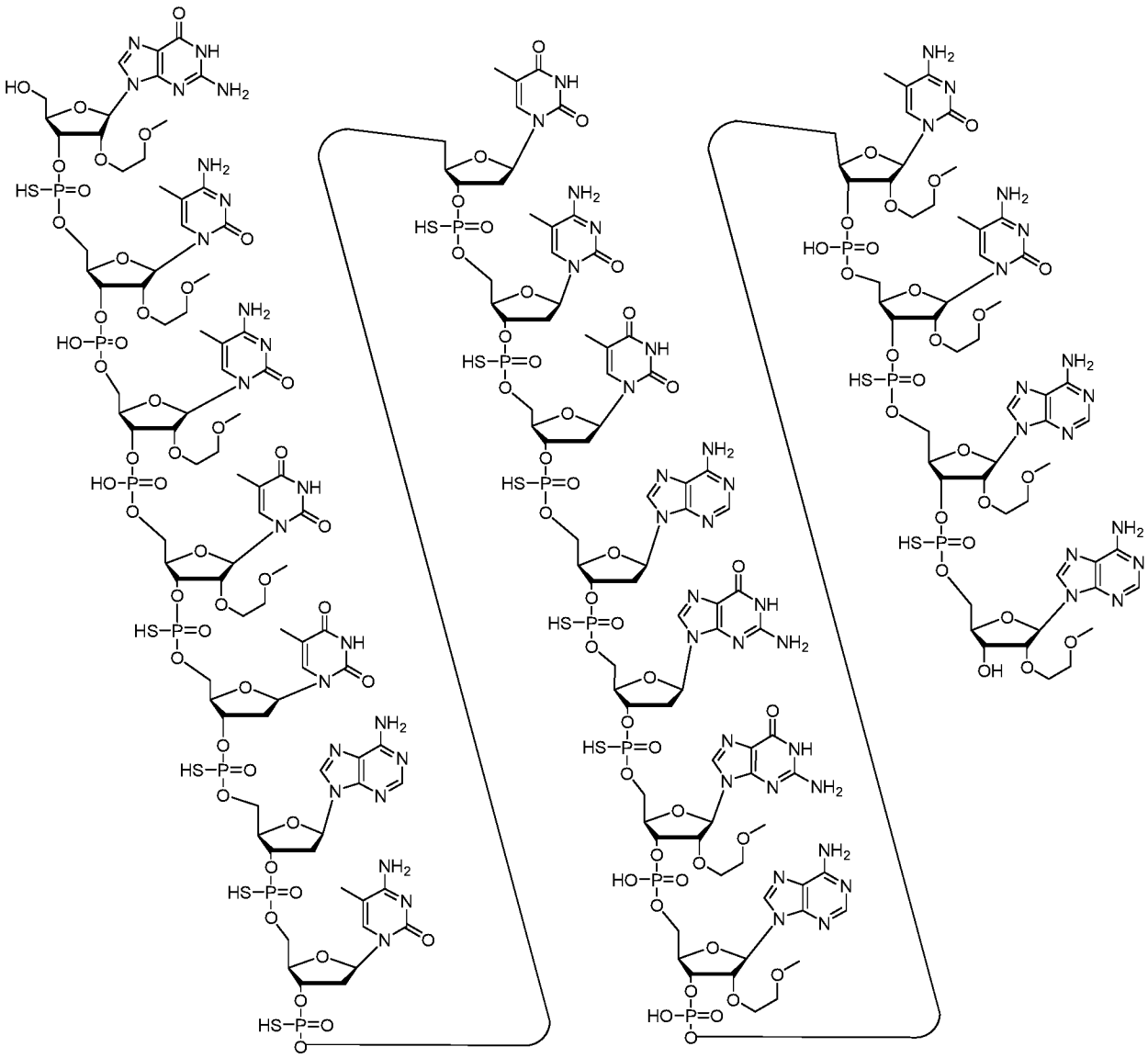
Embodiment 37. A compound consisting of a modified oligonucleotide according to the following formula, or a salt thereof:



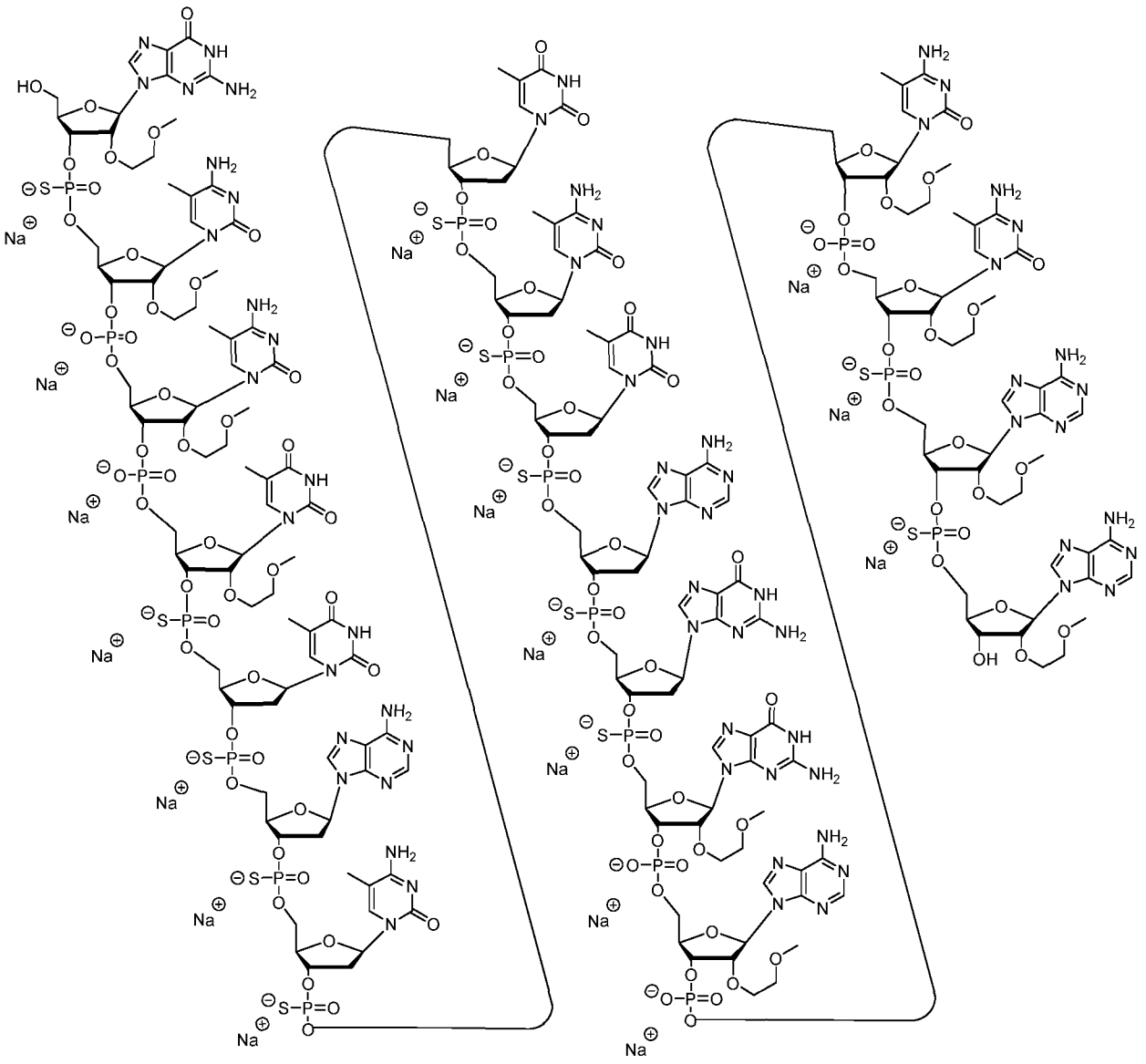
Embodiment 38. A composition consisting of the sodium salt of a modified oligonucleotide according to the following formula:



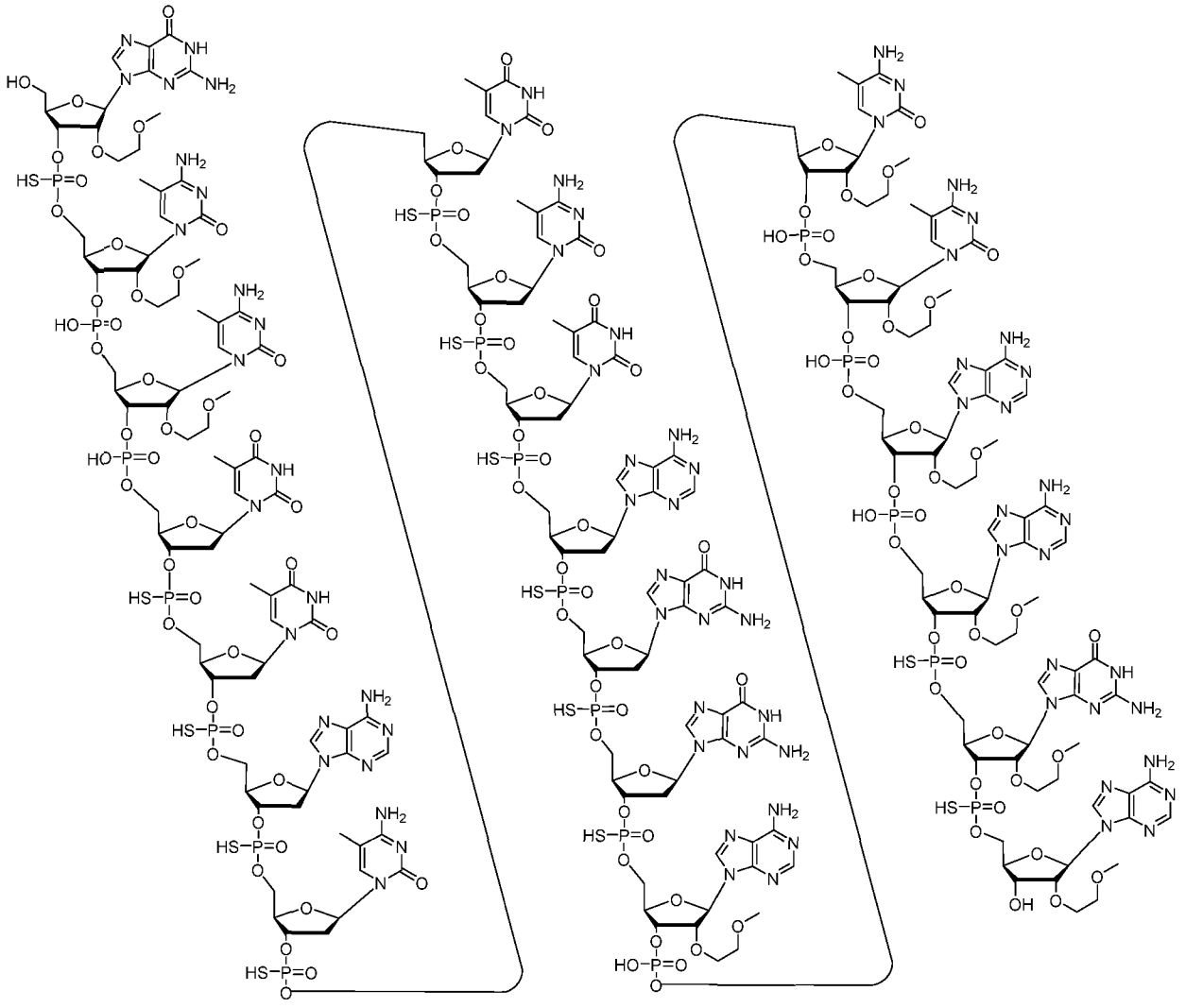
Embodiment 39. A compound consisting of a modified oligonucleotide according to the following formula, or a salt thereof:



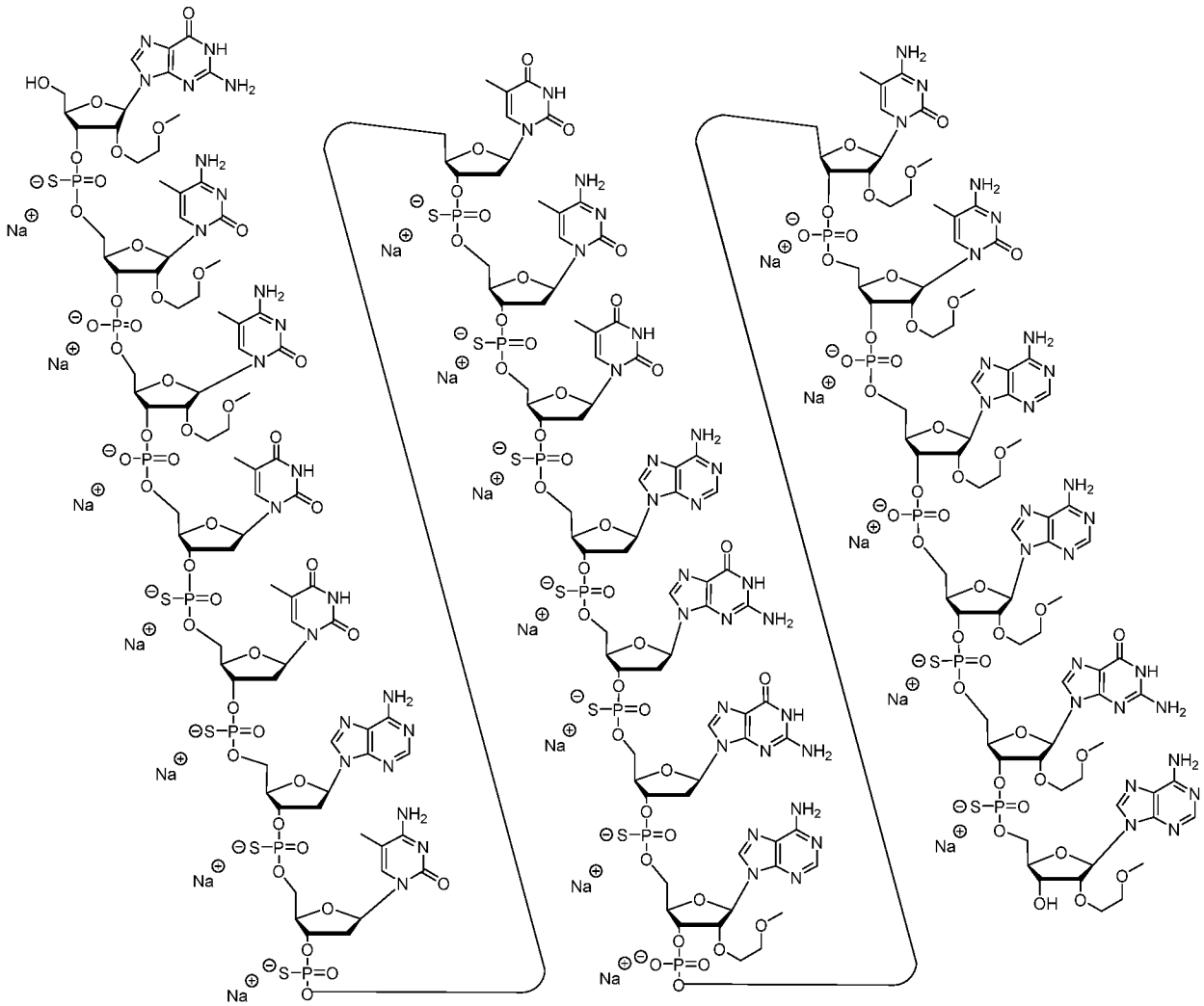
Embodiment 40. A composition consisting of the sodium salt of a modified oligonucleotide according to the following formula:



Embodiment 41. A compound consisting of a modified oligonucleotide according to the following formula, or a salt thereof:



Embodiment 42. A composition consisting of the sodium salt of a modified oligonucleotide according to the following formula:



Embodiment 43. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of reducing human C9ORF72 mRNA or protein expression in a mammal.

Embodiment 44. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of ameliorating at least one symptom of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

Embodiment 45. The compound or composition of embodiment 44, wherein the symptom of ALS is any of motor deficit, anxiety, and denervation.

Embodiment 46. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of delaying progression of disease.

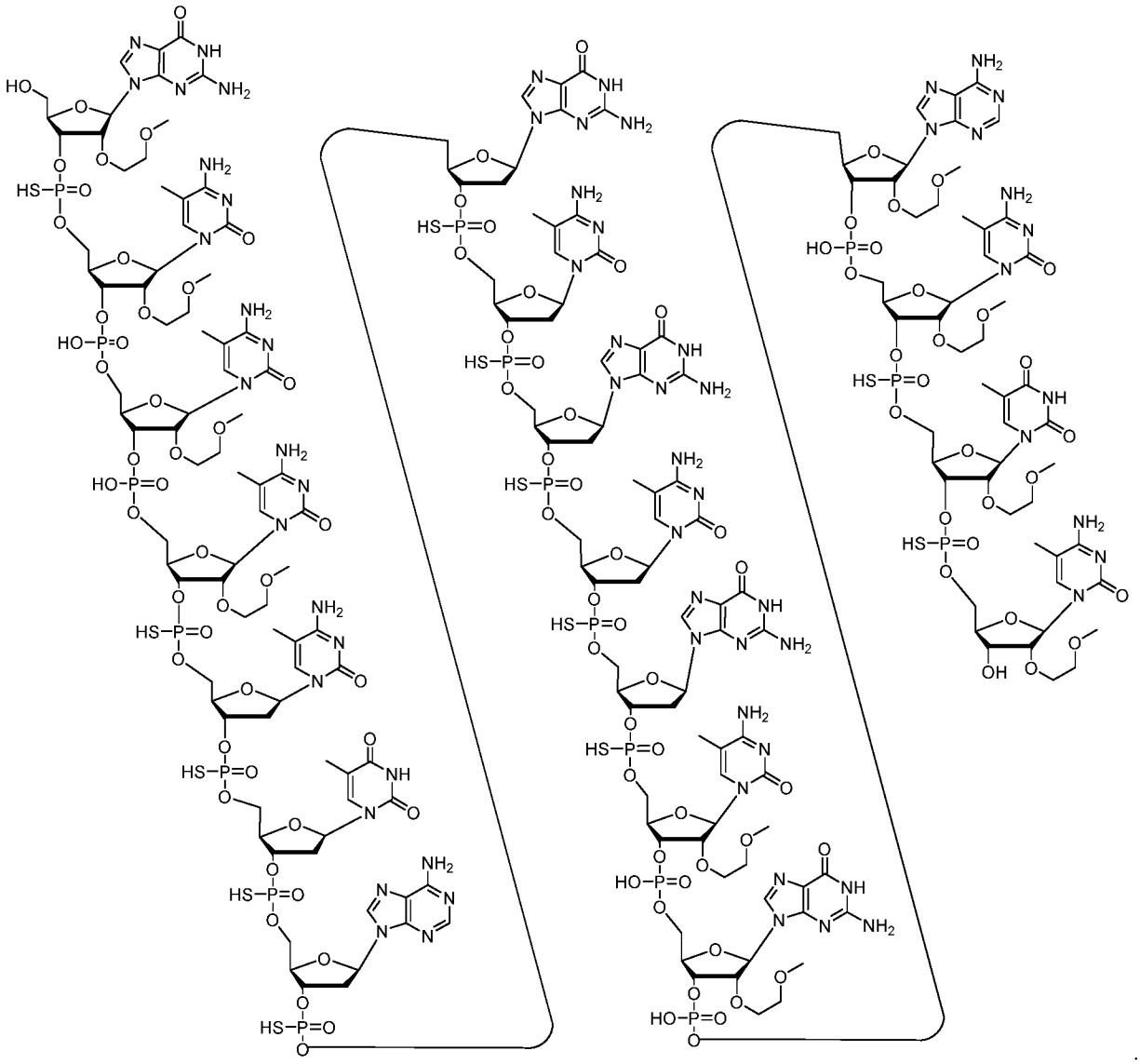
Embodiment 47. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of extending survival.

Embodiment 48. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of reducing C9ORF72 associated RAN translation products.

Embodiment 49. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of selectively reducing C9ORF72 pathogenic associated mRNA variants.

Embodiment 50. The compound or composition of any of embodiments 1-25 or 35-42, wherein the compound or composition is capable of reducing nuclear foci.

Embodiment 51. A compound consisting of a modified oligonucleotide according to the following formula, or a salt thereof:



Embodiment 52. A method comprising administering to an animal the compound of embodiment 51.

Embodiment 53. The method of embodiment 52, wherein the animal is a human.

Embodiment 54. The method of embodiment 53, wherein the administering inhibits C9ORF72.

Embodiment 55. The method of embodiment 53, wherein the administering prevents, treats, ameliorates, or slows progression of a C9ORF72 associated disease.

Embodiment 56. The method of embodiment 55, wherein the C9ORF72 associated disease is caused by a hexanucleotide repeat expansion.

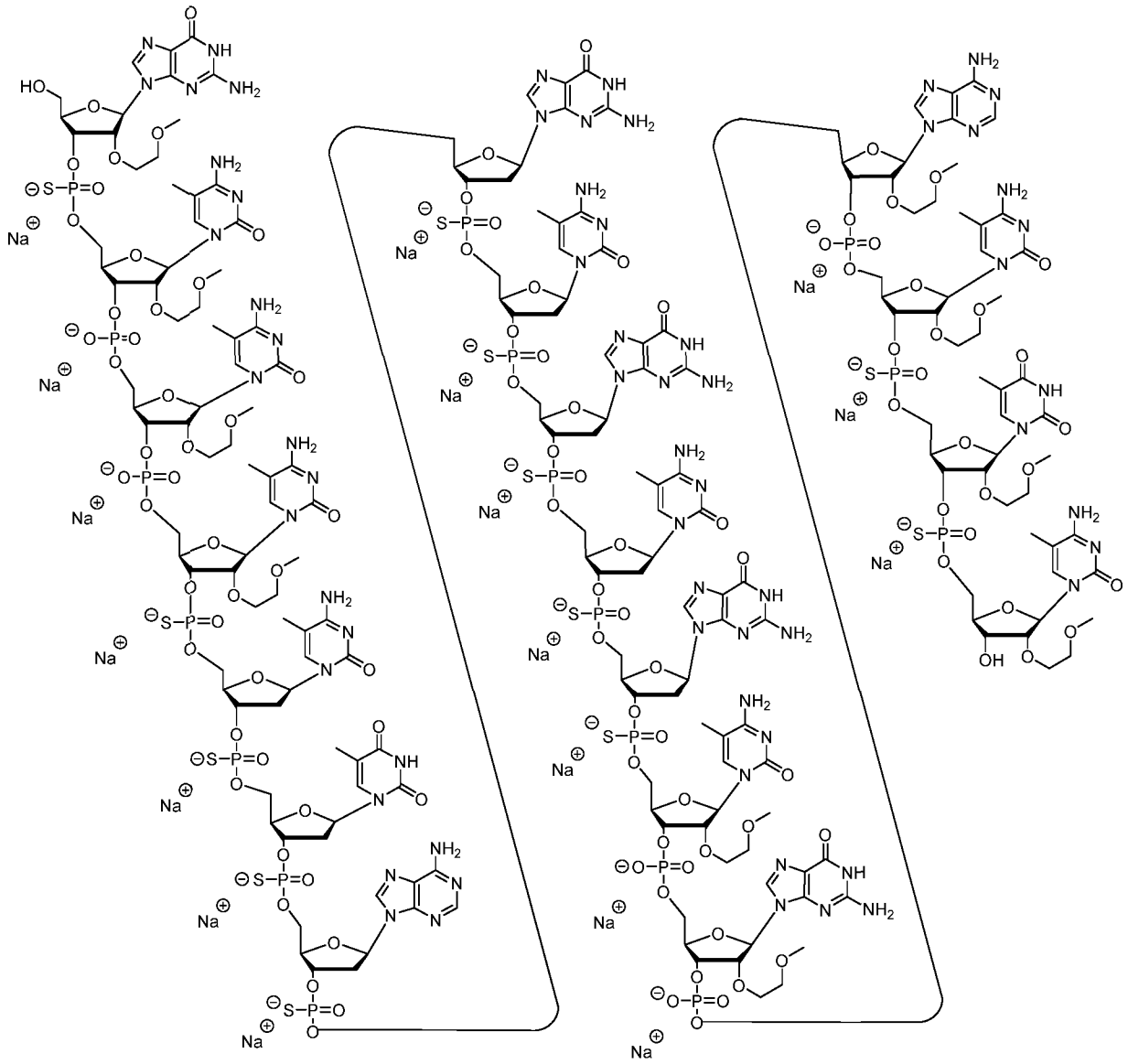
Embodiment 57. The method of embodiment 55, wherein the C9ORF72 associated disease is any of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

Embodiment 58. The method of embodiment 53, wherein the administering reduces nuclear foci.

Embodiment 59. The method of embodiment 53, wherein the administering reduces expression of C9ORF72 associated RAN translation products.

Embodiment 60. The method of embodiment 59, wherein the C9ORF72 associated RAN translation products are any of poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine).

Embodiment 61. A composition consisting of the sodium salt of a modified oligonucleotide according to the following formula:



Embodiment 62. A method comprising administering to an animal the composition of embodiment 60.

Embodiment 63. The method of embodiment 62, wherein the animal is a human.

Embodiment 64. The method of embodiment 63, wherein the administering inhibits C9ORF72.

Embodiment 65. The method of embodiment 62, wherein the administering prevents, treats, ameliorates, or slows progression of a C9ORF72 associated disease.

Embodiment 66. The method of embodiment 65, wherein the C9ORF72 associated disease is caused by a hexanucleotide repeat expansion.

Embodiment 67. The method of embodiment 65, wherein the C9ORF72 associated disease is any of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), corticobasal degeneration syndrome (CBD), atypical Parkinsonian syndrome, or olivopontocerebellar degeneration (OPCD).

Embodiment 68. The method of embodiment 63, wherein the administering reduces nuclear foci.

Embodiment 69. The method of embodiment 63, wherein the administering reduces expression of C9ORF72 associated RAN translation products.

Embodiment 70. The method of embodiment 69, wherein the C9ORF72 associated RAN translation products are any of poly-(glycine-proline), poly-(glycine-alanine), and poly-(glycine-arginine).

Antisense Compounds

Oligomeric compounds include, but are not limited to, oligonucleotides, oligonucleosides, oligonucleotide analogs, oligonucleotide mimetics, antisense compounds, antisense oligonucleotides, and siRNAs. An oligomeric compound may be "antisense" to a target nucleic acid, meaning that is is capable of undergoing hybridization to a target nucleic acid through hydrogen bonding.

In certain embodiments, an antisense compound has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted. In certain such embodiments, an antisense oligonucleotide has a nucleobase sequence that, when written in the 5' to 3' direction, comprises the reverse complement of the target segment of a target nucleic acid to which it is targeted.

In certain embodiments, an antisense compound targeted to a C9ORF72 nucleic acid is 12 to 30 subunits in length. In other words, such antisense compounds are from 12 to 30 linked subunits. In certain embodiments, the antisense compound is 8 to 80, 12 to 50, 15 to 30, 18 to 24, 19 to 22, or 20 linked subunits. In certain embodiments, the antisense compounds are 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, or 80 linked subunits in length, or a range defined by any two of the above values. In some embodiments the antisense compound is an antisense oligonucleotide, and the linked subunits are nucleosides.

In certain embodiments antisense oligonucleotides targeted to a C9ORF72 nucleic acid may be shortened or truncated. For example, a single subunit may be deleted from the 5' end (5' truncation), or alternatively from the 3' end (3' truncation). A shortened or truncated antisense compound targeted to a C9ORF72 nucleic acid may have two subunits deleted from the 5' end, or alternatively may have two subunits deleted from the 3' end, of the antisense compound. Alternatively, the deleted nucleosides may be dispersed throughout the antisense compound, for example, in an antisense compound having one nucleoside deleted from the 5' end and one nucleoside deleted from the 3' end.

When a single additional subunit is present in a lengthened antisense compound, the additional subunit may be located at the 5' or 3' end of the antisense compound. When two or more additional subunits are present, the added subunits may be adjacent to each other, for example, in an antisense compound having two subunits added to the 5' end (5' addition), or alternatively to the 3' end (3' addition), of the antisense compound. Alternatively, the added subunits may be dispersed throughout the antisense compound, for example, in an antisense compound having one subunit added to the 5' end and one subunit added to the 3' end.

It is possible to increase or decrease the length of an antisense compound, such as an antisense oligonucleotide, and/or introduce mismatch bases without eliminating activity. For example, in Woolf et al. (Proc. Natl. Acad. Sci. USA 89:7305-7309, 1992), a series of antisense oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target RNA in an oocyte injection model. Antisense oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the antisense oligonucleotides were able to direct specific cleavage of the target mRNA, albeit to a lesser extent than the antisense oligonucleotides that

contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase antisense oligonucleotides, including those with 1 or 3 mismatches.

Gautschi et al (J. Natl. Cancer Inst. 93:463-471, March 2001) demonstrated the ability of an oligonucleotide having 100% complementarity to the bcl-2 mRNA and having 3 mismatches to the bcl-xL mRNA to reduce the expression of both bcl-2 and bcl-xL *in vitro* and *in vivo*. Furthermore, this oligonucleotide demonstrated potent anti-tumor activity *in vivo*.

Maher and Dolnick (Nuc. Acid. Res. 16:3341-3358,1988) tested a series of tandem 14 nucleobase antisense oligonucleotides, and a 28 and 42 nucleobase antisense oligonucleotides comprised of the sequence of two or three of the tandem antisense oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase antisense oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase antisense oligonucleotides.

Antisense Compound Motifs

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid have chemically modified subunits arranged in patterns, or motifs, to confer to the antisense compounds properties such as enhanced inhibitory activity, increased binding affinity for a target nucleic acid, or resistance to degradation by *in vivo* nucleases.

Chimeric antisense compounds typically contain at least one region modified so as to confer increased resistance to nuclease degradation, increased cellular uptake, increased binding affinity for the target nucleic acid, and/or increased inhibitory activity. A second region of a chimeric antisense compound may optionally serve as a substrate for the cellular endonuclease RNase H, which cleaves the RNA strand of an RNA:DNA duplex.

Antisense compounds having a gapmer motif are considered chimeric antisense compounds. In a gapmer an internal region having a plurality of nucleotides that supports RNaseH cleavage is positioned between external regions having a plurality of nucleotides that are chemically distinct from the nucleosides of the internal region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. In certain embodiments, the regions of a gapmer are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may in some embodiments include β-D-ribonucleosides, β-D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified

nucleosides may include 2'-MOE, and 2'-O-CH_3, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH_2)n-O-2' bridge, where n=1 or n=2 and 4'-CH_2-O-CH_2-2'). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X-Y-Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. As used herein, a gapmer described as "X-Y-Z" has a configuration such that the gap segment is positioned immediately adjacent to each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Any of the antisense compounds described herein can have a gapmer motif. In some embodiments, X and Z are the same, in other embodiments they are different. In a preferred embodiment, Y is between 8 and 15 nucleotides. X, Y or Z can be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more nucleotides. Thus, gapmers described herein include, but are not limited to, for example 5-10-5, 5-10-4, 4-10-4, 4-10-3, 3-10-3, 2-10-2, 5-9-5, 5-9-4, 4-9-5, 5-8-5, 5-8-4, 4-8-5, 5-7-5, 4-7-5, 5-7-4, or 4-7-4.

In certain embodiments, the antisense compound has a "wingmer" motif, having a wing-gap or gap-wing configuration, i.e. an X-Y or Y-Z configuration as described above for the gapmer configuration. Thus, wingmer configurations described herein include, but are not limited to, for example 5-10, 8-4, 4-12, 12-4, 3-14, 16-2, 18-1, 10-3, 2-10, 1-10, 8-2, 2-13, 5-13, 5-8, or 6-8.

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid possess a 5-10-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid possess a 5-8-5 gapmer motif.

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid possess sugar modifications in any of the following patterns: eeekkddddddddkkeee, eekkddddddddkkeee, ekddddddddekekeee, kekeddddddddekeke, and ekekddddddddkekee; wherein,

e = a 2'-O-methoxyethyl modified nucleoside

d = a 2'-deoxynucleoside, and

k = a cEt nucleoside.

In certain embodiments, an antisense compound targeted to a C9ORF72 nucleic acid has a gap-narrowed motif. In certain embodiments, a gap-narrowed antisense oligonucleotide targeted to

a C9ORF72 nucleic acid has a gap segment of 9, 8, 7, or 6 2'-deoxynucleotides positioned immediately adjacent to and between wing segments of 5, 4, 3, 2, or 1 chemically modified nucleosides. In certain embodiments, the chemical modification comprises a bicyclic sugar. In certain embodiments, the bicyclic sugar comprises a 4' to 2' bridge selected from among: 4'-$(CH_2)_n$-O-2' bridge, wherein n is 1 or 2; and 4'-CH_2-O-CH_2-2'. In certain embodiments, the bicyclic sugar is comprises a 4'-$CH(CH_3)$-O-2' bridge. In certain embodiments, the chemical modification comprises a non-bicyclic 2'-modified sugar moiety. In certain embodiments, the non-bicyclic 2'-modified sugar moiety comprises a 2'-O-methylethyl group or a 2'-O-methyl group.

Target Nucleic Acids, Target Regions and Nucleotide Sequences

Nucleotide sequences that encode C9ORF72 include, without limitation, the following: the complement of GENBANK Accession No. NM_001256054.1 (incorporated herein as SEQ ID NO: 1), the complement of GENBANK Accession No. NT_008413.18 truncated from nucleobase 27535000 to 27565000 (incorporated herein as SEQ ID NO: 2), GENBANK Accession No. BQ068108.1 (incorporated herein as SEQ ID NO: 3), GENBANK Accession No. NM_018325.3 (incorporated herein as SEQ ID NO: 4), GENBANK Accession No. DN993522.1 (incorporated herein as SEQ ID NO: 5), GENBANK Accession No. NM_145005.5 (incorporated herein as SEQ ID NO: 6), GENBANK Accession No. DB079375.1 (incorporated herein as SEQ ID NO: 7), GENBANK Accession No. BU194591.1 (incorporated herein as SEQ ID NO: 8), Sequence Identifier 4141_014_A (incorporated herein as SEQ ID NO: 9), and Sequence Identifier 4008_73_A (incorporated herein as SEQ ID NO: 10), and GENBANK Accession No. NW_001101662.1 truncated from nucleosides 8522000 to 8552000 (incorporated herein as SEQ ID NO: 11).

It is understood that the sequence set forth in each SEQ ID NO in the Examples contained herein is independent of any modification to a sugar moiety, an internucleoside linkage, or a nucleobase. As such, antisense compounds defined by a SEQ ID NO may comprise, independently, one or more modifications to a sugar moiety, an internucleoside linkage, or a nucleobase. Antisense compounds described by Isis Number (Isis No) indicate a combination of nucleobase sequence and motif.

In certain embodiments, a target region is a structurally defined region of the target nucleic acid. For example, a target region may encompass a 3' UTR, a 5' UTR, an exon, an intron, an exon/intron junction, a coding region, a translation initiation region, translation termination region, or other defined nucleic acid region. The structurally defined regions for C9ORF72 can be obtained

by accession number from sequence databases such as NCBI and such information is incorporated herein by reference. In certain embodiments, a target region may encompass the sequence from a 5' target site of one target segment within the target region to a 3' target site of another target segment within the same target region.

Targeting includes determination of at least one target segment to which an antisense compound hybridizes, such that a desired effect occurs. In certain embodiments, the desired effect is a reduction in mRNA target nucleic acid levels. In certain embodiments, the desired effect is reduction of levels of protein encoded by the target nucleic acid or a phenotypic change associated with the target nucleic acid.

A target region may contain one or more target segments. Multiple target segments within a target region may be overlapping. Alternatively, they may be non-overlapping. In certain embodiments, target segments within a target region are separated by no more than about 300 nucleotides. In certain emodiments, target segments within a target region are separated by a number of nucleotides that is, is about, is no more than, is no more than about, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 nucleotides on the target nucleic acid, or is a range defined by any two of the preceeding values. In certain embodiments, target segments within a target region are separated by no more than, or no more than about, 5 nucleotides on the target nucleic acid. In certain embodiments, target segments are contiguous. Contemplated are target regions defined by a range having a starting nucleic acid that is any of the 5' target sites or 3' target sites listed herein.

Suitable target segments may be found within a 5' UTR, a coding region, a 3' UTR, an intron, an exon, or an exon/intron junction. Target segments containing a start codon or a stop codon are also suitable target segments. A suitable target segment may specifcally exclude a certain structurally defined region such as the start codon or stop codon.

The determination of suitable target segments may include a comparison of the sequence of a target nucleic acid to other sequences throughout the genome. For example, the BLAST algorithm may be used to identify regions of similarity amongst different nucleic acids. This comparison can prevent the selection of antisense compound sequences that may hybridize in a non-specific manner to sequences other than a selected target nucleic acid (i.e., non-target or off-target sequences).

There may be variation in activity (e.g., as defined by percent reduction of target nucleic acid levels) of the antisense compounds within a target region. In certain embodiments, reductions in C9ORF72 mRNA levels are indicative of inhibition of C9ORF72 expression. Reductions in levels of a C9ORF72 protein are also indicative of inhibition of target mRNA expression. Reduction in the

presence of expanded C9ORF72 RNA foci are indicative of inhibition of C9ORF72 expression. Further, phenotypic changes are indicative of inhibition of C9ORF72 expression. For example, improved motor function and respiration may be indicative of inhibition of C9ORF72 expression.

Hybridization

In some embodiments, hybridization occurs between an antisense compound disclosed herein and a C9ORF72 nucleic acid. The most common mechanism of hybridization involves hydrogen bonding (e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules.

Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Methods of determining whether a sequence is specifically hybridizable to a target nucleic acid are well known in the art. In certain embodiments, the antisense compounds provided herein are specifically hybridizable with a C9ORF72 nucleic acid.

Complementarity

An antisense compound and a target nucleic acid are complementary to each other when a sufficient number of nucleobases of the antisense compound can hydrogen bond with the corresponding nucleobases of the target nucleic acid, such that a desired effect will occur (e.g., antisense inhibition of a target nucleic acid, such as a C9ORF72 nucleic acid).

Non-complementary nucleobases between an antisense compound and a C9ORF72 nucleic acid may be tolerated provided that the antisense compound remains able to specifically hybridize to a target nucleic acid. Moreover, an antisense compound may hybridize over one or more segments of a C9ORF72 nucleic acid such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure, mismatch or hairpin structure).

In certain embodiments, the antisense compounds provided herein, or a specified portion thereof, are, or are at least, 70%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% complementary to a C9ORF72 nucleic acid, a target region, target segment, or specified portion thereof. Percent complementarity of an antisense compound with a target nucleic acid can be determined using routine methods.

For example, an antisense compound in which 18 of 20 nucleobases of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. In this example, the remaining noncomplementary nucleobases may be clustered or interspersed with complementary nucleobases and need not be contiguous to each other or to complementary nucleobases. As such, an antisense compound which is 18 nucleobases in length having 4 (four) noncomplementary nucleobases which are flanked by two regions of complete complementarity with the target nucleic acid would have 77.8% overall complementarity with the target nucleic acid and would thus fall within the scope of the present invention. Percent complementarity of an antisense compound with a region of a target nucleic acid can be determined routinely using BLAST programs (basic local alignment search tools) and PowerBLAST programs known in the art (Altschul et al., J. Mol. Biol., 1990, 215, 403 410; Zhang and Madden, Genome Res., 1997, 7, 649 656). Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482 489).

In certain embodiments, the antisense compounds provided herein, or specified portions thereof, are fully complementary (*i.e.*, 100% complementary) to a target nucleic acid, or specified portion thereof. For example, an antisense compound may be fully complementary to a C9ORF72 nucleic acid, or a target region, or a target segment or target sequence thereof. As used herein, "fully complementary" means each nucleobase of an antisense compound is capable of precise base pairing with the corresponding nucleobases of a target nucleic acid. For example, a 20 nucleobase antisense compound is fully complementary to a target sequence that is 400 nucleobases long, so long as there is a corresponding 20 nucleobase portion of the target nucleic acid that is fully complementary to the antisense compound. Fully complementary can also be used in reference to a specified portion of the first and /or the second nucleic acid. For example, a 20 nucleobase portion of a 30 nucleobase antisense compound can be "fully complementary" to a target sequence that is 400 nucleobases long. The 20 nucleobase portion of the 30 nucleobase oligonucleotide is fully complementary to the target sequence if the target sequence has a corresponding 20 nucleobase portion wherein each nucleobase is complementary to the 20 nucleobase portion of the antisense compound. At the same time, the entire 30 nucleobase antisense compound may or may not be fully

complementary to the target sequence, depending on whether the remaining 10 nucleobases of the antisense compound are also complementary to the target sequence.

The location of a non-complementary nucleobase may be at the 5' end or 3' end of the antisense compound. Alternatively, the non-complementary nucleobase or nucleobases may be at an internal position of the antisense compound. When two or more non-complementary nucleobases are present, they may be contiguous (i.e., linked) or non-contiguous. In one embodiment, a non-complementary nucleobase is located in the wing segment of a gapmer antisense oligonucleotide.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length comprise no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a C9ORF72 nucleic acid, or specified portion thereof.

In certain embodiments, antisense compounds that are, or are up to 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleobases in length comprise no more than 6, no more than 5, no more than 4, no more than 3, no more than 2, or no more than 1 non-complementary nucleobase(s) relative to a target nucleic acid, such as a C9ORF72 nucleic acid, or specified portion thereof.

The antisense compounds provided herein also include those which are complementary to a portion of a target nucleic acid. As used herein, "portion" refers to a defined number of contiguous (i.e. linked) nucleobases within a region or segment of a target nucleic acid. A "portion" can also refer to a defined number of contiguous nucleobases of an antisense compound. In certain embodiments, the antisense compounds, are complementary to at least an 8 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 9 nucleobase portion of a target segment. In certain embodiments, the antisense compounds are complementary to at least a 10 nucleobase portion of a target segment. In certain embodiments, the antisense compounds, are complementary to at least an 11 nucleobase portion of a target segment. In certain embodiments, the antisense compounds, are complementary to at least a 12 nucleobase portion of a target segment. In certain embodiments, the antisense compounds, are complementary to at least a 13 nucleobase portion of a target segment. In certain embodiments, the antisense compounds, are complementary to at least a 14 nucleobase portion of a target segment. In certain embodiments, the antisense compounds, are complementary to at least a 15 nucleobase portion of a target segment. Also contemplated are antisense compounds that are complementary to at least a 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nucleobase portion of a target segment, or a range defined by any two of these values.

Identity

The antisense compounds provided herein may also have a defined percent identity to a particular nucleotide sequence, SEQ ID NO, or compound represented by a specific Isis number, or portion thereof. As used herein, an antisense compound is identical to the sequence disclosed herein if it has the same nucleobase pairing ability. For example, a RNA which contains uracil in place of thymidine in a disclosed DNA sequence would be considered identical to the DNA sequence since both uracil and thymidine pair with adenine. Shortened and lengthened versions of the antisense compounds described herein as well as compounds having non-identical bases relative to the antisense compounds provided herein also are contemplated. The non-identical bases may be adjacent to each other or dispersed throughout the antisense compound. Percent identity of an antisense compound is calculated according to the number of bases that have identical base pairing relative to the sequence to which it is being compared.

In certain embodiments, the antisense compounds, or portions thereof, are at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to one or more of the antisense compounds or SEQ ID NOs, or a portion thereof, disclosed herein.

In certain embodiments, a portion of the antisense compound is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

In certain embodiments, a portion of the antisense oligonucleotide is compared to an equal length portion of the target nucleic acid. In certain embodiments, an 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleobase portion is compared to an equal length portion of the target nucleic acid.

Modifications

A nucleoside is a base-sugar combination. The nucleobase (also known as base) portion of the nucleoside is normally a heterocyclic base moiety. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', 3' or 5'

hydroxyl moiety of the sugar. Oligonucleotides are formed through the covalent linkage of adjacent nucleosides to one another, to form a linear polymeric oligonucleotide. Within the oligonucleotide structure, the phosphate groups are commonly referred to as forming the internucleoside linkages of the oligonucleotide.

Modifications to antisense compounds encompass substitutions or changes to internucleoside linkages, sugar moieties, or nucleobases. Modified antisense compounds are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target, increased stability in the presence of nucleases, or increased inhibitory activity.

Chemically modified nucleosides may also be employed to increase the binding affinity of a shortened or truncated antisense oligonucleotide for its target nucleic acid. Consequently, comparable results can often be obtained with shorter antisense compounds that have such chemically modified nucleosides.

Modified Internucleoside Linkages

The naturally occuring internucleoside linkage of RNA and DNA is a 3' to 5' phosphodiester linkage. Antisense compounds having one or more modified, i.e. non-naturally occurring, internucleoside linkages are often selected over antisense compounds having naturally occurring internucleoside linkages because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for target nucleic acids, and increased stability in the presence of nucleases.

Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom as well as internucleoside linkages that do not have a phosphorus atom. Representative phosphorus containing internucleoside linkages include, but are not limited to, phosphodiesters, phosphotriesters, methylphosphonates, phosphoramidate, and phosphorothioates. Methods of preparation of phosphorous-containing and non-phosphorous-containing linkages are well known.

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid comprise one or more modified internucleoside linkages. In certain embodiments, the modified internucleoside linkages are interspersed throughout the antisense compound. In certain embodiments, the modified internucleoside linkages are phosphorothioate linkages. In certain embodiments, each internucleoside linkage of an antisense compound is a phosphorothioate

internucleoside linkage. In certain embodiments, the antisense compounds targeted to a C9ORF72 nucleic acid comprise at least one phosphodiester linkage and at least one phosphorothioate linkage.

In certain embodiments, antisense compounds targeted to a C9ORF72 nucleic acid possess internucleoside linkages in any of the following patterns: soooosssssssssssooss, sooosssssssssooss, soosssssssssooss, and sosssssssssoooss; wherein,

s = a phosphorothioate linkage, and

o = a phosphodiester linkage.

Modified Sugar Moieties

Antisense compounds of the invention can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. In certain embodiments, nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include without limitation, addition of substitutent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or $C(R_1)(R_2)$ (R, R_1 and R_2 are each independently H, C_1-C_{12} alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (see PCT International Application WO 2008/101157 Published on 8/21/08 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a BNA (see PCT International Application WO 2007/134181 Published on 11/22/07 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

Examples of nucleosides having modified sugar moieties include without limitation nucleosides comprising 5'-vinyl, 5'-methyl (*R* or *S*), 4'-S, 2'-F, 2'-OCH_3, 2'-OCH_2CH_3, 2'-OCH_2CH_2F and 2'-$O(CH_2)_2OCH_3$ substituent groups. The substituent at the 2' position can also be selected from allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, OCF_3, OCH_2F, $O(CH_2)_2SCH_3$, $O(CH_2)_2$-O-$N(R_m)(R_n)$, O-CH_2-C(=O)-$N(R_m)(R_n)$, and O-CH_2-C(=O)-$N(R_l)$-$(CH_2)_2$-$N(R_m)(R_n)$, where each R_l, R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl.

As used herein, "bicyclic nucleosides" refer to modified nucleosides comprising a bicyclic sugar moiety. Examples of bicyclic nucleic acids (BNAs) include without limitation nucleosides

comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, antisense compounds provided herein include one or more BNA nucleosides wherein the bridge comprises one of the formulas: 4'-(CH_2)-O-2' (LNA); 4'-(CH_2)-S-2'; 4'-(CH_2)$_2$-O-2' (ENA); 4'-CH(CH_3)-O-2' and 4'-CH(CH_2OCH_3)-O-2' (and analogs thereof see U.S. Patent 7,399,845, issued on July 15, 2008); 4'-C(CH_3)(CH_3)-O-2' (and analogs thereof see PCT/US2008/068922 published as WO/2009/006478, published January 8, 2009); 4'-CH_2-N(OCH_3)-2' (and analogs thereof see PCT/US2008/064591 published as WO/2008/150729, published December 11, 2008); 4'-CH_2-O-N(CH_3)-2' (see published U.S. Patent Application US2004-0171570, published September 2, 2004); 4'-CH_2-N(R)-O-2', wherein R is H, C_1-C_{12} alkyl, or a protecting group (see U.S. Patent 7,427,672, issued on September 23, 2008); 4'-CH_2-C(H)(CH_3)-2' (see Chattopadhyaya *et al., J. Org. Chem.,* 2009, *74*, 118-134); and 4'-CH_2-C(=CH_2)-2' (and analogs thereof see PCT/US2008/066154 published as WO 2008/154401, published on December 8, 2008).

Further bicyclic nucleosides have been reported in published literature (see for example: Srivastava et al., *J. Am. Chem. Soc.*, 2007, *129(26)* 8362-8379; Frieden *et al., Nucleic Acids Research,* 2003, *21*, 6365-6372; Elayadi *et al., Curr. Opinion Invens. Drugs,* 2001, *2*, 558-561; Braasch *et al., Chem. Biol.,* 2001, *8*, 1-7; Orum *et al., Curr. Opinion Mol. Ther.,* 2001, *3*, 239-243; Wahlestedt et al., *Proc. Natl. Acad. Sci. U. S. A.*, 2000, *97*, 5633-5638; Singh et al., *Chem. Commun.*, 1998, *4*, 455-456; Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630; Kumar et al., *Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222; Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039; U.S. Patents Nos.: 7,399,845; 7,053,207; 7,034,133; 6,794,499; 6,770,748; 6,670,461; 6,525,191; 6,268,490; U.S. Patent Publication Nos.: US2008-0039618; US2007-0287831; US2004-0171570; U.S. Patent Applications, Serial Nos.: 12/129,154; 61/099,844; 61/097,787; 61/086,231; 61/056,564; 61/026,998; 61/026,995; 60/989,574; International applications WO 2007/134181; WO 2005/021570; WO 2004/106356; and PCT International Applications Nos.: PCT/US2008/068922; PCT/US2008/066154; and PCT/US2008/064591). Each of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see PCT international application PCT/DK98/00393, published on March 25, 1999 as WO 99/14226).

As used herein, "monocylic nucleosides" refer to nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

As used herein, "4'-2' bicyclic nucleoside" or "4' to 2' bicyclic nucleoside" refers to a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

In certain embodiments, bicyclic sugar moieties of BNA nucleosides include, but are not limited to, compounds having at least one bridge between the 4' and the 2' carbon atoms of the pentofuranosyl sugar moiety including without limitation, bridges comprising 1 or from 1 to 4 linked groups independently selected from $-[C(R_a)(R_b)]_n-$, $-C(R_a)=C(R_b)-$, $-C(R_a)=N-$, $-C(=NR_a)-$, $-C(=O)-$, $-C(=S)-$, $-O-$, $-Si(R_a)_2-$, $-S(=O)_x-$, and $-N(R_a)-$; wherein: x is 0, 1, or 2; n is 1, 2, 3, or 4; each R_a and R_b is, independently, H, a protecting group, hydroxyl, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C_5-C_7 alicyclic radical, substituted C_5-C_7 alicyclic radical, halogen, OJ_1, NJ_1J_2, SJ_1, N_3, $COOJ_1$, acyl ($C(=O)$-H), substituted acyl, CN, sulfonyl ($S(=O)_2$-J_1), or sulfoxyl ($S(=O)$-J_1); and

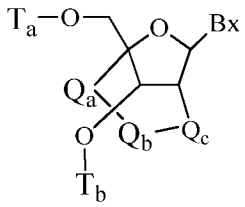
each J_1 and J_2 is, independently, H, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_5-C_{20} aryl, substituted C_5-C_{20} aryl, acyl ($C(=O)$-H), substituted acyl, a heterocycle radical, a substituted heterocycle radical, C_1-C_{12} aminoalkyl, substituted C_1-C_{12} aminoalkyl or a protecting group.

In certain embodiments, the bridge of a bicyclic sugar moiety is , $-[C(R_a)(R_b)]_n-$, $-[C(R_a)(R_b)]_n-O-$, $-C(R_aR_b)-N(R)-O-$ or $-C(R_aR_b)-O-N(R)-$. In certain embodiments, the bridge is 4'-CH_2-2', 4'-$(CH_2)_2$-2', 4'-$(CH_2)_3$-2', 4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2' and 4'-CH_2-N(R)-O-2'- wherein each R is, independently, H, a protecting group or C_1-C_{12} alkyl.

In certain embodiments, bicyclic nucleosides are further defined by isomeric configuration. For example, a nucleoside comprising a 4'-(CH_2)-O-2' bridge, may be in the α-L configuration or in the β-D configuration. Previously, α-L-methyleneoxy (4'-CH_2-O-2') BNA's have been incorporated into antisense oligonucleotides that showed antisense activity (Frieden *et al., Nucleic Acids Research,* 2003, *21*, 6365-6372).

In certain embodiments, bicyclic nucleosides include those having a 4' to 2' bridge wherein such bridges include without limitation, α-L-4'-(CH_2)-O-2', β-D-4'-CH_2-O-2', 4'-$(CH_2)_2$-O-2', 4'-CH_2-O-N(R)-2', 4'-CH_2-N(R)-O-2', 4'-$CH(CH_3)$-O-2', 4'-CH_2-S-2', 4'-CH_2-N(R)-2', 4'-CH_2-$CH(CH_3)$-2', and 4'-$(CH_2)_3$-2', wherein R is H, a protecting group or C_1-C_{12} alkyl.

In certain embodiment, bicyclic nucleosides have the formula:



wherein:

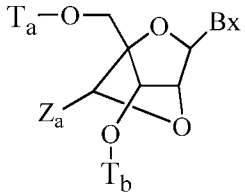
Bx is a heterocyclic base moiety;

$-Q_a-Q_b-Q_c-$ is $-CH_2-N(R_c)-CH_2-$, $-C(=O)-N(R_c)-CH_2-$, $-CH_2-O-N(R_c)-$, $-CH_2-N(R_c)-O-$ or $-N(R_c)-O-CH_2$;

R_c is C_1-C_{12} alkyl or an amino protecting group; and

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium.

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

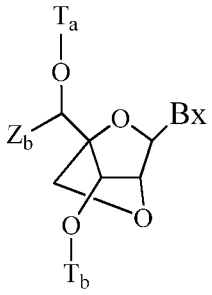
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_a is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl, acyl, substituted acyl, substituted amide, thiol or substituted thiol.

In one embodiment, each of the substituted groups, is, independently, mono or poly substituted with substituent groups independently selected from halogen, oxo, hydroxyl, OJ_c, NJ_cJ_d, SJ_c, N_3, $OC(=X)J_c$, and $NJ_eC(=X)NJ_cJ_d$, wherein each J_c, J_d and J_e is, independently, H, C_1-C_6 alkyl, or substituted C_1-C_6 alkyl and X is O or NJ_c.

In certain embodiments, bicyclic nucleosides have the formula:



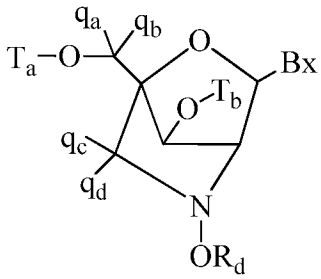
wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

Z_b is C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_1-C_6 alkyl, substituted C_2-C_6 alkenyl, substituted C_2-C_6 alkynyl or substituted acyl (C(=O)-).

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

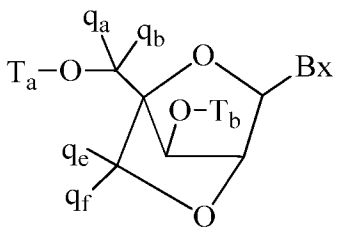
Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

R_d is C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl;

each q_a, q_b, q_c and q_d is, independently, H, halogen, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl, C_1-C_6 alkoxyl, substituted C_1-C_6 alkoxyl, acyl, substituted acyl, C_1-C_6 aminoalkyl or substituted C_1-C_6 aminoalkyl;

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

Bx is a heterocyclic base moiety;

T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

q_a, q_b, q_e and q_f are each, independently, hydrogen, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxy, substituted C_1-C_{12} alkoxy, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, $O\text{-}C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$;

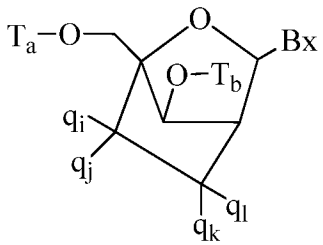
or q_e and q_f together are $=C(q_g)(q_h)$;

q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

The synthesis and preparation of adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil bicyclic nucleosides having a 4'-CH_2-O-2' bridge, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., *Tetrahedron*, 1998, *54*, 3607-3630). The synthesis of bicyclic nucleosides has also been described in WO 98/39352 and WO 99/14226.

Analogs of various bicyclic nucleosides that have 4' to 2' bridging groups such as 4'-CH_2-O-2' and 4'-CH_2-S-2', have also been prepared (Kumar *et al., Bioorg. Med. Chem. Lett.*, 1998, *8*, 2219-2222). Preparation of oligodeoxyribonucleotide duplexes comprising bicyclic nucleosides for use as substrates for nucleic acid polymerases has also been described (Wengel et al., WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., *J. Org. Chem.*, 1998, *63*, 10035-10039). In addition, 2'-amino- and 2'-methylamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported.

In certain embodiments, bicyclic nucleosides have the formula:



wherein:

Bx is a heterocyclic base moiety;

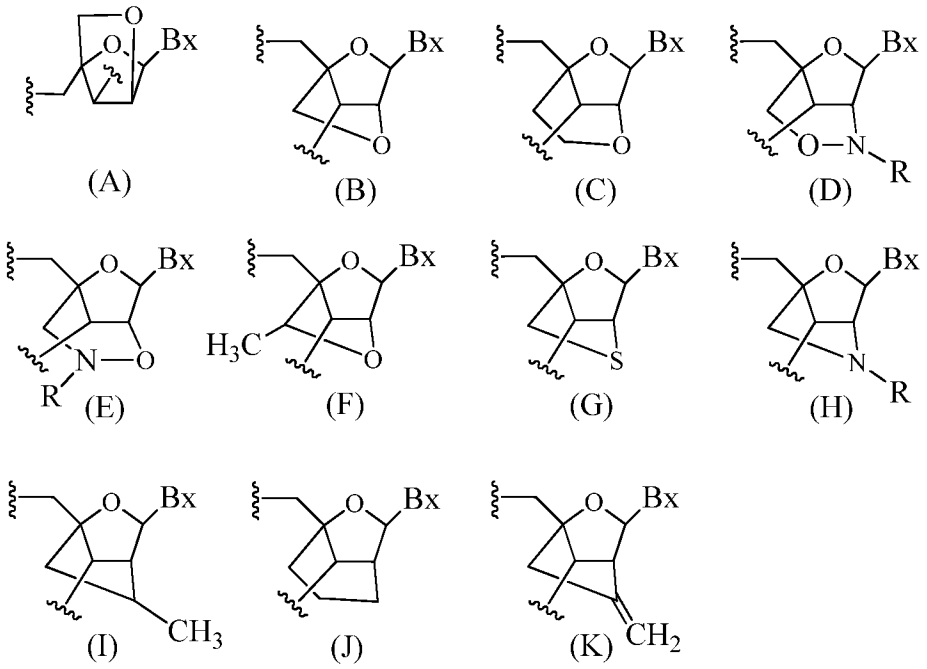
T_a and T_b are each, independently H, a hydroxyl protecting group, a conjugate group, a reactive phosphorus group, a phosphorus moiety or a covalent attachment to a support medium;

each q_i, q_j, q_k and q_l is, independently, H, halogen, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, C_2-C_{12} alkenyl, substituted C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, substituted C_2-C_{12} alkynyl, C_1-C_{12} alkoxyl, substituted C_1-C_{12} alkoxyl, OJ_j, SJ_j, SOJ_j, SO_2J_j, NJ_jJ_k, N_3, CN, $C(=O)OJ_j$, $C(=O)NJ_jJ_k$, $C(=O)J_j$, O-$C(=O)NJ_jJ_k$, $N(H)C(=NH)NJ_jJ_k$, $N(H)C(=O)NJ_jJ_k$ or $N(H)C(=S)NJ_jJ_k$; and

q_i and q_j or q_l and q_k together are $=C(q_g)(q_h)$, wherein q_g and q_h are each, independently, H, halogen, C_1-C_{12} alkyl or substituted C_1-C_{12} alkyl.

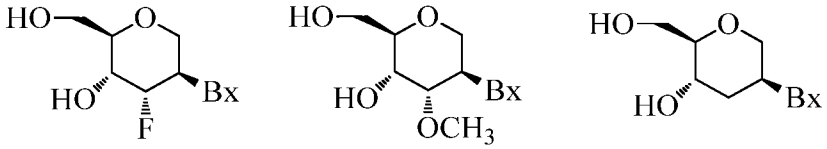
One carbocyclic bicyclic nucleoside having a 4'-$(CH_2)_3$-2' bridge and the alkenyl analog bridge 4'-CH=CH-CH_2-2' have been described (Frier *et al., Nucleic Acids Research*, 1997, *25*(*22*), 4429-4443 and Albaek *et al., J. Org. Chem.*, 2006, *71*, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

In certain embodiments, bicyclic nucleosides include, but are not limited to, (A) α-L-methyleneoxy (4'-CH_2-O-2') BNA , (B) β-D-methyleneoxy (4'-CH_2-O-2') BNA , (C) ethyleneoxy (4'-$(CH_2)_2$-O-2') BNA, (D) aminooxy (4'-CH_2-O-N(R)-2') BNA, (E) oxyamino (4'-CH_2-N(R)-O-2') BNA, (F) methyl(methyleneoxy) (4'-$CH(CH_3)$-O-2') BNA (also referred to as constrained ethyl or cEt), (G) methylene-thio (4'-CH_2-S-2') BNA, (H) methylene-amino (4'-CH_2-N(R)-2') BNA, (I) methyl carbocyclic (4'-CH_2-$CH(CH_3)$-2') BNA, (J) propylene carbocyclic (4'-$(CH_2)_3$-2') BNA, and (K) vinyl BNA as depicted below.

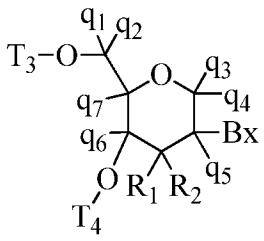


wherein Bx is the base moiety and R is, independently, H, a protecting group, C_1-C_6 alkyl or C_1-C_6 alkoxy.

As used herein, the term "modified tetrahydropyran nucleoside" or "modified THP nucleoside" means a nucleoside having a six-membered tetrahydropyran "sugar" substituted for the pentofuranosyl residue in normal nucleosides and can be referred to as a sugar surrogate. Modified THP nucleosides include, but are not limited to, what is referred to in the art as hexitol nucleic acid (HNA), anitol nucleic acid (ANA), manitol nucleic acid (MNA) (see Leumann, *Bioorg. Med. Chem.*, 2002, *10*, 841-854) or fluoro HNA (F-HNA) having a tetrahydropyranyl ring system as illustrated below.



In certain embodiment, sugar surrogates are selected having the formula:



wherein:

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the tetrahydropyran nucleoside analog to the oligomeric compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an oligomeric compound or oligonucleotide and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group or a 5' or 3'-terminal group;

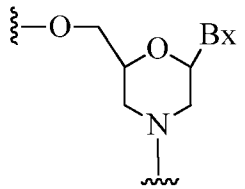
q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl or substituted C_2-C_6 alkynyl; and

one of R_1 and R_2 is hydrogen and the other is selected from halogen, substituted or unsubstituted alkoxy, NJ_1J_2, SJ_1, N_3, $OC(=X)J_1$, $OC(=X)NJ_1J_2$, $NJ_3C(=X)NJ_1J_2$ and CN, wherein X is O, S or NJ_1 and each J_1, J_2 and J_3 is, independently, H or C_1-C_6 alkyl.

In certain embodiments, q_1, q_2, q_3, q_4, q_5, q_6 and q_7 are each H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is other than H. In certain embodiments, at least one of q_1, q_2, q_3, q_4, q_5, q_6 and q_7 is methyl. In certain embodiments, THP nucleosides are provided wherein one of R_1 and R_2 is F. In certain embodiments, R_1 is fluoro and R_2 is H; R_1 is methoxy and R_2 is H, and

R_1 is methoxyethoxy and R_2 is H.

In certain embodiments, sugar surrogates comprise rings having more than 5 atoms and more than one heteroatom. For example nucleosides comprising morpholino sugar moieties and their use in oligomeric compounds has been reported (see for example: Braasch *et al.*, *Biochemistry*, 2002, *41*, 4503-4510; and U.S. Patents 5,698,685; 5,166,315; 5,185,444; and 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following formula:

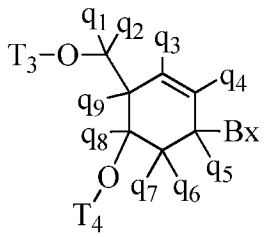


In certain embodiments, morpholinos may be modified, for example by adding or altering various substituent groups from the above morpholino structure. Such sugar surrogates are referred to herein as "modifed morpholinos."

Combinations of modifications are also provided without limitation, such as 2'-F-5'-methyl substituted nucleosides (see PCT International Application WO 2008/101157 published on 8/21/08 for other disclosed 5', 2'-bis substituted nucleosides) and replacement of the ribosyl ring oxygen atom with S and further substitution at the 2'-position (see published U.S. Patent Application US2005-0130923, published on June 16, 2005) or alternatively 5'-substitution of a bicyclic nucleic acid (see PCT International Application WO 2007/134181, published on 11/22/07 wherein a 4'-CH_2-O-2' bicyclic nucleoside is further substituted at the 5' position with a 5'-methyl or a 5'-vinyl group). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (*see, e.g.,* Srivastava *et al., J. Am. Chem. Soc.* 2007, *129(26)*, 8362-8379).

In certain embodiments, antisense compounds comprise one or more modified cyclohexenyl nucleosides, which is a nucleoside having a six-membered cyclohexenyl in place of the pentofuranosyl residue in naturally occurring nucleosides. Modified cyclohexenyl nucleosides include, but are not limited to those described in the art (see for example commonly owned, published PCT Application WO 2010/036696, published on April 10, 2010, Robeyns *et al., J. Am. Chem. Soc.,* 2008, *130(6)*, 1979-1984; Horváth *et al., Tetrahedron Letters,* 2007, *48*, 3621-3623; Nauwelaerts *et al., J. Am. Chem. Soc.*, 2007, *129(30)*, 9340-9348; Gu *et al.,*, *Nucleosides, Nucleotides & Nucleic Acids*, 2005, *24(5-7)*, 993-998; Nauwelaerts *et al., Nucleic Acids Research,* 2005, *33(8)*, 2452-2463; Robeyns et al., *Acta Crystallographica, Section F: Structural Biology and*

Crystallization Communications, 2005, *F61(6)*, 585-586; Gu *et al.*, *Tetrahedron*, 2004, *60(9)*, 2111-2123; Gu *et al.*, *Oligonucleotides*, 2003, *13(6)*, 479-489; Wang *et al.*, *J. Org. Chem.*, 2003, *68*, 4499-4505; Verbeure *et al.*, *Nucleic Acids Research*, 2001, *29(24)*, 4941-4947; Wang *et al.*, *J. Org. Chem.*, 2001, *66*, 8478-82; Wang *et al.*, *Nucleosides, Nucleotides & Nucleic Acids*, 2001, *20(4-7)*, 785-788; Wang *et al.*, *J. Am. Chem.*, 2000, *122*, 8595-8602; Published PCT application, WO 06/047842; and Published PCT Application WO 01/049687; the text of each is incorporated by reference herein, in their entirety). Certain modified cyclohexenyl nucleosides have Formula X.



X

wherein independently for each of said at least one cyclohexenyl nucleoside analog of Formula X:

Bx is a heterocyclic base moiety;

T_3 and T_4 are each, independently, an internucleoside linking group linking the cyclohexenyl nucleoside analog to an antisense compound or one of T_3 and T_4 is an internucleoside linking group linking the tetrahydropyran nucleoside analog to an antisense compound and the other of T_3 and T_4 is H, a hydroxyl protecting group, a linked conjugate group, or a 5'-or 3'-terminal group; and

q_1, q_2, q_3, q_4, q_5, q_6, q_7, q_8 and q_9 are each, independently, H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_2-C_6 alkenyl, substituted C_2-C_6 alkenyl, C_2-C_6 alkynyl, substituted C_2-C_6 alkynyl or other sugar substituent group.

Many other monocyclic, bicyclic and tricyclic ring systems are known in the art and are suitable as sugar surrogates that can be used to modify nucleosides for incorporation into oligomeric compounds as provided herein (see for example review article: Leumann, Christian J. *Bioorg. & Med. Chem.*, 2002, *10*, 841-854). Such ring systems can undergo various additional substitutions to further enhance their activity.

As used herein, "2'-modified sugar" means a furanosyl sugar modified at the 2' position. In certain embodiments, such modifications include substituents selected from: a halide, including, but not limited to substituted and unsubstituted alkoxy, substituted and unsubstituted thioalkyl, substituted and unsubstituted amino alkyl, substituted and unsubstituted alkyl, substituted and unsubstituted allyl, and substituted and unsubstituted alkynyl. In certain embodiments, 2'

modifications are selected from substituents including, but not limited to: $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nF$, $O(CH_2)_nONH_2$, $OCH_2C(=O)N(H)CH_3$, and $O(CH_2)_nON[(CH_2)_nCH_3]_2$, where n and m are from 1 to about 10. Other 2'- substituent groups can also be selected from: C_1-C_{12} alkyl, substituted alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, F, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving pharmacokinetic properties, or a group for improving the pharmacodynamic properties of an antisense compound, and other substituents having similar properties. In certain embodiments, modifed nucleosides comprise a 2'-MOE side chain (Baker *et al.*, *J. Biol. Chem.*, 1997, *272*, 11944-12000). Such 2'-MOE substitution have been described as having improved binding affinity compared to unmodified nucleosides and to other modified nucleosides, such as 2'- *O*-methyl, *O*-propyl, and *O*-aminopropyl. Oligonucleotides having the 2'-MOE substituent also have been shown to be antisense inhibitors of gene expression with promising features for *in vivo* use (Martin, *Helv. Chim. Acta*, 1995, *78*, 486-504; Altmann *et al.*, *Chimia*, 1996, *50*, 168-176; Altmann *et al.*, *Biochem. Soc. Trans.*, 1996, *24*, 630-637; and Altmann *et al.*, *Nucleosides Nucleotides*, 1997, *16*, 917-926).

As used herein, "2'-modified" or "2'-substituted" refers to a nucleoside comprising a sugar comprising a substituent at the 2' position other than H or OH. 2'-modified nucleosides, include, but are not limited to, bicyclic nucleosides wherein the bridge connecting two carbon atoms of the sugar ring connects the 2' carbon and another carbon of the sugar ring; and nucleosides with non-bridging 2'substituents, such as allyl, amino, azido, thio, O-allyl, O-C_1-C_{10} alkyl, -OCF_3, O-$(CH_2)_2$-O-CH_3, 2'-$O(CH_2)_2SCH_3$, O-$(CH_2)_2$-O-$N(R_m)(R_n)$, or O-CH_2-C(=O)-$N(R_m)(R_n)$, where each R_m and R_n is, independently, H or substituted or unsubstituted C_1-C_{10} alkyl. 2'-modifed nucleosides may further comprise other modifications, for example at other positions of the sugar and/or at the nucleobase.

As used herein, "2'-F" refers to a nucleoside comprising a sugar comprising a fluoro group at the 2' position of the sugar ring.

As used herein, "2'-OMe" or "2'-OCH_3", "2'-O-methyl" or "2'-methoxy" each refers to a nucleoside comprising a sugar comprising an -OCH_3 group at the 2' position of the sugar ring.

As used herein, "MOE" or "2'-MOE" or "2'-$OCH_2CH_2OCH_3$" or "2'-O-methoxyethyl" each refers to a nucleoside comprising a sugar comprising a -$OCH_2CH_2OCH_3$ group at the 2' position of the sugar ring.

Methods for the preparations of modified sugars are well known to those skilled in the art. Some representative U.S. patents that teach the preparation of such modified sugars include without limitation, U.S.: 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,670,633; 5,700,920; 5,792,847 and 6,600,032 and International Application PCT/US2005/019219, filed June 2, 2005 and published as WO 2005/121371 on December 22, 2005, and each of which is herein incorporated by reference in its entirety.

As used herein, "oligonucleotide" refers to a compound comprising a plurality of linked nucleosides. In certain embodiments, one or more of the plurality of nucleosides is modified. In certain embodiments, an oligonucleotide comprises one or more ribonucleosides (RNA) and/or deoxyribonucleosides (DNA).

In nucleotides having modified sugar moieties, the nucleobase moieties (natural, modified or a combination thereof) are maintained for hybridization with an appropriate nucleic acid target.

In certain embodiments, antisense compounds comprise one or more nucleosides having modified sugar moieties. In certain embodiments, the modified sugar moiety is 2'-MOE. In certain embodiments, the 2'-MOE modified nucleosides are arranged in a gapmer motif. In certain embodiments, the modified sugar moiety is a bicyclic nucleoside having a (4'-$CH(CH_3)$-O-2') bridging group. In certain embodiments, the (4'-$CH(CH_3)$-O-2') modified nucleosides are arranged throughout the wings of a gapmer motif.

Compositions and Methods for Formulating Pharmaceutical Compositions

Antisense oligonucleotides may be admixed with pharmaceutically acceptable active or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions are dependent upon a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

An antisense compound targeted to a C9ORF72 nucleic acid can be utilized in pharmaceutical compositions by combining the antisense compound with a suitable pharmaceutically acceptable diluent or carrier. A pharmaceutically acceptable diluent includes phosphate-buffered saline (PBS). PBS is a diluent suitable for use in compositions to be delivered parenterally. Accordingly, in one embodiment, employed in the methods described herein is a pharmaceutical composition comprising an antisense compound targeted to a C9ORF72 nucleic acid

and a pharmaceutically acceptable diluent. In certain embodiments, the pharmaceutically acceptable diluent is PBS. In certain embodiments, the antisense compound is an antisense oligonucleotide.

Pharmaceutical compositions comprising antisense compounds encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any other oligonucleotide which, upon administration to an animal, including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, the disclosure is also drawn to pharmaceutically acceptable salts of antisense compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium and potassium salts.

A prodrug can include the incorporation of additional nucleosides at one or both ends of an antisense compound which are cleaved by endogenous nucleases within the body, to form the active antisense compound.

Conjugated Antisense Compounds

Antisense compounds may be covalently linked to one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the resulting antisense oligonucleotides. Typical conjugate groups include cholesterol moieties and lipid moieties. Additional conjugate groups include carbohydrates, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes.

Antisense compounds can also be modified to have one or more stabilizing groups that are generally attached to one or both termini of antisense compounds to enhance properties such as, for example, nuclease stability. Included in stabilizing groups are cap structures. These terminal modifications protect the antisense compound having terminal nucleic acid from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap), or at the 3'-terminus (3'-cap), or can be present on both termini. Cap structures are well known in the art and include, for example, inverted deoxy abasic caps. Further 3' and 5'-stabilizing groups that can be used to cap one or both ends of an antisense compound to impart nuclease stability include those disclosed in WO 03/004602 published on January 16, 2003.

Cell culture and antisense compounds treatment

The effects of antisense compounds on the level, activity or expression of C9ORF72 nucleic acids can be tested *in vitro* in a variety of cell types. Cell types used for such analyses are

available from commerical vendors (*e.g.* American Type Culture Collection, Manassus, VA; Zen-Bio, Inc., Research Triangle Park, NC; Clonetics Corporation, Walkersville, MD) and are cultured according to the vendor's instructions using commercially available reagents (e.g. Invitrogen Life Technologies, Carlsbad, CA). Illustrative cell types include, but are not limited to, HepG2 cells, Hep3B cells, and primary hepatocytes.

In vitro testing of antisense oligonucleotides

Described herein are methods for treatment of cells with antisense oligonucleotides, which can be modified appropriately for treatment with other antisense compounds.

In general, cells are treated with antisense oligonucleotides when the cells reach approximately 60-80% confluency in culture.

One reagent commonly used to introduce antisense oligonucleotides into cultured cells includes the cationic lipid transfection reagent LIPOFECTIN (Invitrogen, Carlsbad, CA). Antisense oligonucleotides are mixed with LIPOFECTIN in OPTI-MEM 1 (Invitrogen, Carlsbad, CA) to achieve the desired final concentration of antisense oligonucleotide and a LIPOFECTIN concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another reagent used to introduce antisense oligonucleotides into cultured cells includes LIPOFECTAMINE (Invitrogen, Carlsbad, CA). Antisense oligonucleotide is mixed with LIPOFECTAMINE in OPTI-MEM 1 reduced serum medium (Invitrogen, Carlsbad, CA) to achieve the desired concentration of antisense oligonucleotide and a LIPOFECTAMINE concentration that typically ranges 2 to 12 ug/mL per 100 nM antisense oligonucleotide.

Another technique used to introduce antisense oligonucleotides into cultured cells includes electroporation.

Cells are treated with antisense oligonucleotides by routine methods. Cells are typically harvested 16-24 hours after antisense oligonucleotide treatment, at which time RNA or protein levels of target nucleic acids are measured by methods known in the art and described herein. In general, when treatments are performed in multiple replicates, the data are presented as the average of the replicate treatments.

The concentration of antisense oligonucleotide used varies from cell line to cell line. Methods to determine the optimal antisense oligonucleotide concentration for a particular cell line are well known in the art. Antisense oligonucleotides are typically used at concentrations ranging from 1 nM to 300 nM when transfected with LIPOFECTAMINE. Antisense oligonucleotides are

used at higher concentrations ranging from 625 to 20,000 nM when transfected using electroporation.

RNA Isolation

RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. RNA is prepared using methods well known in the art, for example, using the TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommended protocols.

Analysis of inhibition of target levels or expression

Inhibition of levels or expression of a C9ORF72 nucleic acid can be assayed in a variety of ways known in the art. For example, target nucleic acid levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or quantitaive real-time PCR. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation are well known in the art. Northern blot analysis is also routine in the art. Quantitative real-time PCR can be conveniently accomplished using the commercially available ABI PRISM 7600, 7700, or 7900 Sequence Detection System, available from PE-Applied Biosystems, Foster City, CA and used according to manufacturer's instructions.

Quantitative Real-Time PCR Analysis of Target RNA Levels

Quantitation of target RNA levels may be accomplished by quantitative real-time PCR using the ABI PRISM 7600, 7700, or 7900 Sequence Detection System (PE-Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Methods of quantitative real-time PCR are well known in the art.

Prior to real-time PCR, the isolated RNA is subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. The RT and real-time PCR reactions are performed sequentially in the same sample well. RT and real-time PCR reagents are obtained from Invitrogen (Carlsbad, CA). RT real-time-PCR reactions are carried out by methods well known to those skilled in the art.

Gene (or RNA) target quantities obtained by real time PCR are normalized using either the expression level of a gene whose expression is constant, such as cyclophilin A, or by quantifying total RNA using RIBOGREEN (Invitrogen, Inc. Carlsbad, CA). Cyclophilin A expression is

quantified by real time PCR, by being run simultaneously with the target, multiplexing, or separately. Total RNA is quantified using RIBOGREEN RNA quantification reagent (Invetrogen, Inc. Eugene, OR). Methods of RNA quantification by RIBOGREEN are taught in Jones, L.J., et al, (Analytical Biochemistry, 1998, 265, 368-374). A CYTOFLUOR 4000 instrument (PE Applied Biosystems) is used to measure RIBOGREEN fluorescence.

Probes and primers are designed to hybridize to a C9ORF72 nucleic acid. Methods for designing real-time PCR probes and primers are well known in the art, and may include the use of software such as PRIMER EXPRESS Software (Applied Biosystems, Foster City, CA).

Analysis of Protein Levels

Antisense inhibition of C9ORF72 nucleic acids can be assessed by measuring C9ORF72 protein levels. Protein levels of C9ORF72 can be evaluated or quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), enzyme-linked immunosorbent assay (ELISA), quantitative protein assays, protein activity assays (for example, caspase activity assays), immunohistochemistry, immunocytochemistry or fluorescence-activated cell sorting (FACS). Antibodies directed to a target can be identified and obtained from a variety of sources, such as the MSRS catalog of antibodies (Aerie Corporation, Birmingham, MI), or can be prepared via conventional monoclonal or polyclonal antibody generation methods well known in the art. Antibodies useful for the detection of mouse, rat, monkey, and human C9ORF72 are commercially available.

In vivo testing of antisense compounds

Antisense compounds, for example, antisense oligonucleotides, are tested in animals to assess their ability to inhibit expression of C9ORF72 and produce phenotypic changes, such as, improved motor function and respiration. In certain embodiments, motor function is measured by rotarod, grip strength, pole climb, open field performance, balance beam, hindpaw footprint testing in the animal. In certain embodiments, respiration is measured by whole body plethysmograph, invasive resistance, and compliance measurements in the animal. Testing may be performed in normal animals, or in experimental disease models. For administration to animals, antisense oligonucleotides are formulated in a pharmaceutically acceptable diluent, such as phosphate-buffered saline (PBS) or artificial cerebrospinal fluid (aCSF). Administration includes parenteral routes of administration, such as intraperitoneal, intravenous, and subcutaneous, as well as central

routes of administration such as intracerebroventricular or intrathecal. Calculation of antisense oligonucleotide dosage and dosing frequency is within the abilities of those skilled in the art, and depends upon factors such as route of administration and animal body weight. Following a period of treatment with antisense oligonucleotides, RNA is isolated from CNS tissue or CSF and changes in C9ORF72 nucleic acid expression are measured.

Targeting C9ORF72

Antisense oligonucleotides described herein may hybridize to a C9ORF72 nucleic acid in any stage of RNA processing. For example, described herein are antisense oligonucleotides that are complementary to a pre-mRNA or a mature mRNA. Additionally, antisense oligonucleotides described herein may hybridize to any element of a C9ORF72 nucleic acid. For example, described herein are antisense oligonucleotides that are complementary to an exon, an intron, the 5' UTR, the 3' UTR, a repeat region, a hexanucleotide repeat expansion, a splice junction, an exon:exon splice junction, an exonic splicing silencer (ESS), an exonic splicing enhancer (ESE), exon 1a, exon 1b, exon 1c, exon 1d, exon 1e, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon11, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, intron 9, or intron 10 of a C9ORF72 nucleic acid.

In certain embodiments, antisense oligonucleotides described herein hybridize to all variants of C9ORF72. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to certain variants of C9ORF72. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to variants of C9ORF72 containing a hexanucleotide repeat expansion. In certain embodiments, the antisense oligonucleotides described herein selectively hybridize to pre-mRNA variants containing the hexanucleotide repeat. In certain embodiments, pre-mRNA variants of C9ORF72 containing a hexanucleotide repeat expansion include SEQ ID NO: 1-3 and 6-10. In certain embodiments, such hexanucleotide repeat expansion comprises at least 24 repeats of any of GGGGCC, GGGGGG, GGGGGC, or GGGGCG.

In certain embodiments, the antisense oligonucleotides described herein inhibit expression of all variants of C9ORF72. In certain embodiments, the antisense oligonucleotides described herein inhibit expression of all variants of C9ORF72 equally. In certain embodiments, the antisense oligonucleotides described herein preferentially inhibit expression of one or more variants of C9ORF72. In certain embodiments, the antisense oligonucleotides described herein preferentially inhibit expression of variants of C9ORF72 containing a hexanucleotide repeat expansion. In certain

embodiments, the antisense oligonucleotides described herein selectively inhibit expression of pre-mRNA variants containing the hexanucleotide repeat. In certain embodiments, the antisense oligonucleotides described herein selectively inhibit expression of C9ORF72 pathogenic associated mRNA variants. In certain embodiments, pre-mRNA variants of C9ORF72 containing a hexanucleotide repeat expansion include SEQ ID NO: 1-3 and 6-10. In certain embodiments, such hexanucleotide repeat expansion comprises at least 24 repeats of any of GGGGCC, GGGGGG, GGGGGC, or GGGGCG. In certain embodiments, the hexanucleotide repeat expansion forms nuclear foci. In certain embodiments, antisense oligonucleotides described herein are useful for reducing nuclear foci. Nuclear foci may be reduced in terms of percent of cells with foci as well as number of foci per cell.

Selective Inhibition of Certain Pathogenic Associated Variants

In certain examples herein, primer probe set RTS3905 detects an mRNA variant (e.g. NM_001256054.1) processed from a pre-mRNA variant containing the hexanucleotide repeat. The mRNA variant processed from a pre-mRNA variant containing the hexanucleotide repeat (i.e., the "C9ORF72 pathogenic associated mRNA variant"). A pre-mRNA contains the hexanucleotide repeat when transcription of the pre-mRNA begins in the region from the start site of exon 1A to the start site of exon 1B, e.g., nucleotides 1107 to 1520 of the genomic sequence (SEQ ID NO: 2, the complement of GENBANK Accession No. NT_008413.18 truncated from nucleosides 27535000 to 27565000). Oligonucleotides were designed in this region to selectively target the pre-mRNA variant containing the hexanucleotide repeat. RTS3905 measures an mRNA product (i.e. the C9ORF72 pathogenic associated mRNA variant) of the pre-mRNA variant containing the hexanucleotide repeat and, therefore, measures the reduction of the pre-mRNA variant containing the hexanucleotide repeat.

C9ORF72 Features

Antisense oligonucleotides described herein may hybridize to any C9ORF72 variant at any state of processing within any element of the C9ORF72 gene. For example, antisense oligonucleotides described herein may hybridize to an exon, an intron, the 5' UTR, the 3' UTR, a repeat region, a hexanucleotide repeat expansion, a splice junction, an exon:exon splice junction, an exonic splicing silencer (ESS), an exonic splicing enhancer (ESE), exon 1a, exon 1b, exon 1c, exon 1d, exon 1e, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, exon 10, exon 11,

intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, intron 9, or intron 10. For example, antisense oligonucleotides may target any of the exons characterized below in Tables 1-5 for the various C9ORF72 variants described below. Antisense oligonucleotides described herein may also target variants not characterized below and such variants are characterized in GENBANK. Moreover, antisense oligonucleotides described herein may also target elements other than exons and such elements are characterized in GENBANK.

Table 1
Functional Segments for NM_001256054.1 (SEQ ID NO: 1)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1C	1	158	1137	1294
exon 2	159	646	7839	8326
exon 3	647	706	9413	9472
exon 4	707	802	12527	12622
exon 5	803	867	13354	13418
exon 6	868	940	14704	14776
exon 7	941	1057	16396	16512
exon 8	1058	1293	18207	18442
exon 9	1294	1351	24296	24353
exon 10	1352	1461	26337	26446
exon 11	1462	3339	26581	28458

Table 2
Functional Segments for NM_018325.3 (SEQ ID NO: 4)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1B	1	63	1510	1572
exon 2	64	551	7839	8326
exon 3	552	611	9413	9472
exon 4	612	707	12527	12622
exon 5	708	772	13354	13418
exon 6	773	845	14704	14776
exon 7	846	962	16396	16512
exon 8	963	1198	18207	18442

exon 9	1199	1256	24296	24353
exon 10	1257	1366	26337	26446
exon 11	1367	3244	26581	28458

Table 3
Functional Segments for NM_145005.5 (SEQ ID NO: 6)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1A	1	80	1137	1216
exon 2	81	568	7839	8326
exon 3	569	628	9413	9472
exon 4	629	724	12527	12622
exon 5B (exon 5 into intron 5)	725	1871	13354	14500

Table 4
Functional Segments for DB079375.1 (SEQ ID NO: 7)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1E	1	35	1135	1169
exon 2	36	524	7839	8326
exon 3 (EST ends before end of full exon)	525	562	9413	9450

Table 5
Functional Segments for BU194591.1 (SEQ ID NO: 8)

Exon Number	mRNA start site	mRNA stop site	Start site in reference to SEQ ID NO: 2	Stop site in reference to SEQ ID NO: 2
exon 1D	1	36	1241	1279
exon 2	37	524	7839	8326
exon 3	525	584	9413	9472
exon 4	585	680	12527	12622
exon 5B (exon 5 into intron 5)	681	798	13354	13465

Certain Indications

In certain embodiments, provided herein are methods of treating an individual comprising administering one or more pharmaceutical compositions described herein. In certain embodiments, the individual has a neurodegenerative disease. In certain embodiments, the individual is at risk for developing a neurodegenerative disease, including, but not limited to, ALS or FTD. In certain embodiments, the individual has been identified as having a C9ORF72 associated disease. In certain embodiments, provided herein are methods for prophylactically reducing C9ORF72 expression in an individual. Certain embodiments include treating an individual in need thereof by administering to an individual a therapeutically effective amount of an antisense compound targeted to a C9ORF72 nucleic acid.

In one embodiment, administration of a therapeutically effective amount of an antisense compound targeted to a C9ORF72 nucleic acid is accompanied by monitoring of C9ORF72 levels in an individual, to determine an individual's response to administration of the antisense compound. An individual's response to administration of the antisense compound may be used by a physician to determine the amount and duration of therapeutic intervention.

In certain embodiments, administration of an antisense compound targeted to a C9ORF72 nucleic acid results in reduction of C9ORF72 expression by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values. In certain embodiments, administration of an antisense compound targeted to a C9ORF72 nucleic acid results in improved motor function and respiration in an animal. In certain embodiments, administration of a C9ORF72 antisense compound improves motor function and respiration by at least 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99%, or a range defined by any two of these values.

In certain embodiments, pharmaceutical compositions comprising an antisense compound targeted to C9ORF72 are used for the preparation of a medicament for treating a patient suffering or susceptible to a neurodegenerative disease including ALS and FTD.

Certain Combination Therapies

In certain embodiments, one or more pharmaceutical compositions described herein are co-administered with one or more other pharmaceutical agents. In certain embodiments, such one or more other pharmaceutical agents are designed to treat the same disease, disorder, or condition as the one or more pharmaceutical compositions described herein. In certain embodiments, such one or

more other pharmaceutical agents are designed to treat a different disease, disorder, or condition as the one or more pharmaceutical compositions described herein. In certain embodiments, such one or more other pharmaceutical agents are designed to treat an undesired side effect of one or more pharmaceutical compositions described herein. In certain embodiments, one or more pharmaceutical compositions described herein are co-administered with another pharmaceutical agent to treat an undesired effect of that other pharmaceutical agent. In certain embodiments, one or more pharmaceutical compositions described herein are co-administered with another pharmaceutical agent to produce a combinational effect. In certain embodiments, one or more pharmaceutical compositions described herein are co-administered with another pharmaceutical agent to produce a synergistic effect.

In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are administered at the same time. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are administered at different times. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are prepared together in a single formulation. In certain embodiments, one or more pharmaceutical compositions described herein and one or more other pharmaceutical agents are prepared separately.

In certain embodiments, pharmaceutical agents that may be co-administered with a pharmaceutical composition described herein include Riluzole (Rilutek), Lioresal (Lioresal), and Dexpramipexole.

In certain embodiments, pharmaceutical agents that may be co-administered with a C9ORF72 specific inhibitor described herein include, but are not limited to, an additional C9ORF72 inhibitor.

In certain embodiments, pharmaceutical agents that may be co-administered with a C9ORF72 specific inhibitor described herein include, but are not limited to, a C9ORF72 antisense transcript specific inhibitor. In certain embodiments, the C9ORF72 antisense transcript specific inhibitor is an antisense compound. In certain embodiments, the antisense compound is a modified oligonucleotide. In certain embodiments, the modified oligonucleotide is single-stranded.

In certain embodiments, the co-administered pharmaceutical agent is administered prior to administration of a pharmaceutical composition described herein. In certain embodiments, the co-administered pharmaceutical agent is administered following administration of a pharmaceutical composition described herein. In certain embodiments the co-administered pharmaceutical agent is

administered at the same time as a pharmaceutical composition described herein. In certain embodiments the dose of a co-administered pharmaceutical agent is the same as the dose that would be administered if the co-administered pharmaceutical agent was administered alone. In certain embodiments the dose of a co-administered pharmaceutical agent is lower than the dose that would be administered if the co-administered pharmaceutical agent was administered alone. In certain embodiments the dose of a co-administered pharmaceutical agent is greater than the dose that would be administered if the co-administered pharmaceutical agent was administered alone.

In certain embodiments, the co-administration of a second compound enhances the effect of a first compound, such that co-administration of the compounds results in an effect that is greater than the effect of administering the first compound alone. In other embodiments, the co-administration results in effects that are additive of the effects of the compounds when administered alone. In certain embodiments, the co-administration results in effects that are supra-additive of the effects of the compounds when administered alone. In certain embodiments, the first compound is an antisense compound. In certain embodiments, the second compound is an antisense compound.

Certain Comparator Compositions

In certain embodiments, compounds described herein are more tolerable than ISIS 576816, ISIS 576974, ISIS 577061, ISIS 577065, and/or ISIS 577083. ISIS 576816, ISIS 576974, ISIS 577061, ISIS 577065, and ISIS 577083 were selected as comparator compounds because they exhibited high levels of dose-dependent reduction of C9ORF72 mRNA in various studies described in WO2014/062691. Thus, ISIS 576816, ISIS 576974, ISIS 577061, ISIS 577065, and ISIS 577083 were deemed highly efficacious and potent compounds. Additionally, ISIS 577065, ISIS 577056, and ISIS 576816 described in WO2014/062691 are structurally similar as compounds described herein. For example, ISIS 577065 has a 16 nucleobase overlap with ISIS 801287; ISIS 577056 has a 16 nucleobase overlap with ISIS 806679; ISIS 576816 has an 18 nucleobase overlap with ISIS 802473 (18-mer); and ISIS 576816 has an 18 nucleobase overlap with ISIS 802459.

In certain embodiments, ISIS 576816, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') GCCTTACTCTAGGACCAAGA (incorporated herein as SEQ ID NO: 21), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 576816 achieved an average FOB score of 7.00 in a study of acute tolerability in mice (see Example 3

hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS 802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 576816.

In certain embodiments, ISIS 576974, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') GGGACACTACAAGGTAGTAT (incorporated herein as SEQ ID NO: 56), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 576974 achieved an average FOB score of 5.67 in a study of acute tolerability in mice (see Example 3 hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS 802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 576974.

In certain embodiments, ISIS 577061, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') TACAGGCTGCGGTTGTTTCC (incorporated herein as SEQ ID NO: 57), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 577061 achieved an average FOB score of 7.00 in a study of acute tolerability in mice (see Example 3 hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS 802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 577061.

In certain embodiments, ISIS 577065, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') CCCGGCCCCTAGCGCGCGAC (incorporated herein as SEQ ID NO: 58), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 577065 achieved an average FOB score of 6.00 in a study of acute tolerability in mice (see Example 3 hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS

802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 577065.

In certain embodiments, ISIS 577083, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') GGTAACTTCAAACTCTTGGG (incorporated herein as SEQ ID NO: 59), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 577083 achieved an average FOB score of 7.00 in a study of acute tolerability in mice (see Example 3 hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS 802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 577083.

In certain embodiments, ISIS 577056, a 5-10-5 MOE gapmer, having a sequence of (from 5' to 3') AATCTTTATCAGGTCTTTTC (incorporated herein as SEQ ID NO: 60), wherein each internucleoside linkage is a phosphorothioate linkage, each cytosine is a 5-methylcytosine, and each of nucleosides 1-5 and 16-20 comprise a 2'-O-methoxyethyl group, which was previously described in WO2014/062691, incorporated herein by reference, is a comparator compound. ISIS 577056 achieved an average FOB score of 6.5 in a study of acute tolerability in mice (see Example 3 hereinbelow). Certain compounds described herein achieved a lower FOB score in a similar study of acute tolerability in mice (see Example 2 hereinbelow), including ISIS 801287, ISIS 806679, ISIS 802473, and ISIS 802459. Therefore, certain compounds described herein are more tolerable than comparator compound ISIS 577056.

Certain Human Therapeutics

The human C9ORF72 antisense oligonucleotides described herein are human therapeutics. Various parameters of potency, efficacy, and/or tolerability are being examined. Such parameters include in vitro inhibition of total C9ORF72 RNA expression, in vitro inhibition of C9ORF72 pathogenic associated RNA variant expression, in vitro dose response (IC50), in vivo inhibition of total or pathogenic RNA and/or protein in a transgenic animal containing a human C9ORF72 transgene in relevant tissues (e.g., brain and/or spinal cord), tolerability in mouse, tolerability in rat, and/or tolerability in a primate. Tolerability markers that may be measured include blood and serum chemistry parameters, CSF chemistry parameters, body and organ weights, general observations

and/or behavioral tests, and/or biochemical markers such as GFAP and/or AIF1. Acute or long term tolerability may be measured.

Certain Compositions

1. ISIS 801287

In certain embodiments, ISIS 801287 is characterized as a 4-8-6 MOE gapmer, having a sequence of (from 5' to 3') GCCCCTAGCGCGCGACTC (incorporated herein as SEQ ID NO: 33), wherein each of nucleosides 1-4 and 13-18 are 2'-O-methoxyethylribose modified nucleosides, and each of nucleosides 5-12 are 2'-deoxynucleosides, wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 13 to 14, 14 to 15, and 15 to 16 are phosphodiester linkages and the internucleoside linkages between nucleosides 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 16 to 17, and 17 to 18 are phosphorothioate linkages, and wherein each cytosine is a 5'-methylcytosine.

In certain embodiments, ISIS 801287 is described by the following chemical notation: Ges mCeo mCeo mCes mCds Tds Ads Gds mCds Gds mCds Gds mCeo Geo Aeo mCes Tes mCe; wherein,

A = an adenine,

mC = a 5'-methylcytosine

G = a guanine,

T = a thymine,

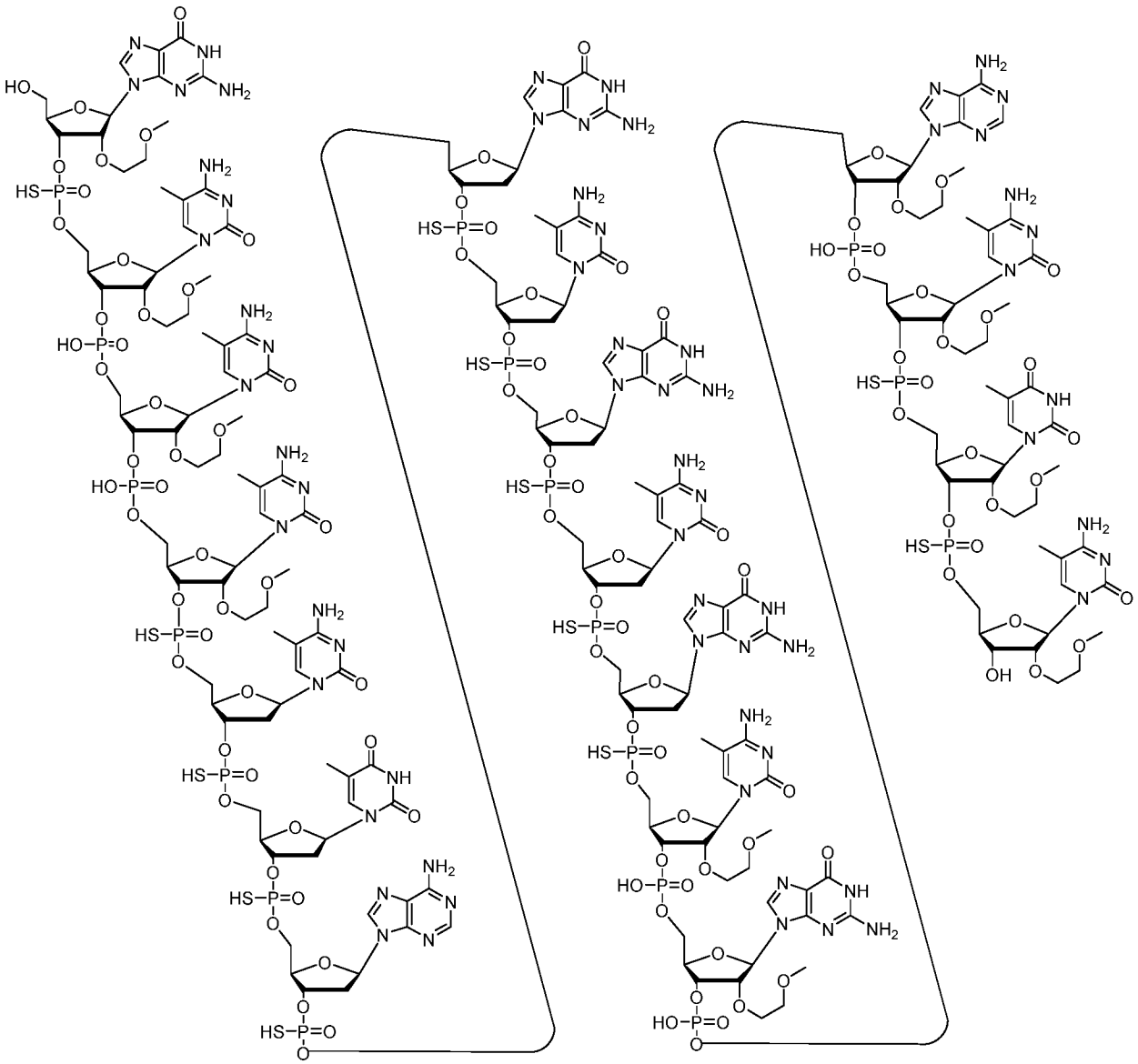
e = a 2'-O-methoxyethylribose modified sugar

d = a 2'-deoxyribose sugar,

s = a phosphorothioate internucleoside linkage, and

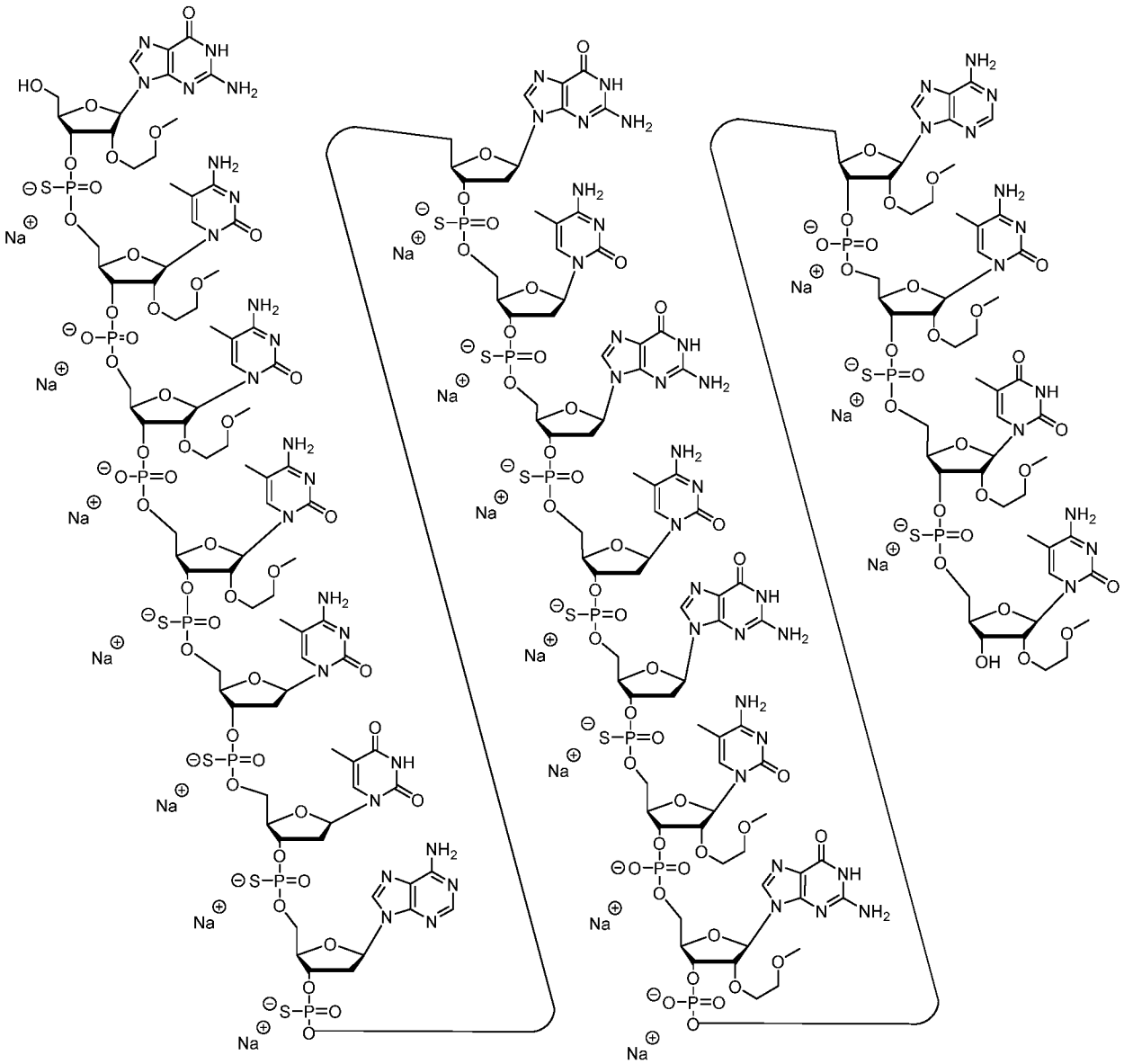
o = a phosphodiester internucleoside linkage.

In certain embodiments, ISIS 801287 is described by the following chemical structure, or a salt thereof:



Structure 1. ISIS 801287

In certain embodiments, the sodium salt of ISIS 801287 is described by the following chemical structure:



Structure 2. The sodium salt of ISIS 801287

2. ISIS 806679

In certain embodiments, ISIS 806679 is characterized as a 6-10-4 MOE gapmer, having a sequence of (from 5' to 3') GGTTAATCTTTATCAGGTCT (incorporated herein as SEQ ID NO: 49), wherein each of nucleosides 1-6 and 17-20 are 2'-O-methoxyethylribose modified nucleosides, and each of nucleosides 7-16 are 2'-deoxynucleosides, wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, and 17 to 18 are phosphodiester linkages and the internucleoside linkages between nucleosides 1 to 2, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, 14 to 15, 15 to 16, 16 to 17, 18 to 19, and 19 to 20 are phosphorothioate linkages, and wherein each cytosine is a 5'-methylcytosine.

In certain embodiments, ISIS 806679 is described by the following chemical notation: Ges Geo Teo Teo Aeo Aeo Tds mCds Tds Tds Tds Ads Tds mCds Ads Gds Geo Tes mCes Te; wherein,

A = an adenine,

mC = a 5'-methylcytosine

G = a guanine,

T = a thymine,

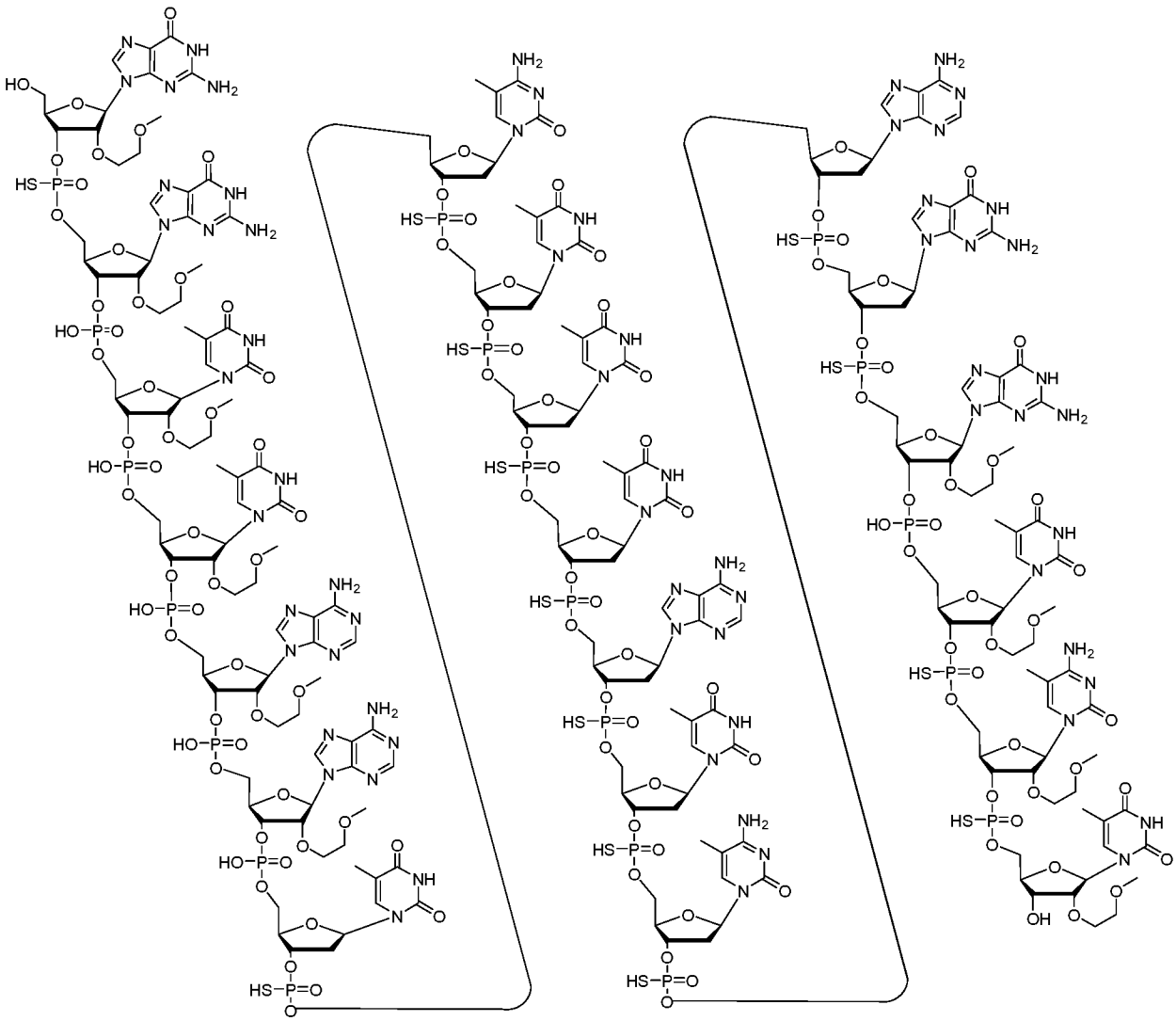
e = a 2'-O-methoxyethylribose modified sugar

d = a 2'-deoxyribose sugar,

s = a phosphorothioate internucleoside linkage, and

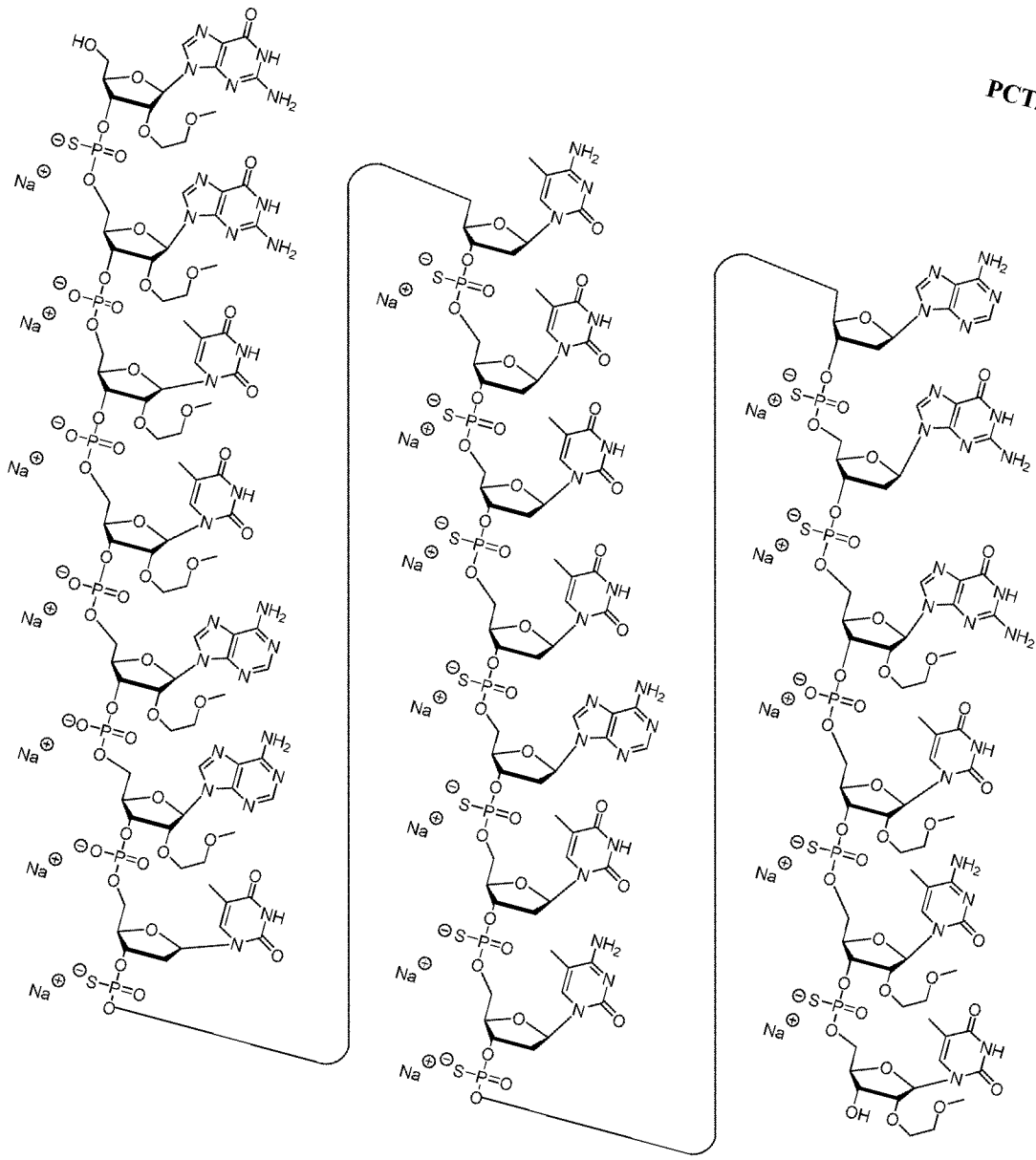
o = a phosphodiester internucleoside linkage.

In certain embodiments, ISIS 806679 is described by the following chemical structure, or a salt thereof:



Structure 3. ISIS 806679

In certain embodiments, the sodium salt of ISIS 806679 is described by the following chemical structure:



Structure 4. The sodium salt of ISIS 806679

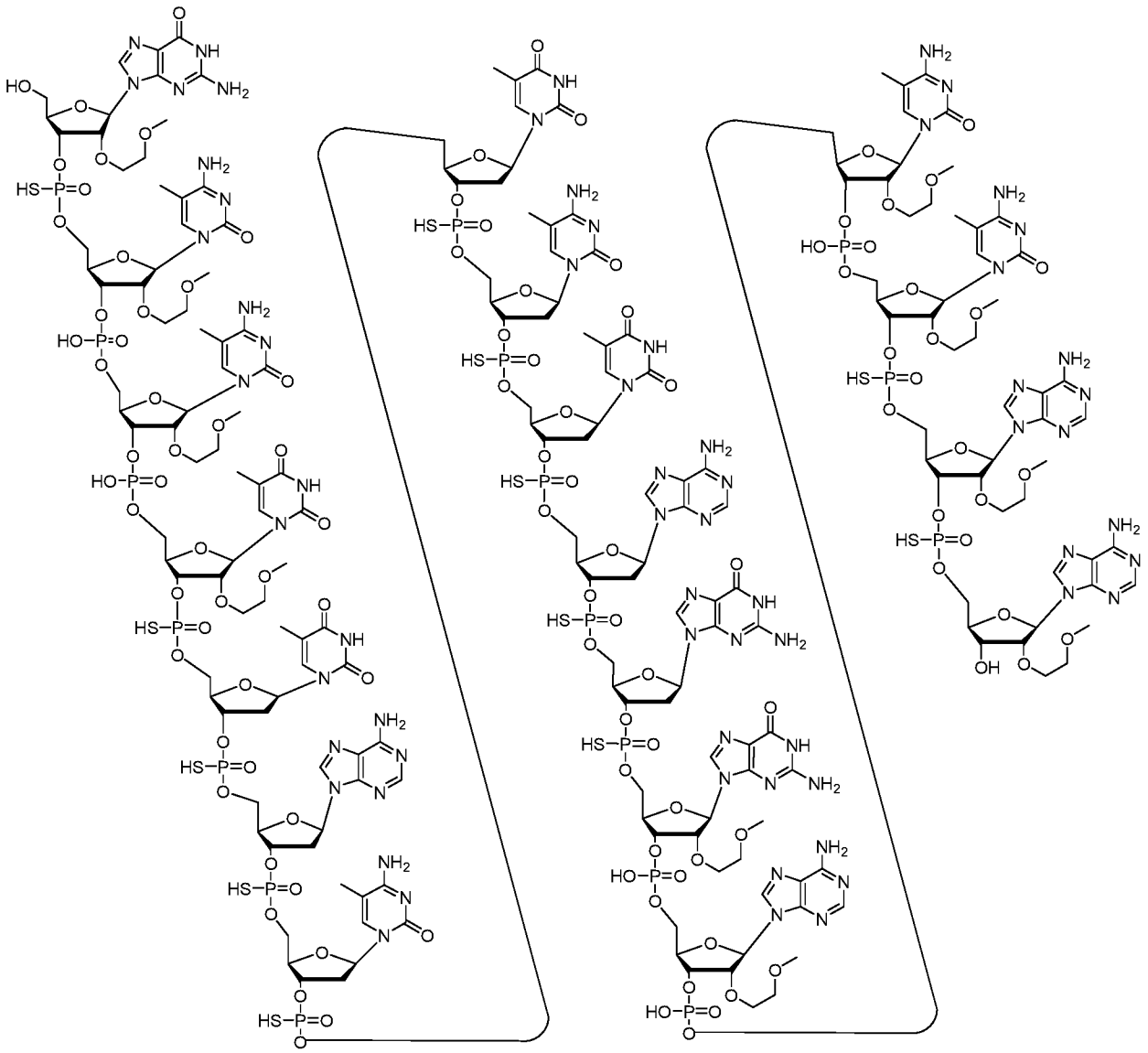
3. ISIS 802473

In certain embodiments, ISIS 802473 is characterized as a 4-8-6 MOE gapmer, having a sequence of (from 5' to 3') GCCTTACTCTAGGACCAA (incorporated herein as SEQ ID NO: 47), wherein each of nucleosides 1-4 and 13-18 are 2'-O-methoxyethylribose modified nucleosides, and each of nucleosides 5-12 are 2'-deoxynucleosides, wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 13 to 14, 14 to 15, and 15 to 16 are phosphodiester linkages and the internucleoside linkages between nucleosides are 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 16 to 17, and 17 to 18 are phosphorothioate linkages, and wherein each cytosine is a 5'-methylcytosine.

In certain embodiments, ISIS 802473 is described by the following chemical notation: Ges mCeo mCeo Tes Tds Ads mCds Tds mCds Tds Ads Gds Geo Aeo mCeo mCes Aes Ae; wherein,

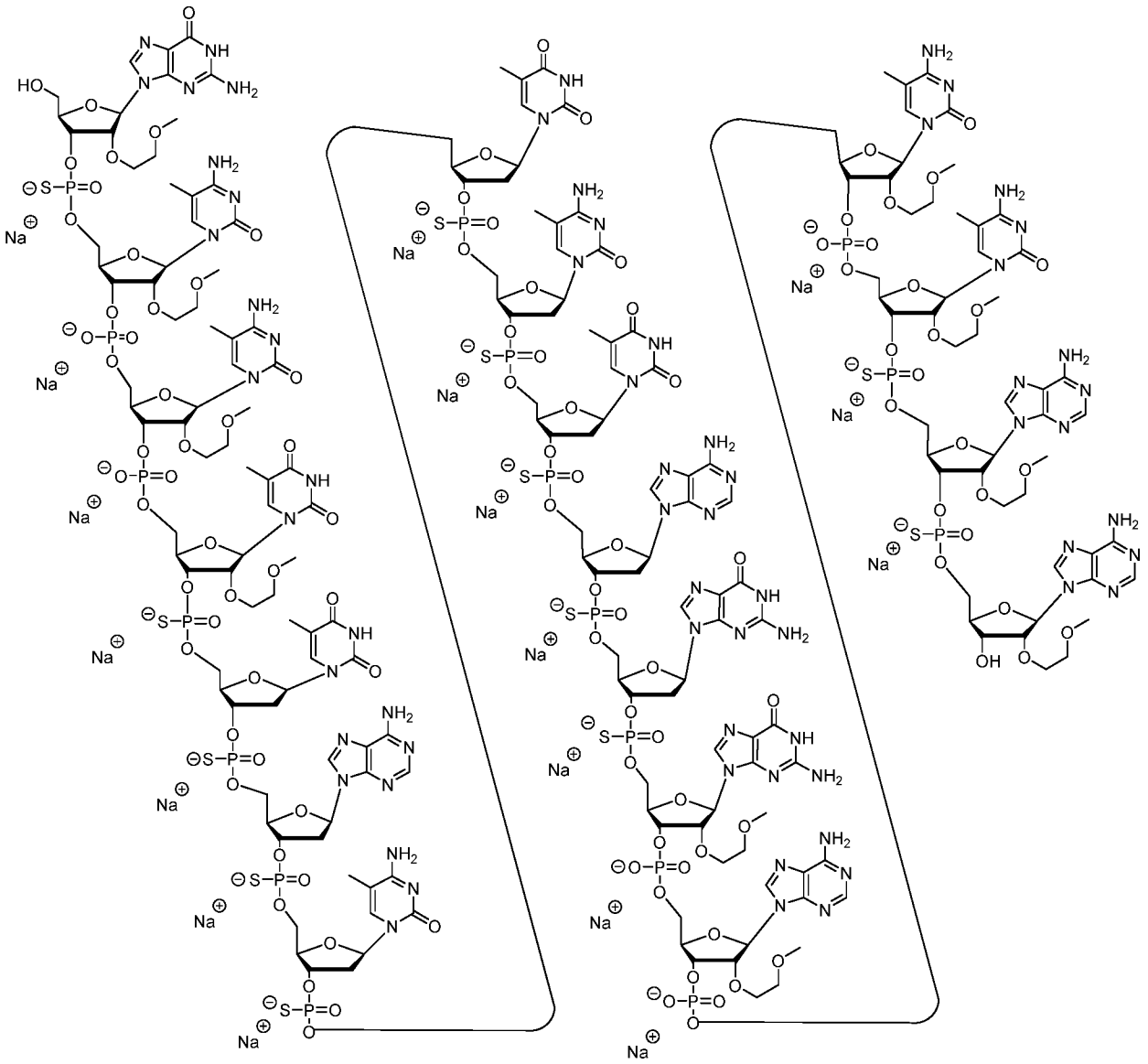
A = an adenine,
mC = a 5'-methylcytosine
G = a guanine,
T = a thymine,
e = a 2'-O-methoxyethylribose modified sugar
d = a 2'-deoxyribose sugar,
s = a phosphorothioate internucleoside linkage, and
o = a phosphodiester internucleoside linkage.

In certain embodiments, ISIS 802473 is described by the following chemical structure, or a salt thereof:



Structure 5. ISIS 802473

In certain embodiments, the sodium salt of ISIS 802473 is described by the following chemical structure:



Structure 6. The sodium salt of ISIS 802473

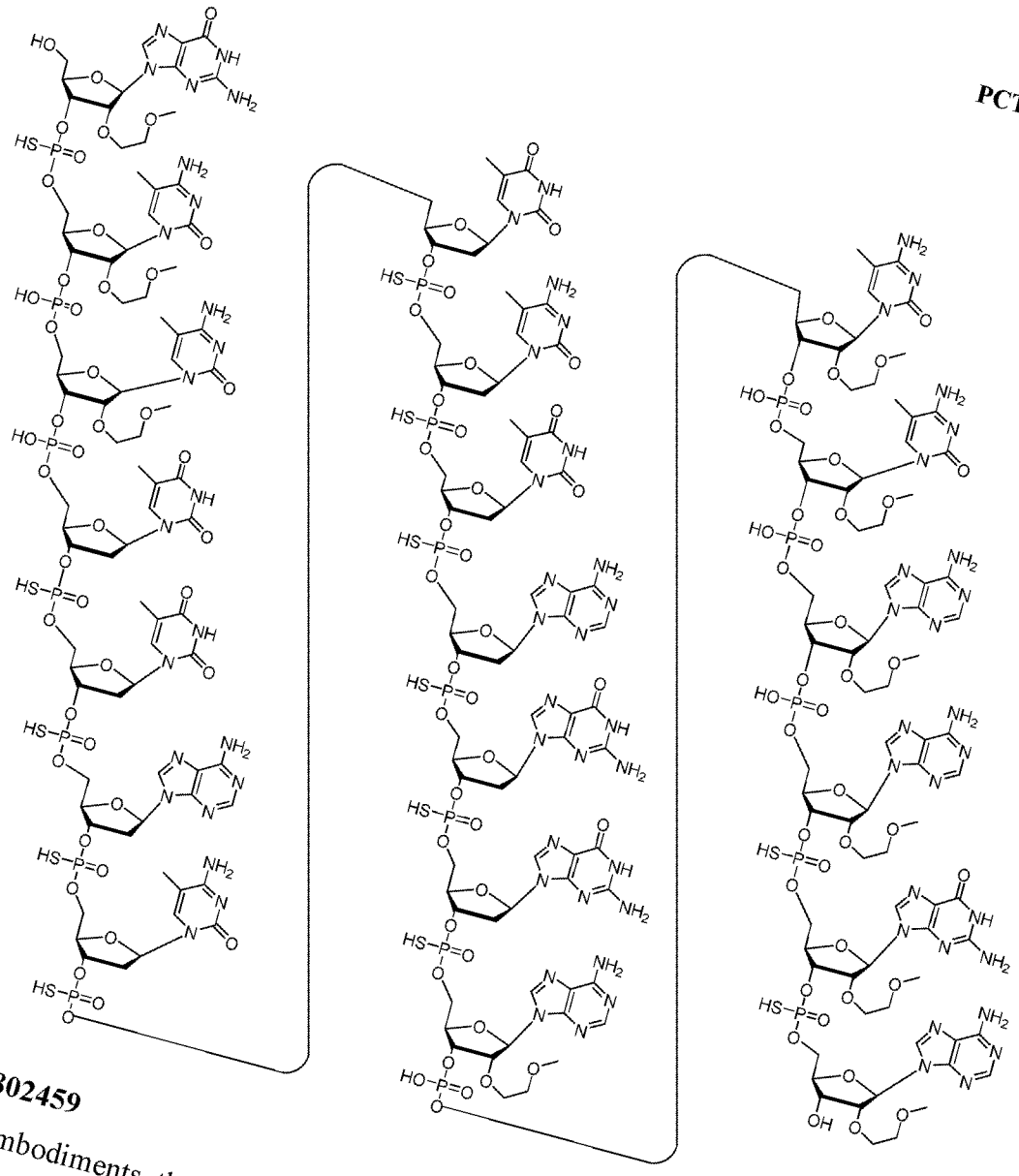
4. ISIS 802459

In certain embodiments, ISIS 802459 is characterized as a 3-10-7 MOE gapmer, having a sequence of (from 5' to 3') GCCTTACTCTAGGACCAAGA (incorporated herein as SEQ ID NO: 21), wherein each of nucleosides 1-3 and 14-20 are 2'-O-methoxyethylribose modified nucleosides, and each of nucleosides 4-13 are 2'-deoxynucleosides, wherein the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 14 to 15, 15 to 16, 16 to 17, and 17 to 18 are phosphodiester linkages and the internucleoside linkages between nucleosides are 1 to 2, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 9, 9 to 10, 10 to 11, 11 to 12, 12 to 13, 13 to 14, 18 to 19, and 19 to 20 are phosphorothioate linkages, and wherein each cytosine is a 5'-methylcytosine.

In certain embodiments, ISIS 802459 is described by the following chemical notation: Ges mCeo mCeo Tds Tds Ads mCds Tds mCds Tds Ads Gds Gds Aeo mCeo mCeo Aeo Aes Ges Ae; wherein,

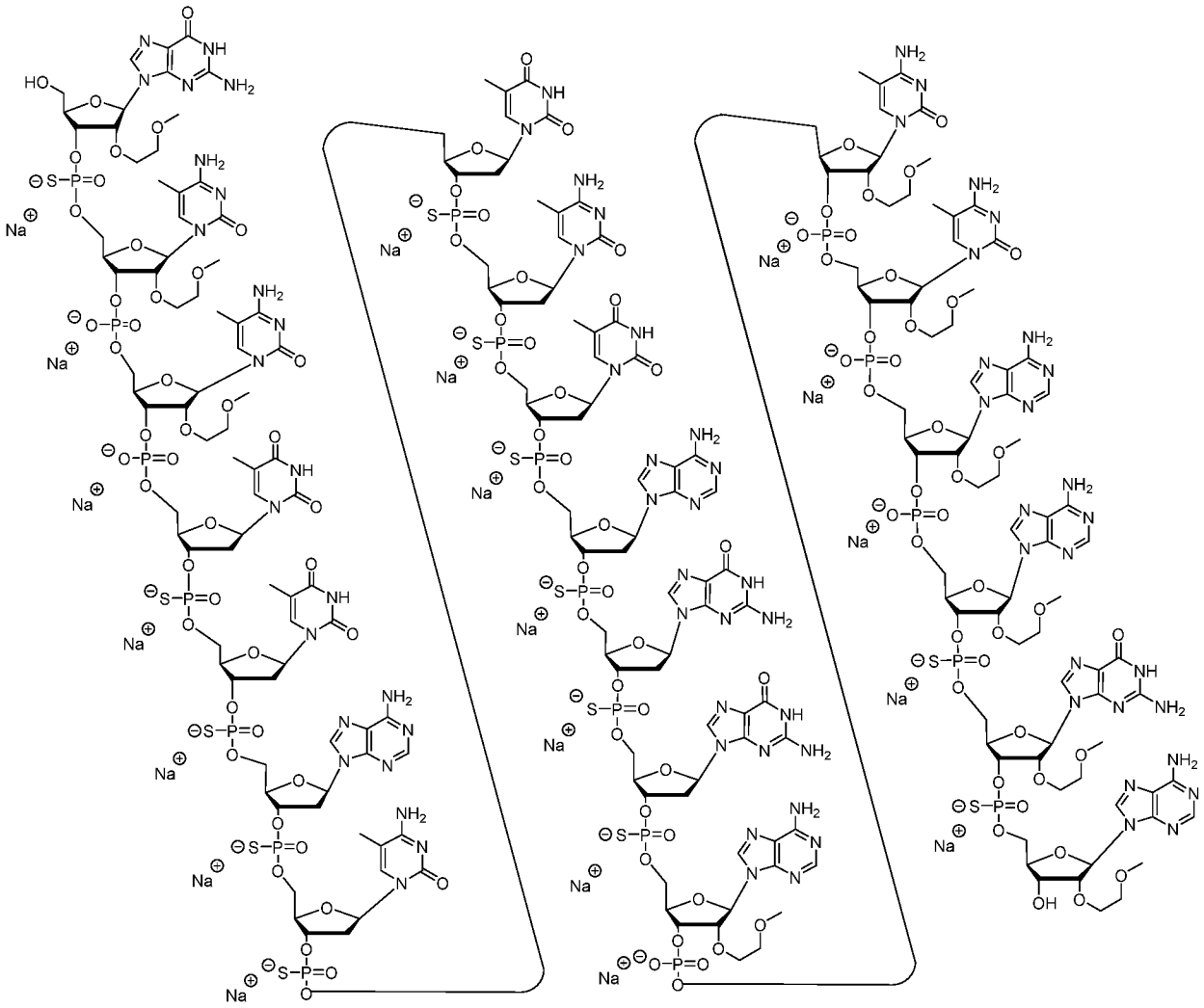
A = an adenine,
mC = a 5'-methylcytosine
G = a guanine,
T = a thymine,
e = a 2'-O-methoxyethylribose modified sugar
d = a 2'-deoxyribose sugar,
s = a phosphorothioate internucleoside linkage, and
o = a phosphodiester internucleoside linkage.

In certain embodiments, ISIS 802459 is described by the following chemical structure, or a salt thereof:



Structure 7. ISIS 802459

In certain embodiments, the sodium salt of ISIS 802459 is described by the following chemical structure:



Structure 8. The sodium salt of ISIS 802459

EXAMPLES

Non-limiting disclosure and incorporation by reference

While certain compounds, compositions, and methods described herein have been described with specificity in accordance with certain embodiments, the following examples serve only to illustrate the compounds described herein and are not intended to limit the same. Each of the references recited in the present application is incorporated herein by reference in its entirety.

Example 1: Antisense oligonucleotides targeting human C9ORF72

The antisense oligonucleotides in the table below were designed as MOE gapmers. The central gap segment of each gapmer contains 2'-deoxynucleosides and is flanked by wing segments on both the 5' end

and on the 3' end containing nucleosides that each comprise a 2'-MOE group. The specific motif of each gapmer is listed in table below, represented by three numbers separated by hyphens. The numbers represent the number of nucleosides in the 5'-wing, the gap, and the 3'-wing, respectively. All cytosine residues throughout each oligonucleotide are 5-methylcytosines. The internucleoside linkages for the gapmers are mixed phosphorothioate and phosphodiester linkages. The internucleoside linkages for each gapmer are presented in the Linkage column, where 'o' indicates a phosphodiester linkage and's' indicates a phosphorothioate linkage.

Each antisense oligonucleotide listed in the table below is targeted to the human C9ORF72 genomic sequence, designated herein as SEQ ID NO: 2 (the complement of GENBANK Accession No. NT_008413.18 truncated from nucleosides 27535000 to 27565000). "Start site" indicates the 5'-most nucleoside to which the gapmer is targeted in the human genomic sequence. "Stop site" indicates the 3'-most nucleoside to which the gapmer is targeted human genomic sequence.

Table 6

Antisense oligonucleotides targeting human C9ORF72

Isis No.	Start Site	Stop Site	Sequence	Linkage	Motif	SEQ ID NO
791656	1445	1464	CCGGCCCCTAGCGCGCGACT	soossssssssssooooss	3-10-7	22
791657	1445	1464	CCGGCCCCTAGCGCGCGACT	sooossssssssssoooss	4-10-6	22
791658	1445	1464	CCGGCCCCTAGCGCGCGACT	sooooossssssssssoss	6-10-4	22
791659	1445	1464	CCGGCCCCTAGCGCGCGACT	soooooossssssssssss	7-10-3	22
791660	1445	1463	CGGCCCCTAGCGCGCGACT	soooossssssssssoss	5-9-5	23
791661	1446	1464	CCGGCCCCTAGCGCGCGAC	soooossssssssssoss	5-9-5	24
791662	1445	1463	CGGCCCCTAGCGCGCGACT	soooossssssssssoooss	4-9-6	23
791663	1446	1464	CCGGCCCCTAGCGCGCGAC	soooossssssssssoooss	4-9-6	24
791664	1445	1463	CGGCCCCTAGCGCGCGACT	soooooossssssssssoss	6-9-4	23
791665	1446	1464	CCGGCCCCTAGCGCGCGAC	soooooossssssssssoss	6-9-4	24
801274	1440	1459	CCCTAGCGCGCGACTCCTGA	soooossssssssssoooss	4-10-6	25
801275	1441	1460	CCCCTAGCGCGCGACTCCTG	soooossssssssssoooss	4-10-6	26
801276	1442	1461	GCCCCTAGCGCGCGACTCCT	soooossssssssssoooss	4-10-6	27
801277	1443	1462	GGCCCCTAGCGCGCGACTCC	soooossssssssssoooss	4-10-6	28
801278	1444	1463	CGGCCCCTAGCGCGCGACTC	soooossssssssssoooss	4-10-6	29
801279	1440	1459	CCCTAGCGCGCGACTCCTGA	soooooossssssssssoss	6-10-4	25
801280	1441	1460	CCCCTAGCGCGCGACTCCTG	soooooossssssssssoss	6-10-4	26
801281	1442	1461	GCCCCTAGCGCGCGACTCCT	soooooossssssssssoss	6-10-4	27
801282	1443	1462	GGCCCCTAGCGCGCGACTCC	soooooossssssssssoss	6-10-4	28
801283	1444	1463	CGGCCCCTAGCGCGCGACTC	soooooossssssssssoss	6-10-4	29
801284	1441	1458	CCTAGCGCGCGACTCCTG	soossssssssoooss	4-8-6	30
801285	1442	1459	CCCTAGCGCGCGACTCCT	soossssssssoooss	4-8-6	31

801286	1443	1460	CCCCTAGCGCGCGACTCC	soossssssssoooss	4-8-6	32
801287	1444	1461	GCCCCTAGCGCGCGACTC	soossssssssoooss	4-8-6	33
801288	1445	1462	GGCCCCTAGCGCGCGACT	soossssssssoooss	4-8-6	34
801289	1446	1463	CGGCCCCTAGCGCGCGAC	soossssssssoooss	4-8-6	35
801290	1441	1458	CCTAGCGCGCGACTCCTG	soooossssssssoss	6-8-4	30
801291	1442	1459	CCCTAGCGCGCGACTCCT	soooossssssssoss	6-8-4	31
801292	1443	1460	CCCCTAGCGCGCGACTCC	soooossssssssoss	6-8-4	32
801293	1444	1461	GCCCCTAGCGCGCGACTC	soooossssssssoss	6-8-4	33
801294	1445	1462	GGCCCCTAGCGCGCGACT	soooossssssssoss	6-8-4	34
801295	1446	1463	CGGCCCCTAGCGCGCGAC	soooossssssssoss	6-8-4	35
801296	1403	1422	AGGCTGCGGTTGTTTCCCTC	soooossssssssooooss	4-10-6	36
801297	1404	1423	CAGGCTGCGGTTGTTTCCCT	soooossssssssooooss	4-10-6	37
801298	1403	1421	GGCTGCGGTTGTTTCCCTC	soooossssssssooss	5-9-5	38
801299	1404	1422	AGGCTGCGGTTGTTTCCCT	soooossssssssooss	5-9-5	39
801300	1405	1423	CAGGCTGCGGTTGTTTCCC	soooossssssssooss	5-9-5	40
801301	1403	1421	GGCTGCGGTTGTTTCCCTC	soooossssssssooooss	4-9-6	38
801302	1404	1422	AGGCTGCGGTTGTTTCCCT	soooossssssssooooss	4-9-6	39
801303	1405	1423	CAGGCTGCGGTTGTTTCCC	soooossssssssooooss	4-9-6	40
801304	1403	1421	GGCTGCGGTTGTTTCCCTC	soooooossssssssoss	6-9-4	38
801305	1404	1422	AGGCTGCGGTTGTTTCCCT	soooooossssssssoss	6-9-4	39
801306	1405	1423	CAGGCTGCGGTTGTTTCCC	soooooossssssssoss	6-9-4	40
801307	1403	1420	GCTGCGGTTGTTTCCCTC	soossssssssoooss	4-8-6	41
801308	1404	1421	GGCTGCGGTTGTTTCCCT	soossssssssoooss	4-8-6	42
801309	1405	1422	AGGCTGCGGTTGTTTCCC	soossssssssoooss	4-8-6	43
801310	1406	1423	CAGGCTGCGGTTGTTTCC	soossssssssoooss	4-8-6	44
801311	1403	1420	GCTGCGGTTGTTTCCCTC	soooossssssssoss	6-8-4	41
801312	1404	1421	GGCTGCGGTTGTTTCCCT	soooossssssssoss	6-8-4	42
801313	1405	1422	AGGCTGCGGTTGTTTCCC	soooossssssssoss	6-8-4	43
801314	1406	1423	CAGGCTGCGGTTGTTTCC	soooossssssssoss	6-8-4	44
801315	1403	1422	AGGCTGCGGTTGTTTCCCTC	soooooossssssssoss	6-10-4	36
801316	1404	1423	CAGGCTGCGGTTGTTTCCCT	soooooossssssssoss	6-10-4	37
802459	7990	8009	GCCTTACTCTAGGACCAAGA	soossssssssoooooss	3-10-7	21
802460	8012	8031	TCTGTCTTTGGAGCCCAAAT	soossssssssoooooss	3-10-7	45
802461	8186	8205	CTGCGATCCCCATTCCAGTT	soossssssssoooooss	3-10-7	46
802462	7990	8009	GCCTTACTCTAGGACCAAGA	soooossssssssoooss	4-10-6	21
802463	8012	8031	TCTGTCTTTGGAGCCCAAAT	soooossssssssoooss	4-10-6	45
802464	8186	8205	CTGCGATCCCCATTCCAGTT	soooossssssssoooss	4-10-6	46
802465	7990	8009	GCCTTACTCTAGGACCAAGA	soooooossssssssoss	6-10-4	21
802466	8012	8031	TCTGTCTTTGGAGCCCAAAT	soooooossssssssoss	6-10-4	45
802467	8186	8205	CTGCGATCCCCATTCCAGTT	soooooossssssssoss	6-10-4	46
802468	7990	8009	GCCTTACTCTAGGACCAAGA	soooooossssssssssss	7-10-3	21
802469	8012	8031	TCTGTCTTTGGAGCCCAAAT	soooooossssssssssss	7-10-3	45
802470	8186	8205	CTGCGATCCCCATTCCAGTT	soooooossssssssssss	7-10-3	46

802471	7992	8009	GCCTTACTCTAGGACCAA	SOSSSSSSSSSOOOOSS	3-8-7	47
802472	8014	8031	TCTGTCTTTGGAGCCCAA	SOSSSSSSSSSOOOOSS	3-8-7	48
802473	7992	8009	GCCTTACTCTAGGACCAA	SOOSSSSSSSSSOOOSS	4-8-6	47
802474	8014	8031	TCTGTCTTTGGAGCCCAA	SOOSSSSSSSSSOOOSS	4-8-6	48
802475	7992	8009	GCCTTACTCTAGGACCAA	SOOOOSSSSSSSSSOSS	6-8-4	47
802476	8014	8031	TCTGTCTTTGGAGCCCAA	SOOOOSSSSSSSSSOSS	6-8-4	48
802477	7992	8009	GCCTTACTCTAGGACCAA	SOOOOOSSSSSSSSSSS	7-8-3	47
802478	8014	8031	TCTGTCTTTGGAGCCCAA	SOOOOOSSSSSSSSSSS	7-8-3	48
806673	1370	1389	GGTTAATCTTTATCAGGTCT	SOOSSSSSSSSSOOOOSS	3-10-7	49
806674	1371	1390	TGGTTAATCTTTATCAGGTC	SOOSSSSSSSSSOOOOSS	3-10-7	50
806675	1372	1391	CTGGTTAATCTTTATCAGGT	SOOSSSSSSSSSOOOOSS	3-10-7	51
806676	1370	1389	GGTTAATCTTTATCAGGTCT	SOOOSSSSSSSSSOOOSS	4-10-6	49
806677	1371	1390	TGGTTAATCTTTATCAGGTC	SOOOSSSSSSSSSOOOSS	4-10-6	50
806678	1372	1391	CTGGTTAATCTTTATCAGGT	SOOOSSSSSSSSSOOOSS	4-10-6	51
806679	1370	1389	GGTTAATCTTTATCAGGTCT	SOOOOOSSSSSSSSSSOSS	6-10-4	49
806680	1371	1390	TGGTTAATCTTTATCAGGTC	SOOOOOSSSSSSSSSSOSS	6-10-4	50
806681	1372	1391	CTGGTTAATCTTTATCAGGT	SOOOOOSSSSSSSSSSOSS	6-10-4	51
806682	1370	1389	GGTTAATCTTTATCAGGTCT	SOOOOOOSSSSSSSSSSS	7-10-3	49
806683	1371	1390	TGGTTAATCTTTATCAGGTC	SOOOOOOSSSSSSSSSSS	7-10-3	50
806684	1372	1391	CTGGTTAATCTTTATCAGGT	SOOOOOOSSSSSSSSSSS	7-10-3	51
806685	1371	1388	GTTAATCTTTATCAGGTC	SOOSSSSSSSSSOOOSS	4-8-6	52
806686	1372	1389	GGTTAATCTTTATCAGGT	SOOSSSSSSSSSOOOSS	4-8-6	53
806687	1373	1390	TGGTTAATCTTTATCAGG	SOOSSSSSSSSSOOOSS	4-8-6	54
806688	1440	1457	CTAGCGCGCGACTCCTGA	SOOSSSSSSSSSOOOSS	4-8-6	55
806689	1371	1388	GTTAATCTTTATCAGGTC	SOOOOSSSSSSSSSOSS	6-8-4	52
806690	1372	1389	GGTTAATCTTTATCAGGT	SOOOOSSSSSSSSSOSS	6-8-4	53
806691	1373	1390	TGGTTAATCTTTATCAGG	SOOOOSSSSSSSSSOSS	6-8-4	54
806692	1440	1457	CTAGCGCGCGACTCCTGA	SOOOOSSSSSSSSSOSS	6-8-4	55

Example 2: Tolerability of antisense oligonucleotides targeting human C9ORF72 in mice

Antisense oligonucleotides described above were tested in mice to assess tolerability of the oligonucleotides. Wild type C57/Bl6 mice each received a single ICV dose of 700 μg of an antisense oligonucleotide listed in the table below. Each treatment group consisted of 4 mice. At 3 hours post injection, each mouse was evaluated according to 7 different criteria. The 7 criteria are (1) the mouse was bright, alert, and responsive; (2) the mouse was standing or hunched without stimuli; (3) the mouse showed any movement without stimuli; (4) the mouse demonstrated forward movement after it was lifted; (5) the mouse demonstrated any movement after it was lifted; (6) the mouse responded to a tail pinch; (7) regular breathing. For each of the 7 different criteria, each mouse was given a sub-score of 0 if it met the criteria or 1 if it did not. After all of the 7 criteria were evaluated, the sub-scores were summed for each mouse and then averaged for each group. For example, if a mouse was bright, alert, and responsive 3 hours after the 700 μg

ICV dose, and met all other criteria, it would get a summed score of 0. If another mouse was not bright, alert, and responsive 3 hours after the 700 μg ICV dose but met all other criteria, it would receive a score of 1. The results are presented as the average score for each treatment group.

Table 7

Acute tolerability scores

Isis No.	Score
791656	3.25
791657	4.25
791658	3.50
791659	2.00
791660	4.00
791661	4.50
791662	5.25
791663	6.00
791664	3.00
791665	5.75
801274	1.75
801275	3.75
801276	1.25
801277	1.50
801278	1.75
801279	1.25
801280	6.00
801281	0.00
801282	1.25
801283	1.25
801284	3.00
801285	1.00
801286	1.00
801287	1.50
801288	2.50
801289	7.00
801290	6.00
801291	3.00
801292	2.00
801293	1.00
801294	3.25
801295	4.50
801296	6.25

801297	4.75
801298	5.50
801299	6.25
801300	5.00
801301	6.00
801302	6.50
801303	4.00
801304	5.25
801305	6.00
801306	6.00
801307	5.00
801308	6.00
801309	7.00
801310	3.50
801311	5.50
801312	2.50
801313	5.25
801314	4.50
801315	4.00
801316	2.00
802459	2.00
802460	6.75
802461	1.75
802462	5.75
802463	6.75
802464	1.75
802465	2.25
802466	4.25
802467	0.25
802468	3.25
802469	2.00
802470	0.25
802471	1.25
802472	4.00
802473	0.25
802474	5.25
802475	1.00
802476	5.50
802477	2.50
802478	6.25
806673	0.00
806674	0.25
806675	0.00

806676	0.00
806677	1.00
806678	0.00
806679	1.50
806680	1.00
806681	0.00
806682	5.75
806683	3.75
806684	2.25
806685	1.00
806686	1.00
806687	3.25
806688	3.25
806689	3.00
806690	1.25
806691	6.25

Example 3: Tolerability of oligonucleotides from WO 2014/062691

Oligonucleotides described in WO 2014/062691 were tested in an acute tolerability study in mice. Groups of 3 wild type C57/Bl6 mice were treated and analyzed as described in Example 2. The tested oligonucleotides include those listed in the table below, which are 5-10-5 MOE gapmers with a full phosphorothioate backbone and each cytosine is a 5-methylcytosine. The start and stop sites on SEQ ID NO: 2 that each oligonucleotide is targeted to are shown. The results are presented as the average score for each treatment group in the table below. These results demonstrate that ISIS 576816, ISIS 576974, ISIS 577061, ISIS 577065, ISIS 577083, and ISIS 577056 were poorly tolerated.

Table 8

Acute tolerability scores 3h after treatment with antisense oligonucleotides from WO 2014/062691

Isis No.	Start site	Stop site	Sequence	Score	SEQ ID NO
576816	7990	8009	GCCTTACTCTAGGACCAAGA	7.00	21
576974	28251	28270	GGGACACTACAAGGTAGTAT	5.67	56
577061	1406	1425	TACAGGCTGCGGTTGTTTCC	7.00	57
577065	1446	1465	CCCGGCCCCTAGCGCGCGAC	6.00	58
577083	3452	3471	GGTAACTTCAAACTCTTGGG	7.00	59

Table 9

Acute tolerability scores 3h after treatment with antisense oligonucleotides from WO 2014/062691

Isis No.	Start site	Stop site	Sequence	Score	SEQ ID NO
577056	1366	1385	AATCTTTATCAGGTCTTTTC	6.5	60

Example 4: Antisense inhibition of a human C9ORF72 mRNA variant in HepG2 cells

Antisense oligonucleotides described above are tested for their effects on C9ORF72 mRNA *in vitro*. Cultured HepG2 cells at a density of 20,000 cells per well are electroporated with an antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA is isolated from the cells and C9ORF72 mRNA levels are measured by quantitative real-time PCR. Human primer probe set RTS3905 (forward primer sequence GGGTCTAGCAAGAGCAGGTG, designated herein as SEQ ID NO: 12; reverse primer sequence GTCTTGGCAACAGCTGGAGAT, designated herein as SEQ ID NO: 13; probe sequence TGATGTCGACTCTTTGCCCACCGC, designated herein as SEQ ID NO: 14 – a TAQ-man primer probe set) are used. RTS3905 detects an mRNA variant (e.g. NM_001256054.1) processed from a pre-mRNA variant containing the hexanucleotide repeat. The mRNA variant processed from a pre-mRNA variant containing the hexanucleotide repeat is herein the "C9ORF72 pathogenic associated mRNA variant." A pre-mRNA contains the hexanucleotide repeat when transcription of the pre-mRNA begins in the region from the start site of exon 1A to the start site of exon 1B (generally nucleotides 1107 to 1520 of the genomic sequence: SEQ ID NO: 2, the complement of GENBANK Accession No. NT_008413.18 truncated from nucleosides 27535000 to 2756500. Therefore, oligonucleotides designed in this region selectively target the pre-mRNA variant containing the hexanucleotide repeat. RTS3905 measures an mRNA product (i.e. the C9ORF72 pathogenic associated mRNA variant) of the pre-mRNA variant containing the hexanucleotide repeat and, therefore, measures the reduction of the pre-mRNA variant containing the hexanucleotide repeat. The levels of the C9ORF72 pathogenic associated mRNA variant are normalized to the total RNA content of the cell, as measured by RIBOGREEN®, then the normalized mRNA variant levels are compared to those of cells that were not treated with antisense oligonucleotide.

Example 5: Dose-dependent antisense inhibition of a human C9ORF72 mRNA variant

Antisense oligonucleotides described above are tested at various doses in HepG2 cells. Cells are plated at a density of 20,000 cells per well and electroporated with antisense oligonucleotide. After a treatment period of approximately 16 hours or 24 hours, RNA is isolated from the cells and C9ORF72 mRNA levels are measured by quantitative real-time PCR. Human C9ORF72 primer probe set RTS3905 is used to measure the C9ORF72 pathogenic associated mRNA variant. The levels of the C9ORF72 pathogenic associated mRNA variant are adjusted according to total RNA content, as measured by RIBOGREEN®. The

half maximal inhibitory concentration (IC_{50}) of each oligonucleotide is calculated based on the inhibition of mRNA variant levels observed at each individual dose of antisense oligonucleotide.

Example 6: Antisense inhibition of C9ORF72 by human-rhesus cross-reactive antisense oligonucleotides in LLC-MK2 cells

Antisense oligonucleotides described above that are fully cross-reactive with a rhesus C9ORF72 nucleic acid are tested for their effects on rhesus C9ORF72 mRNA *in vitro*. Cultured rhesus LLC-MK2 cells at a density of 20,000 cells per well are electroporated with antisense oligonucleotide. After a treatment period of approximately 24 hours, RNA is isolated from the cells and C9ORF72 mRNA levels are measured by quantitative real-time PCR. Primer probe set RTS3750 (forward sequence TGTGACAGTTGGAATGCAGTGA, designated herein as SEQ ID NO: 15; reverse sequence GCCACTTAAAGCAATCTCTGTCTTG, designated herein as SEQ ID NO: 16; probe sequence TCGACTCTTTGCCCACCGCCA, designated herein as SEQ ID NO: 17 – a TAQ-man primer probe set) is used to measure total C9ORF72 mRNA levels. RTS3750 targets exon 2 of the mRNA transcripts and, therefore, measures total mRNA transcripts. C9ORF72 mRNA levels are adjusted according to total RNA content, as measured by RIBOGREEN®, then the normalized mRNA variant levels are compared to those of cells that were not treated with antisense oligonucleotide.

Example 7: Dose-dependent antisense inhibition of human C9ORF72 mRNA in LLC-MK2

Antisense oligonucleotides described above are tested at various doses in LLC-MK2 cells. Cells are plated at a density of 20,000 cells per well and electroporated with antisense oligonucleotide. After a treatment period of approximately 16 hours or 24 hours, RNA is isolated from the cells and C9ORF72 mRNA levels are measured by quantitative real-time PCR. Primer probe set RTS3750 is used to measure total C9ORF72 mRNA levels. C9ORF72 mRNA levels are adjusted according to total RNA content, as measured by RIBOGREEN®, then the normalized mRNA variant levels are compared to those of cells that were not treated with antisense oligonucleotide.

Example 8: Antisense inhibition of human C9ORF72 mRNA in a transgenic mouse model

Antisense oligonucleotides described above are tested in two BAC transgenic mouse lines, designated herein as C9B41 and C9B183, that each express a truncated human C9ORF72 gene comprising exons 1-5. The truncated human C9ORF72 genes of the C9B41 and C9B183 mouse lines comprise 110 and 450 hexanucleotide repeats, respectively. Each mouse in each treatment group receives 350 μg of an antisense oligonucleotide, then expression levels of human C9ORF72 RNA are analyzed by RT-PCR. Dose responses are also performed.

Example 9: Antisense inhibition of C9ORF72 mRNA in patient fibroblasts

Antisense oligonucleotides described above were tested for their effects on C9ORF72 mRNA *in vitro*. The antisense oligonucleotides listed in the table below were added to a plate before patient fibroblasts F09-152 were added at a density of 20,000 cells per well. The final concentrations of the antisense oligonucleotides after the addition of the cells are listed in the table below. After 30 seconds of shaking, the cells were electroporated then transferred to a Primaria coated culture plate. After 16 hours, RNA was isolated from the cells, and levels of total C9ORF72 mRNA (i.e., mRNA starting from exon 1A and mRNA starting from exon 1B) and levels of the pathogenic associated mRNA (see Example 4) were measured by RT-qPCR. Human primer probe sets RTS 3750 (see Example 6) and RTS3905 (see Example 4) were used to detect the total C9ORF72 mRNA and C9ORF72 pathogenic associated mRNA, respectively. The levels of the C9ORF72 total mRNA and C9ORF72 pathogenic associated mRNA were normalized to the total RNA content of the cell, as measured by RIBOGREEN®, then the normalized mRNA levels were compared to those of cells that were not treated with antisense oligonucleotide. The results are shown in the table below. An entry of "nd" means not determined. IC_{50} values listed as "nd" were not determined, because the target was not inhibited sufficiently to determine an IC_{50}. The results show that all of the oligonucleotides listed below inhibited the pathogenic associated C9ORF72 mRNA variant. Oligonucleotides that do not specifically target the repeat variant pre-mRNA inhibited both the pathogenic associated C9ORF72 mRNA and total C9ORF72 mRNA. Oligonucleotides that specifically target the repeat variant pre-mRNA selectively inhibited the pathogenic associated C9ORF72 mRNA.

Table 10

C9ORF72 mRNA levels following antisense inhibition in patient fibroblasts

Isis No.	Concentration (μM)	Total C9ORF72 mRNA		Pathogenic associated C9ORF72 mRNA	
		Level (% UTC)	IC_{50} (μM)	Level (% UTC)	IC_{50} (μM)
791658	0.12	87	nd	26	< 0.1
	0.60	80		9	
	3.00	87		nd	
	15.00	105		4	
791664	0.12	85	nd	13	< 0.1
	0.60	101		7	
	3.00	125		4	
	15.00	114		3	
801278	0.12	91	nd	19	< 0.1
	0.60	71		7	
	3.00	85		nd	

	15.00	90		nd	
801279	0.12	98	nd	57	0.12
	0.60	77		27	
	3.00	77		3	
	15.00	86		5	
801282	0.12	100	nd	51	0.08
	0.60	84		12	
	3.00	97		6	
	15.00	190		nd	
801283	0.12	87	nd	40	< 0.1
	0.60	75		8	
	3.00	94		4	
	15.00	111		4	
801285	0.12	103	nd	35	< 0.1
	0.60	78		23	
	3.00	75		7	
	15.00	79		6	
801286	0.12	94	nd	33	< 0.1
	0.60	85		14	
	3.00	97		5	
	15.00	88		3	
801287	0.12	85	nd	31	< 0.1
	0.60	76		11	
	3.00	77		9	
	15.00	87		2	
801288	0.12	77	nd	13	< 0.1
	0.60	86		11	
	3.00	123		5	
	15.00	177		3	
801292	0.12	69	nd	29	< 0.1
	0.60	71		12	
	3.00	82		3	
	15.00	71		nd	
801293	0.12	77	nd	51	0.09

	0.60	70		10	
	3.00	74		3	
	15.00	81		1	
801294	0.12	75	nd	27	< 0.1
	0.60	67		9	
	3.00	107		7	
	15.00	146		2	
801316	0.12	73	nd	35	< 0.1
	0.60	68		7	
	3.00	65		1	
	15.00	78		1	
802459	0.12	58	0.10	75	0.18
	0.60	15		6	
	3.00	2		11	
	15.00	1		6	
802464	0.12	81	0.39	69	0.21
	0.60	31		23	
	3.00	6		7	
	15.00	2		3	
802465	0.12	40	< 0.1	35	< 0.1
	0.60	7		7	
	3.00	2		nd	
	15.00	1		nd	
802468	0.12	52	0.10	40	< 0.1
	0.60	15		13	
	3.00	3		7	
	15.00	2		nd	
802469	0.12	69	0.17	57	~ 0.5
	0.60	16		9	
	3.00	4		nd	
	15.00	1		nd	
802471	0.12	71	0.27	54	0.12
	0.60	29		20	
	3.00	6		6	

	15.00	7		nd	
802473	0.12	63	0.18	48	< 0.1
	0.60	27		14	
	3.00	8		11	
	15.00	4		nd	
802477	0.12	54	0.12	32	< 0.1
	0.60	16		12	
	3.00	4		11	
	15.00	3		3	
806676	0.12	66	nd	20	< 0.1
	0.60	66		4	
	3.00	76		2	
	15.00	64		nd	
806679	0.12	71	nd	23	< 0.1
	0.60	68		2	
	3.00	78		1	
	15.00	84		1	
806680	0.12	89	nd	41	< 0.1
	0.60	76		13	
	3.00	65		7	
	15.00	84		nd	
806690	0.12	99	nd	44	< 0.1
	0.60	88		17	
	3.00	85		2	
	15.00	77		1	

Example 10: Antisense inhibition of human C9ORF72 mRNA in a transgenic mouse model

Antisense oligonucleotides described above were tested in a BAC transgenic mouse line, C9B41 (see Example 8), that expresses a human C9ORF72 gene comprising the promoter region through exon 5 and 110 hexanucleotide repeats. Each treatment group consisted of 2-3 mice. Each mouse received a single ICVB of 350 μg of an antisense oligonucleotide listed in the tables below or PBS. Two weeks later, the mice were euthanized, and expression levels of the human pathogenic associated C9ORF72 mRNA variant and/or total human C9ORF72 mRNA were analyzed by RT-qPCR as described in Example 9. Analysis of the pathogenic associated C9ORF72 variant mRNA levels was not completed for the oligonucleotides that do not

specifically target the C9ORF72 repeat variant pre-mRNA. The results in the tables below show the average percent normalized human C9ORF72 mRNA levels relative to the normalized average for the PBS treated group.

Table 11

Human C9ORF72 mRNA levels following antisense inhibition in transgenic mice

Isis No.	Spinal Cord (% PBS treated)		Cortex (% PBS treated)	
	Pathogenic variant	Total	Pathogenic variant	Total
791658	6	69	12	47
791659	18	72	27	57
791664	13	55	15	39
801274	49	65	27	37
801276	10	47	12	29
801277	9	44	10	28
801278	8	39	10	27
801279	33	55	40	47
801281	16	48	17	32
801282	18	49	31	42
801283	15	46	17	31
801285	29	51	24	36
801286	37	61	37	47
801287	13	47	30	40
801288	18	52	39	48
801292	25	49	33	40
801293	20	50	29	38
801310	70	80	68	70
801312	43	61	46	55
801315	39	59	41	51
801316	27	56	50	58
806673	38	66	66	66
806674	78	88	98	96
806675	67	86	81	85
806676	29	64	52	58
806677	61	77	69	68
806678	83	96	95	102

806679	24	63	26	44
806680	29	62	41	56
806681	39	68	36	54
806684	44	69	62	64
806685	69	87	56	58
806686	59	75	57	68
806687	74	88	77	80
806688	34	62	42	51
806689	67	92	87	88
806690	28	61	57	68
806692	45	64	44	50

Table 12

Human C9ORF72 mRNA levels following antisense inhibition in transgenic mice

Isis No.	Total human C9ORF72 mRNA (% PBS)	
	Spinal Cord	Cortex
802459	28	30
802461	22	19
802464	30	28
802465	25	25
802467	13	14
802468	24	26
802469	24	20
802470	18	20
802471	36	44
802473	28	38
802475	15	15
802477	14	15

Example 11: Dose dependent antisense inhibition of human C9ORF72 mRNA in a transgenic mouse model

Antisense oligonucleotides described above were tested in two BAC transgenic mouse lines, C9B41 and C9B183, that each express a truncated human C9ORF72 gene comprising exons 1-5. The truncated human C9ORF72 genes of the C9B41 and C9B183 mouse lines comprise 110 and 450 hexanucleotide repeats, respectively (see Example 8). Each treatment group consisted of 2-4 mice. Each mouse received a single ICVB of 30 μg, 100 μg, 300 μg, or 700 μg of an antisense oligonucleotide as listed in the tables below or PBS. Two weeks later, the mice were euthanized, and expression levels of the human pathogenic associated C9ORF72 mRNA variant and/or total human C9ORF72 mRNA were analyzed by RT-qPCR as described in Example 9. The results in the tables below show the average percent normalized human C9ORF72 mRNA levels relative to the normalized average for the PBS treated group. A value of 100 or greater means the antisense oligonucleotide did not reduce mRNA or increased the amount of mRNA.

Table 13

Human C9ORF72 mRNA levels following dose dependnent antisense inhibition in C9B41 transgenic mice

Isis No.	Concentration (μg)	Spinal Cord (% PBS treated)	Cortex (% PBS treated)
		Total C9	Total C9
802459	30	84	85
	100	63	61
	300	33	26
	700	29	17
802473	30	74	90
	100	60	77
	300	37	39
	700	28	26

Table 14

Human C9ORF72 mRNA levels following dose dependnent antisense inhibition in C9B183 transgenic mice

Isis No.	Concentration (μg)	Spinal Cord (% PBS treated)	Cortex (% PBS treated)
		Pathogenic variant	Pathogenic variant
801287	30	76	10
	100	37	75
	300	21	32

	700	11	15
806679	30	72	13
	100	59	100
	300	21	61
	700	10	16
806680	30	94	118
	100	72	97
	300	34	89
	700	30	56
806690	30	52	125
	100	60	131
	300	43	119
	700	19	49

Example 12: Tolerability of antisense oligonucleotides targeting human C9Orf72in mice

Wild type C57/Bl6 mice each received a single ICV dose of 700µg of an antisense oligonucleotide listed in the table below, as described in Example 2. Each treatment group consisted of 4 mice. At 8 weeks post-injection, mice were evaluated according to 7 different criteria. The criteria are (1) the mouse was bright, alert, and responsive; (2) the mouse was standing or hunched without stimuli; (3) the mouse showed any movement without stimuli; (4) the mouse demonstrated forward movement after it was lifted; (5) the mouse demonstrated any movement after it was lifted; (6) the mouse responded to tail pinching; (7) regular breathing. For each of the 7 criteria, a mouse was given a subscore of 0 if it met the criteria and 1 if it did not. After all 7 criteria were evaluated, the scores were summed for each mouse and averaged within each treatment group. The results are presented in the table below.

Animals were sacrificed at 8 weeks. The cortex and spinal cord were collected from each animal, and RT-PCR was performed. Expression levels of allograft inflammatory factor (AIF1) were determined as a measure of inflammation. Expression levels of glial fibrillary acidic protein (GFAP) were also determined as a measure of glial cell activation. Results were normalized to Gpadh and are presented relative to PBS control (1.0) in the table below. "N.D." indicates there was no data because the experiment was not performed. An asterisk indicates that the corresponding result is the average of 1-3 mice.

Table 15: Tolerabilty of antisense oligonucleotides targeting C9Orf72 in mice

ISIS No.	Score 8 weeks after injection	AIF1 (spinal cord)	AIF1 (cerebellum)	GFAP (spinal cord)	GFAP (cortex)	SEQ ID NO.
791656	3.5	1.1*	1.3*	1.0*	1.3*	22
791657	3.5	2.3*	1.0*	1.4*	0.8*	22
791658	0.0	1.9	1.2	1.4	1.3	22
791659	0.0	1.8	1.3	1.5	2.5	22
791660	1.8	2.5*	1.4*	1.9*	0.8*	23
791661	1.8	1.7*	1.3*	1.5*	1.1*	24
791662	5.3	1.1*	1.2*	1.1*	0.8*	23
791663	7.0	N.D.	N.D.	N.D.	N.D.	24
791664	0.0	1.6	1.6	1.2	0.9	23
791665	3.5	1.9*	1.5*	1.9*	0.9*	24
801274	0.0	1.2	1.3	1.3	1.1	25
801275	3.5	1.4*	1.7*	1.6*	2.1*	26
801276	0.0	13.1	2.6	3.7	1.2	27
801277	0.0	2.9	1.4	2.3	1.5	28
801278	0.0	2.0	1.3	1.8	1.1	29
801279	0.0	1.2	1.4	1.3	1.0	25
801280	5.3	1.5*	1.5*	1.4*	1.2*	26
801281	0.0	3.6	2.2	2.4	1.6	27
801282	0.0	1.2	1.2	1.2	1.0	28
801283	0.0	1.4	1.4	1.0	0.9	29
801284	1.8	1.2*	1.5*	0.9*	1.0*	30
801285	0.0	1.2	1.2	1.1	0.8	31
801286	0.0	1.1	1.2	1.0	1.1	32
801287	0.0	1.3	1.2	1.1	1.4	33
801288	0.0	1.3	1.3	1.1	0.9	34
801289	7.0	N.D.	N.D.	N.D.	N.D.	35
801290	3.5	1.2*	1.3*	1.0*	1.1*	30
801291	0.0	2.2	2.2	1.5	1.1	31
801292	0.0	1.3	1.3	1.2	0.9	32
801293	0.0	1.6	1.6	1.3	1.5	33
801294	0.0	1.2	1.2	1.0	1.1	34
801295	1.8	1.4*	1.4*	1.0*	0.9*	35
801296	7.0	N.D.	N.D.	N.D.	N.D.	36
801297	3.5	1.0*	1.0*	0.9*	0.8*	37
801307	5.3	1.2*	0.9*	1.1*	0.8*	41
801308	5.3	1.2*	1.2*	0.9*	1.1*	42
801309	7.0	N.D.	N.D.	N.D.	N.D.	43
801310	1.8	1.2*	1.2*	1.0*	1.0*	44
801311	7.0	N.D.	N.D.	N.D.	N.D.	41
801312	0.0	2.0	1.3	1.0	1.4	42
801313	3.5	1.7*	1.1*	0.9*	0.9*	43

801314	0.0	1.9	1.2	1.0	2.9	44
801315	1.8	1.4*	1.3*	1.0*	1.1*	36
801316	0.0	1.5	1.4	1.0	1.2	37
801298	3.5	1.4*	1.0*	1.1*	1.3*	38
801299	5.3	1.2*	1.2*	1.0*	1.0*	39
801300	3.5	1.2*	1.1*	0.9*	1.0*	40
801301	7.0	N.D.	N.D.	N.D.	N.D.	38
801302	5.3	1.0*	1.1*	0.8*	0.8*	39
801303	1.8	1.1*	1.0*	1.0*	1.1*	40
801304	4.8	14.3*	5.4*	2.2*	3.5*	38
801305	5.3	3.6*	1.7*	1.6*	1.6*	39
801306	0.0	4.7	1.8	1.8	1.8	40
806673	0.0	1.1	1.0	1.0	9.1	49
806674	0.0	1.0	1.0	0.8	8.2	50
806675	0.0	1.0	1.0	0.8	10.3	51
806676	0.0	1.0	1.0	0.8	1.5	49
806677	0.0	0.9	1.0	0.9	1.5	50
806678	0.0	1.0	0.9	1.1	1.2	51
806679	0.0	1.2	1.1	1.1	1.3	49
806680	0.0	1.0	1.1	0.8	1.0	50
806681	0.0	1.0	1.0	0.9	1.4	51
806682	1.8	1.4*	1.2*	1.0*	1.2*	49
806683	0.0	1.2	1.2	0.9	1.2	50
806684	0.0	1.2	1.1	0.9	2.0	51
806685	0.0	1.0	0.9	0.8	1.5	52
806686	0.0	0.9	0.9	0.8	1.1	53
806687	0.0	1.0	1.0	0.8	1.0	54
806688	1.8	1.1*	1.0*	0.8*	1.8*	55
806689	0.0	1.1	1.0	0.8	1.3	52
806690	0.0	1.0	1.1	0.9	1.1	53
806691	5.3	N.D.	N.D.	N.D.	N.D.	54
802459	0.0	0.9	0.9	0.9	1.2	21
802460	5.3	1.1*	1.1*	1.0*	1.4*	45
802461	3.5	1.4*	1.4*	0.9*	0.9*	46
802462	3.5	0.8*	0.9*	0.9*	1.1*	21
802463	7.0	N.D.	N.D.	N.D.	N.D.	45
802464	1.8	1.4*	1.2*	1.5*	1.4*	46
802465	0.0	0.9	1.1	1.1	1.6	21
802466	1.8	1.0*	1.0*	1.0*	1.4*	45
802467	1.3	4.9	2.4	2.7	2.8	46
802468	0.0	1.4	1.3	1.2	2.2	21
802469	0.0	1.0	1.0	1.0	1.2	45
802470	1.5	3.2	3.4	2.6	3.4	46
802471	0.0	0.9	0.9	0.9	1.2	47
802472	3.5	1.6*	1.4*	0.9*	1.5*	48
802473	0.0	1.0	0.9	0.9	1.3	47

802474	1.8	1.3*	1.2*	1.0*	1.8*	48
802475	7.0	N.D.	N.D.	N.D.	N.D.	47
802476	1.8	1*	1.2*	0.8*	1.8*	48
802477	0.0	1.9	1.7	1.3	2.1	47
802478	3.5	1.4*	1.1*	1.1*	1.3*	48

Example 13: Tolerability of antisense oligonucleotides targeting human C9Orf72in rats

Sprague Dawley rats were separated into groups of 4 or 6 rats. Each rat in each group of rats was administered a single 3 mg intrathecal (IT) dose of the oligonucleotide indicated in the table below. At 3 hours and at 8 weeks following the IT dose, the movement of 7 different parts of the body was evaluated for each rat. The 7 body parts are (1) the rat's tail; (2) the rat's posterior posture; (3) the rat's hind limbs; (4) the rat's hind paws; (5) the rat's forepaws; (6) the rat's anterior posture; (7) the rat's head. For each of the 7 different body parts, each rat was given a sub-score of 0 if the body part was moving or 1 if the body part was paralyzed. After each of the 7 body parts were evaluated, the sub-scores were summed for each rat and then averaged for each group. For example, if a rat's tail, head, and all other evaluated body parts were moving 3 hours after the 3 mg IT dose, it would get a summed score of 0. If another rat was not moving its tail 3 hours after the 3 mg IT dose but all other evaluated body parts were moving, it would receive a score of 1. Saline treated rats generally receive a score of 0. A score of at the top end of the range would be suggestive of acute toxicity. Results are presented in the table below as the average score for each treatment group.

Animals were sacrificed at 8 weeks. The cortex and spinal cord were collected from each animal, and RT-PCR was performed. Expression levels of AIF1were determined as a measure of inflammation. Expression levels of (GFAP) were also determined as a measure of glial cell activation. An asterisk indicates that the corresponding result is the average of 2-3 mice. Results were normalized to Gapdh and are presented relative to PBS control (1.0) in the table below.

Table 16: Tolerability of antisense oligonucleotides targeting C9Orf72 in rats

ISIS No.	Score 3 hours after injection	Score 8 weeks after injection	AIF1 (spinal cord)	AIF1 (cortex)	GFAP (spinal cord)	GFAP (cortex)	SEQ ID NO.
801287	2.5	0.0	1.9	1.2	1.3	1.3	33
801288	4.0	3.5	1.3*	1.3*	1.2*	1.3*	34
806676	2.0	1.8	1.6*	1.5*	1.3*	2.5*	49
806679	1.2	0.0	1.4	1.3	1.3	1.8	49

806680	2.0	0.3	1.4	1.4	1.3	1.3	50
806690	1.3	0.0	1.5	1.4	1.3	2.0	53
802459	2.0	0.0	1.4	1.2	1.5	1.5	21
802473	2.0	2.3	1.8*	1.6*	1.7*	1.6*	47

Example 14: Tolerability of antisense oligonucleotides targeting human C9ORF72 in non-human primates

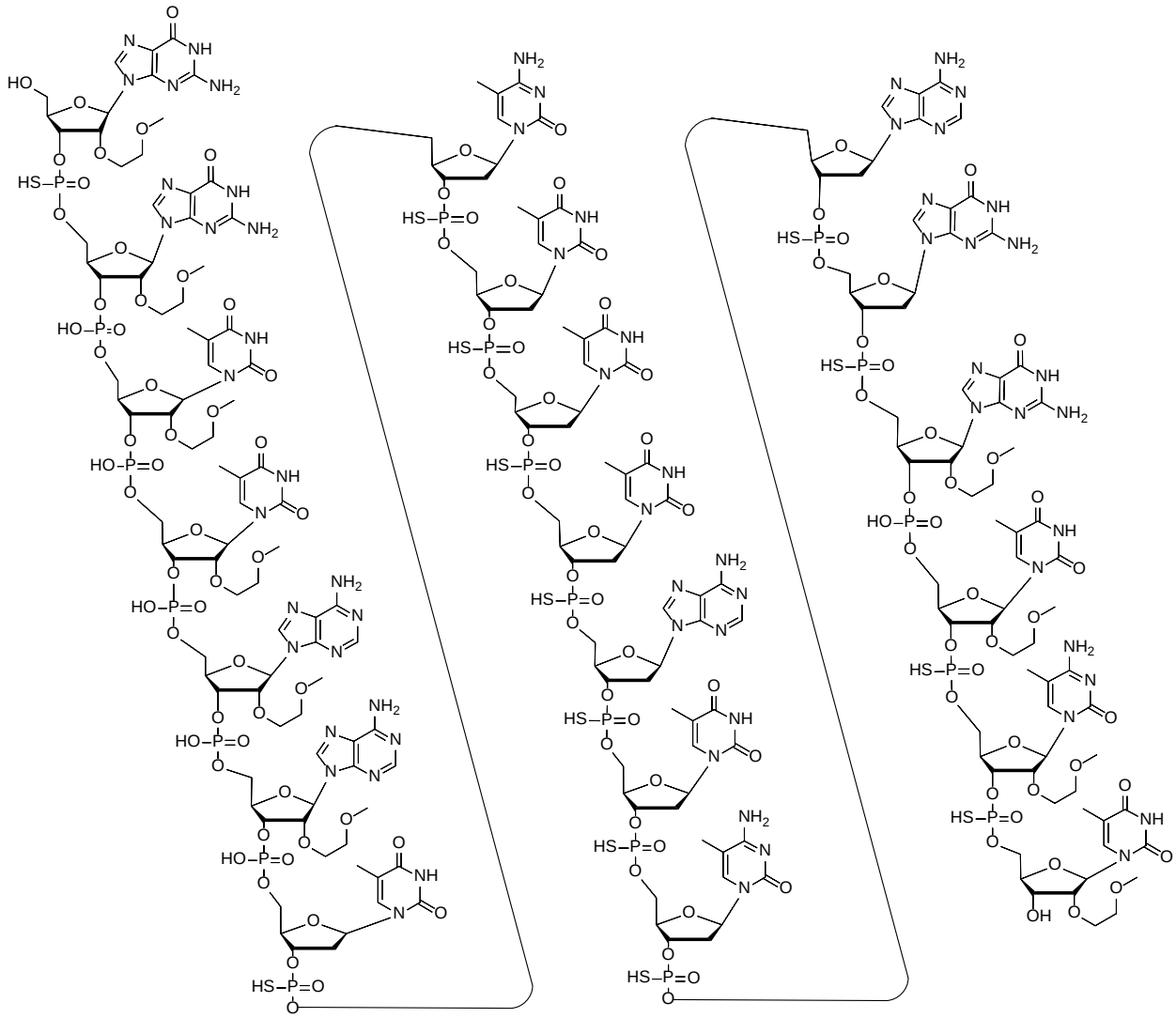
Female cynomolgus monkeys (2-6kg) were given 3 doses of 35mg of antisense oligonucleotide on days 1, 14, and 28 via intrathecal bolus injection (1mL slow bolus followed by 0.25mL flush). Each treatment group contained four monkeys. Two weeks after the final dose, animals were sacrificed and RT-PCR was performed on various CNS tissues. Expression levels of AIF1 were determined as a measure of inflammation and expression levels of GFAP were determined as a measure of glial cell activation. Results were normalized to GADPH and are presented relative to PBS control (1.0) in the table below for ISIS No. 801287, 802459, and 806679.

Table 17: Tolerability of antisense oligonucleotides targeting C9ORF72 in cynomolgus monkeys

	AIF1			GFAP		
Brain Region	801287	802459	806679	801287	802459	806679
Cervical spinal cord	1.0	0.9	0.9	1.1	1.0	1.1
Thoracic spinal cord	1.1	0.9	0.9	1.2	1.2	1.2
Temporal cortex	1.0	1.0	1.5	1.2	1.1	1.7
Motor cortex	1.0	1.0	1.2	1.2	0.7	1.3
Lumbar spinal cord	1.2	0.9	0.9	1.3	0.9	1.1
Hippocampus	1.0	1.0	1.3	1.9	1.9	1.7
Frontal cortex	1.3	0.9	1.3	1.0	0.7	1.3

Claims

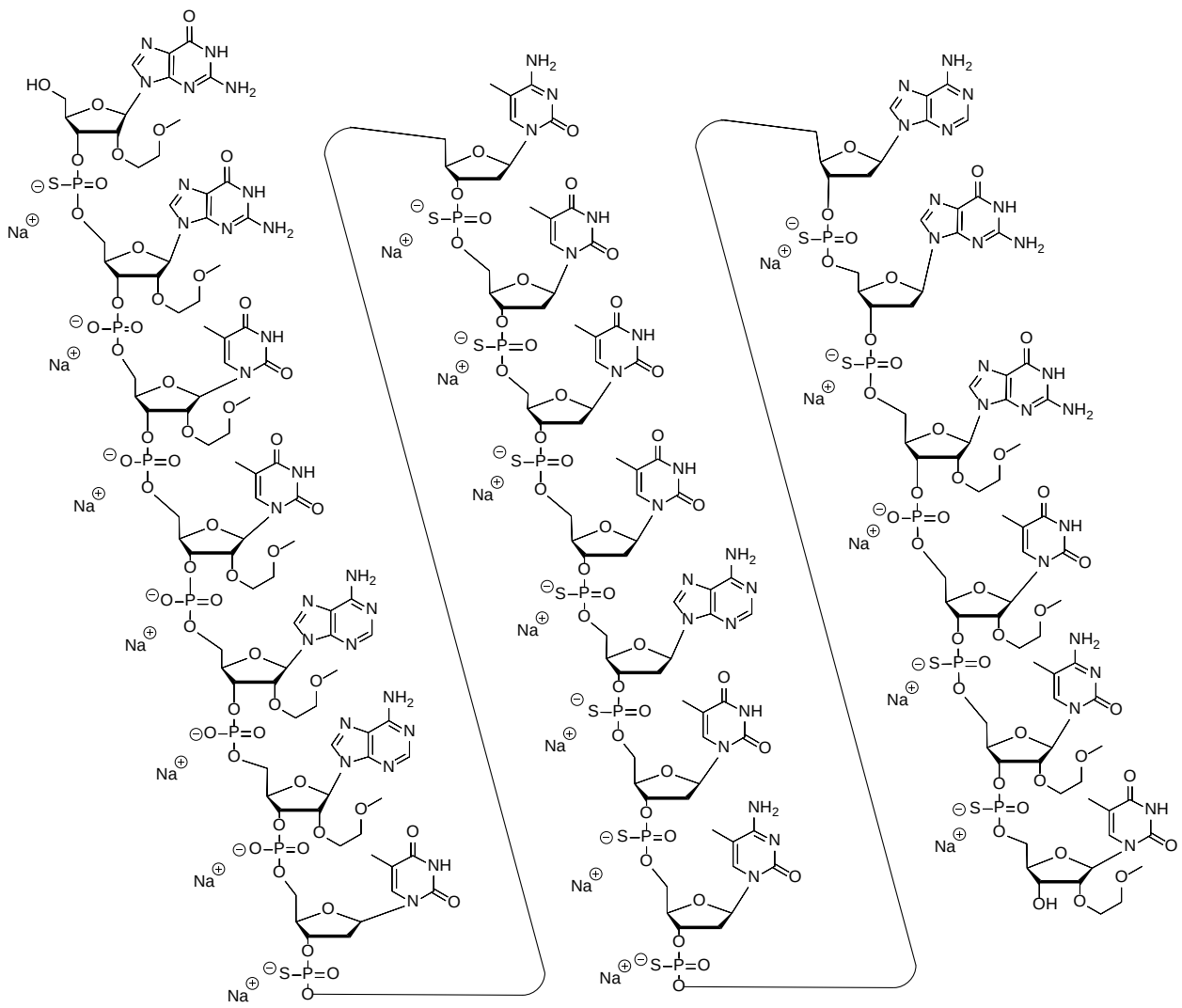
1. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 49)

or a salt thereof.

2. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 49).

3. The modified oligonucleotide of claim 1, which is the sodium salt or the potassium salt.

4. A pharmaceutical composition comprising the modified oligonucleotide of claim 1 and a pharmaceutically acceptable diluent or carrier.

5. The pharmaceutical composition of claim 4, comprising a pharmaceutically acceptable diluent, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

6. The pharmaceutical composition of claim 4, wherein the pharmaceutical composition consists essentially of the modified oligonucleotide and phosphate-buffered saline (PBS).

7. A pharmaceutical composition comprising the modified oligonucleotide of claim 3 and a pharmaceutically acceptable diluent or carrier.

8. The pharmaceutical composition of claim 7, comprising a pharmaceutically acceptable diluent, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

9. The pharmaceutical composition of claim 7, wherein the pharmaceutical composition consists essentially of the modified oligonucleotide and phosphate-buffered saline (PBS).

10. A pharmaceutical composition comprising the modified oligonucleotide of claim 2 and a pharmaceutically acceptable diluent or carrier.

11. The pharmaceutical composition of claim 10, comprising a pharmaceutically acceptable diluent, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

12. The pharmaceutical composition of claim 10, wherein the pharmaceutical composition consists essentially of the modified oligonucleotide and phosphate-buffered saline (PBS).7.

13. A compound comprising a modified oligonucleotide, wherein the modified oligonucleotide is a gapmer consisting of a 5’ wing segment, a central gap segment, and a 3’ wing segment, wherein:

the 5’ wing segment consists of six 2’-O-methoxyethyl nucleosides,

the central gap segment consists of ten β-D-deoxyribonucleosides, and

the 3’ wing segment consists of four 2’-O-methoxyethyl nucleosides;

wherein the modified oligonucleotide has the nucleobase sequence 5’-GGTTAATCTTTATCAGGTCT-3’ (SEQ ID NO: 49), wherein each cytosine is a 5-methylcytosine; and wherein the internucleoside linkages of the modified oligonucleotide are, from 5’ to 3’, sooooossssssssssoss, wherein each s is a phosphorothioate linkage and each o is a phosphodiester linkage.

14. The compound of claim 13, comprising the modified oligonucleotide covalently linked to a conjugate group.

15. A pharmaceutical composition comprising the compound of claim 13 and a pharmaceutically acceptable diluent or carrier.

16. The pharmaceutical composition of claim 15, comprising a pharmaceutically acceptable diluent, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

17. The pharmaceutical composition of claim 15, wherein the pharmaceutical composition consists essentially of the compound and phosphate-buffered saline (PBS).

18. A pharmaceutical composition comprising the compound of claim 14 and a pharmaceutically acceptable diluent or carrier.

19. The pharmaceutical composition of claim 18, comprising a pharmaceutically acceptable diluent, wherein the pharmaceutically acceptable diluent is phosphate-buffered saline (PBS).

20. The pharmaceutical composition of claim 18, wherein the pharmaceutical composition consists essentially of the compound and phosphate-buffered saline (PBS).