

COMPOUNDS AND METHODS FOR MODULATING SMN2

Sequence Listing

5 The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled BIOL0367WOSEQ_ST25.txt, created on February 26, 2021, which is 44 KB in size. The information in the electronic format of the sequence listing is incorporated herein by reference in its entirety.

Field

10 Provided are compounds, methods, and pharmaceutical compositions for modulating SMN2 RNA in a cell or subject. Such compounds, methods, and pharmaceutical compositions are useful to ameliorate at least one symptom of a neurodegenerative disorder. Such symptoms include reduced muscle strength; inability or reduced ability to sit upright, to stand, and/or walk; reduced neuromuscular activity; reduced electrical activity in one or more muscles; reduced respiration; inability or reduced ability to eat, drink, and/or breathe without assistance; loss of weight or reduced weight gain; and/or decreased survival.

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Background

 Proximal spinal muscular atrophy (SMA) is a genetic neurodegenerative disorder characterized by the loss of spinal motor neurons. SMA is an autosomal recessive disease of early onset and is a leading genetic cause of death among infants. The severity of SMA varies among patients and it has thus been classified into four types. Type I SMA is the most severe form with onset at birth or within 6 months, and typically results in death within 2 years. Children with Type I SMA are unable to sit or walk. Type II SMA is the intermediate form and patients are able to sit, but cannot stand or walk. Patients with Type III SMA, a chronic form of the disease, typically develop SMA after 18 months of age (Lefebvre et al., *Hum. Mol. Genet.*, 1998, 7, 1531-1536). Type IV SMA is a milder form and typically has an onset after 18 years of age, sometimes after 10 years of age; patients with Type IV SMA experience limited mild motor impairment, are able to walk in adulthood and generally do not have respiratory or nutritional problems (Farrar et al., *Ann. Neurol.*, 2017, 81, 355-368; D'Amico et al., *Orphanet J. of Rare Diseases*, 2011, 6: 71).

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 The molecular basis of SMA is the loss of both copies of survival motor neuron gene 1 (SMN1), which may also be known as SMN Telomeric, and encodes a protein that is part of a multi-protein complex thought to be involved in snRNP biogenesis and recycling. A nearly identical gene, SMN2, which may also be known as SMN Centromeric, exists in a duplicated region on chromosome 5q13 and modulates disease severity. Although SMN1 and SMN2 have the potential to code for the same protein, expression of the normal SMN1 gene results solely in expression of full-length survival motor neuron (SMN) protein, while expression of the SMN2 gene results in two different protein forms, full-length SMN2 protein, and a truncated SMN2 protein, SMN Δ 7 protein. SMN2 contains a translationally silent mutation at position +6 of exon 7, which results in inefficient inclusion of exon 7 in SMN2 transcripts. Thus, the predominant form of SMN2 is a truncated version, lacking exon 7, which is unstable and inactive (Cartegni and Krainer, *Nat. Genet.*, 2002, 30, 377-384). Expression of the SMN2 gene results in approximately 10-20% of the full-length SMN protein and 80-90% of the unstable/non-functional SMN Δ 7 protein. SMN protein plays a well-established role in assembly of the spliceosome and may also mediate mRNA trafficking in the axon and nerve terminus of neurons.

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It is an object herein to provide compounds, methods, and pharmaceutical compositions for the treatment of SMA.

Summary of the Invention

5 Provided herein are compounds, methods, and pharmaceutical compositions for modulating splicing of SMN2 RNA in a cell or subject. In certain embodiments, compounds useful for modulating splicing of SMN2 RNA are oligomeric compounds. In certain embodiments, oligomeric compounds increase the amount of SMN2 RNA including exon 7. In certain embodiments, oligomeric compounds increase full-length SMN2 protein expression. In certain
10 embodiments, the oligomeric compound comprises a modified oligonucleotide. In certain embodiments, the subject has a neurodegenerative disease. In certain embodiments, the subject has Spinal Muscular Atrophy (SMA).

 Also provided are methods useful for ameliorating at least one symptom of a neurodegenerative disease. In certain embodiments, the neurodegenerative disease is SMA. In certain embodiments, symptoms include reduced muscle strength; inability or reduced ability to sit upright, to stand, and/or walk; reduced neuromuscular activity; reduced electrical activity in one or more muscles; reduced respiration; inability or reduced ability to eat, drink, and/or
15 breathe without assistance; loss of weight or reduced weight gain; and/or decreased survival. In certain embodiments, provided herein are modified oligonucleotides for treating SMA.

Detailed Description of the Invention

 It is to be understood that both the foregoing general description and the following detailed description are
20 exemplary and explanatory only and are not restrictive. 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. 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.

25 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 application, including, but not limited to, patents, patent applications, articles, books, and treatises, and GenBank and NCBI reference sequence records are hereby expressly incorporated-by-reference for the portions of the document discussed herein, as well as in their entirety.

30 DEFINITIONS

 Unless specific definitions are provided, the nomenclature used 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. Where permitted, all patents, applications, published applications and other publications and other data referred to throughout in the disclosure are incorporated by reference
35 herein in their entirety.

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

 As used herein, “2'-deoxyribonucleoside” means a nucleoside comprising a 2'-H(H) deoxyribosyl sugar moiety. In certain embodiments, a 2'-deoxyribonucleoside is a 2'-β-D deoxyribonucleoside and comprises a 2'-β-D-deoxyribosyl sugar moiety, which has the β-D configuration as found in naturally occurring deoxyribonucleic acids

(DNA). In certain embodiments, a 2'-deoxyribonucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (uracil).

As used herein, "2'-MOE" means a 2'-OCH₂CH₂OCH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. A "2'-MOE sugar moiety" is a sugar moiety with a 2'-OCH₂CH₂OCH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. Unless otherwise indicated, a 2'-MOE sugar moiety is in the β-D configuration. "MOE" means O-methoxyethyl.

As used herein, "2'-MOE nucleoside" means a nucleoside comprising a 2'-MOE sugar moiety.

As used herein, "2'-NMA" means a -O-CH₂-C(=O)-NH-CH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. A "2'-NMA sugar moiety" is a sugar moiety with a 2'-O-CH₂-C(=O)-NH-CH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. Unless otherwise indicated, a 2'-NMA sugar moiety is in the β-D configuration. "NMA" means O-N-methyl acetamide.

As used herein, "2'-NMA nucleoside" means a nucleoside comprising a 2'-NMA sugar moiety.

As used herein, "2'-OMe" means a 2'-OCH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. A "2'-OMe sugar moiety" is a sugar moiety with a 2'-OCH₃ group in place of the 2'-OH group of a ribosyl sugar moiety. Unless otherwise indicated, a 2'-OMe sugar moiety is in the β-D configuration. "OMe" means O-methyl.

As used herein, "2'-OMe nucleoside" means a nucleoside comprising a 2'-OMe sugar moiety.

As used herein, "2'-substituted nucleoside" means a nucleoside comprising a 2'-substituted sugar moiety. As used herein, "2'-substituted" in reference to a sugar moiety means a sugar moiety comprising at least one 2'-substituent group other than H or OH.

As used herein, "5-methyl cytosine" means a cytosine modified with a methyl group attached to the 5 position. A 5-methyl cytosine is a modified nucleobase.

As used herein, "administering" means providing a pharmaceutical agent to a subject.

As used herein, "ameliorate" in reference to a treatment means improvement in at least one symptom relative to the same symptom in the absence of the treatment. In certain embodiments, amelioration is the reduction in the severity or frequency of a symptom, or the delayed onset or slowing of progression in the severity or frequency of a symptom. In certain embodiments, the symptom is reduced muscle strength; inability or reduced ability to sit upright, to stand, and/or walk; reduced neuromuscular activity; reduced electrical activity in one or more muscles; reduced respiration; inability or reduced ability to eat, drink, and/or breathe without assistance; loss of weight or reduced weight gain; and/or decreased survival.

As used herein, "antisense activity" means any detectable and/or measurable change attributable to the hybridization of an antisense compound to its target nucleic acid.

As used herein, "antisense compound" means an oligomeric compound or oligomeric duplex capable of achieving at least one antisense activity.

As used herein, "bicyclic nucleoside" or "BNA" means a nucleoside comprising a bicyclic sugar moiety.

As used herein, "bicyclic sugar" or "bicyclic sugar moiety" means a modified sugar moiety comprising two rings, wherein the second ring is formed via a bridge connecting two of the atoms in the first ring thereby forming a bicyclic structure. In certain embodiments, the first ring of the bicyclic sugar moiety is a furanosyl moiety. In certain embodiments, the furanosyl moiety is a ribosyl moiety. In certain embodiments, the bicyclic sugar moiety does not comprise a furanosyl moiety.

As used herein, "cerebrospinal fluid" or "CSF" means the fluid filling the space around the brain and spinal cord. "Artificial cerebrospinal fluid" or "aCSF" means a prepared or manufactured fluid that has certain properties of cerebrospinal fluid.

As used herein, "cEt" means a 4' to 2' bridge in place of the 2'OH-group of a ribosyl sugar moiety, wherein the bridge has the formula of 4'-CH(CH₃)-O-2', and wherein the methyl group of the bridge is in the *S* configuration. A "cEt sugar moiety" is a bicyclic sugar moiety with a 4' to 2' bridge in place of the 2'OH-group of a ribosyl sugar moiety, wherein the bridge has the formula of 4'-CH(CH₃)-O-2', and wherein the methyl group of the bridge is in the *S* configuration. "cEt" means constrained ethyl.

As used herein, "cEt nucleoside" means a nucleoside comprising a cEt sugar moiety.

As used herein, "chirally enriched population" means a plurality of molecules of identical molecular formula, wherein the number or percentage of molecules within the population that contain a particular stereochemical configuration at a particular chiral center is greater than the number or percentage of molecules expected to contain the same particular stereochemical configuration at the same particular chiral center within the population if the particular chiral center were stereorandom. Chirally enriched populations of molecules having multiple chiral centers within each molecule may contain one or more stereorandom chiral centers. In certain embodiments, the molecules are modified oligonucleotides. In certain embodiments, the molecules are compounds comprising modified oligonucleotides.

As used herein, "complementary" in reference to an oligonucleotide means that at least 70% of the nucleobases of the oligonucleotide or one or more portions thereof and the nucleobases of another nucleic acid or one or more portions thereof are capable of hydrogen bonding with one another when the nucleobase sequence of the oligonucleotide and the other nucleic acid are aligned in opposing directions. Complementary nucleobases means nucleobases that are capable of forming hydrogen bonds with one another. Complementary nucleobase pairs include adenine (A) with thymine (T), adenine (A) with uracil (U), cytosine (C) with guanine (G), and 5-methyl cytosine (mC) with guanine (G). Complementary oligonucleotides and/or target nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. As used herein, "fully complementary" or "100% complementary" in reference to an oligonucleotide, or a portion thereof, means that the oligonucleotide, or portion thereof, is complementary to another oligonucleotide or target nucleic acid at each nucleobase of the shorter of the two oligonucleotides, or at each nucleoside if the oligonucleotides are the same length.

As used herein, "contiguous" in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, "contiguous nucleobases" means nucleobases that are immediately adjacent to each other in a sequence.

As used herein, "hybridization" means the pairing or annealing of complementary oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

As used herein, "internucleoside linkage" means the covalent linkage between contiguous nucleosides in an oligonucleotide. As used herein, "modified internucleoside linkage" means any internucleoside linkage other than a phosphodiester internucleoside linkage. "Phosphorothioate internucleoside linkage" is a modified internucleoside linkage in which one of the non-bridging oxygen atoms of a phosphodiester internucleoside linkage is replaced with a sulfur atom.

As used herein, “mismatch” or “non-complementary” means a nucleobase of a first oligonucleotide that is not complementary with the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotide are aligned.

As used herein, “motif” means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

As used herein, “non-bicyclic modified sugar moiety” means a modified sugar moiety that comprises a modification, such as a substituent, that does not form a bridge between two atoms of the sugar to form a second ring.

As used herein, “nucleobase” means an unmodified nucleobase or a modified nucleobase. As used herein an “unmodified nucleobase” is adenine (A), thymine (T), cytosine (C), uracil (U), or guanine (G). As used herein, a “modified nucleobase” is a group of atoms other than unmodified A, T, C, U, or G capable of pairing with at least one unmodified nucleobase. A “5-methyl cytosine” is a modified nucleobase. A universal base is a modified nucleobase that can pair with any one of the five unmodified nucleobases. As used herein, “nucleobase sequence” means the order of contiguous nucleobases in a target nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage modification.

As used herein, “nucleoside” means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. As used herein, “modified nucleoside” means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. “Linked nucleosides” are nucleosides that are connected in a contiguous sequence (i.e., no additional nucleosides are presented between those that are linked).

As used herein, “oligomeric compound” means an oligonucleotide and optionally one or more additional features, such as a conjugate group or terminal group. An oligomeric compound may be paired with a second oligomeric compound that is complementary to the first oligomeric compound or may be unpaired. A “singled-stranded oligomeric compound” is an unpaired oligomeric compound. The term “oligomeric duplex” means a duplex formed by two oligomeric compounds having complementary nucleobase sequences. Each oligomeric compound of an oligomeric duplex may be referred to as a “duplexed oligomeric compound.”

As used herein, “oligonucleotide” means a strand of linked nucleosides connected via internucleoside linkages, wherein each nucleoside and internucleoside linkage may be modified or unmodified. Unless otherwise indicated, oligonucleotides consist of 8-50 linked nucleosides. As used herein, “modified oligonucleotide” means an oligonucleotide, wherein at least one nucleoside or internucleoside linkage is modified. As used herein, “unmodified oligonucleotide” means an oligonucleotide that does not comprise any nucleoside modifications or internucleoside modifications.

As used herein, “pharmaceutical composition” means a mixture of substances suitable for administering to a subject. For example, a pharmaceutical composition may comprise an oligomeric compound and a sterile aqueous solution.

As used herein, “pharmaceutically acceptable carrier or diluent” means any substance suitable for use in administering to a subject. Certain such carriers enable pharmaceutical compositions to be formulated as, for example, tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspension, and lozenges for the oral ingestion by a subject. In certain embodiments, a pharmaceutically acceptable carrier or diluent is sterile water, sterile saline, sterile buffer solution, or sterile artificial cerebrospinal fluid.

As used herein, “pharmaceutically acceptable salts” means physiologically and pharmaceutically acceptable salts of compounds. Pharmaceutically acceptable salts retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

5 As used herein, “RNA” means an RNA transcript and includes pre-mRNA and mature mRNA unless otherwise specified.

As used herein, “stereorandom chiral center” in the context of a population of molecules of identical molecular formula means a chiral center having a random stereochemical configuration. For example, in a population of molecules comprising a stereorandom chiral center, the number of molecules having the (*S*) configuration of the stereorandom chiral center may be but is not necessarily the same as the number of molecules having the (*R*) configuration of the stereorandom chiral center. The stereochemical configuration of a chiral center is considered random when it is the result of a synthetic method that is not designed to control the stereochemical configuration. In certain embodiments, a stereorandom chiral center is a stereorandom phosphorothioate internucleoside linkage.

As used herein, “subject” means a human or non-human animal.

As used herein, “sugar moiety” means an unmodified sugar moiety or a modified sugar moiety. As used herein, “unmodified sugar moiety” means a 2'-OH(H) β-D ribosyl moiety, as found in RNA (an “unmodified RNA sugar moiety”), or a 2'-H(H) β-D deoxyribosyl moiety, as found in DNA (an “unmodified DNA sugar moiety”). Unmodified sugar moieties have one hydrogen at each of the 1', 3', and 4' positions, an oxygen at the 3' position, and two hydrogens at the 5' position. As used herein, “modified sugar moiety” or “modified sugar” means a modified furanosyl sugar moiety or a sugar surrogate.

20 As used herein, “sugar surrogate” means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group in an oligonucleotide. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide and such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or target nucleic acids.

25 As used herein, “standard *in vivo* assay” means the assay described in Example 2 and reasonable variations thereof.

As used herein, “symptom” means any physical feature or test result that indicates the existence or extent of a disease or disorder. In certain embodiments, a symptom is apparent to a subject or to a medical professional examining or testing the subject.

30 As used herein, “target nucleic acid” means a nucleic acid that an antisense compound is designed to affect.

As used herein, “target region” means a portion of a target nucleic acid to which an oligomeric compound is designed to hybridize.

As used herein, “terminal group” means a chemical group or group of atoms that is covalently linked to a terminus of an oligonucleotide.

35 As used herein, “therapeutically effective amount” means an amount of a pharmaceutical agent that provides a therapeutic benefit to a subject. For example, a therapeutically effective amount improves a symptom of a disease.

CERTAIN EMBODIMENTS

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

Embodiment 1. An oligomeric compound comprising a modified oligonucleotide consisting of 16, 17, 18, 19, or 20 linked nucleosides and having a nucleobase sequence comprising at least 15 or at least 16 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 20-50, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

- 5 Embodiment 2. An oligomeric compound comprising a modified oligonucleotide consisting of 17, 18, 19, or 20 linked nucleosides and having a nucleobase sequence comprising at least 15, at least 16, or at least 17 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 20-27, 29-30, or 32-50, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

- 10 Embodiment 3. An oligomeric compound comprising a modified oligonucleotide consisting of 18, 19, or 20 linked nucleosides and having a nucleobase sequence comprising at least 15, at least 16, or at least 17, or at least 18 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 20-27, 30, or 33-50, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

- 15 Embodiment 4. An oligomeric compound comprising a modified oligonucleotide consisting of 19 or 20 linked nucleosides and having a nucleobase sequence comprising at least 15, at least 16, or at least 17, at least 18, or at least 19 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 20, 22, 24-27, 30, 33-50, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

- 20 Embodiment 5. An oligomeric compound comprising a modified oligonucleotide consisting of 20 linked nucleosides and having a nucleobase sequence comprising at least 15, at least 16, or at least 17, at least 18, at least 19, or at least 20 contiguous nucleobases of any of the nucleobase sequences of SEQ ID NOs: 20, 22, 25, 27, 35, 39-46, or 49, wherein the modified oligonucleotide comprises at least one modification selected from a modified sugar moiety and a modified internucleoside linkage.

- 25 Embodiment 6. The oligomeric compound of any of embodiments 1-5, wherein the modified oligonucleotide has a nucleobase sequence that is at least 80%, 85%, 87.5%, 88.2%, 89%, 89.4%, 90%, 93.7%, 94%, 94.7%, 95%, or is 100% complementary to the nucleobase sequence of SEQ ID NO: 1 when measured across the entire nucleobase sequence of the modified oligonucleotide.

- Embodiment 7. The oligomeric compound of any of embodiments 1-6, wherein the modified oligonucleotide has an internucleoside linkage motif (5' to 3') selected from: sososssssssssss, ssosssssssssoss, ssosssssssssoss,
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Embodiment 8. The oligomeric compound of any of embodiments 1-6, wherein the modified oligonucleotide has an internucleoside linkage motif selected from: ssssssssssssssx and ssssssssssssssx, wherein 's' represents a phosphorothioate internucleoside linkage, 'o' represents a phosphodiester internucleoside linkage, and "x" represents a methoxypropylphosphonate internucleoside linkage.

Embodiment 9. The oligomeric compound of any of embodiments 1-6, wherein the modified oligonucleotide has an internucleoside linkage motif selected from: *zzzzzzzzzzzzzzzzzzzz*, *sssssssssszzzzzz*, *sssszzzzzzssssss*, *zzzzzzzzzzzzzzzzzzzzzz*, *zzzzzzzzzzzzzzzzzzzzzz*, *zzzzzzzzzzzzzzzzzzzzzz*, *zzzzzzzzzzzzzzzzzzzzzz*, and *sszzzzzzzzzzzzzzzzzzzz*, wherein 's' represents a phosphorothioate internucleoside linkage, 'o' represents a phosphodiester internucleoside linkage, and "z" represents a mesyl phosphoramidate internucleoside linkage.

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eeeeeeeeeeeeeeeeed, eeeeeeeeeeeeeeeey, ennnnnnnnnnnnnnnnnnn, and ennnnnnnnnnnnnnnnnne; wherein 'e' represents a 2'-MOE sugar moiety, 'n' represents a 2'-NMA sugar moiety, 'k' represents a cEt sugar moiety, 'd' represents a 2'-β-D-deoxyribose sugar moiety, and 'y' represents a 2'-OMe sugar moiety.

5 Embodiment 11. The oligomeric compound of any of embodiments 1-9, wherein the modified oligonucleotide has a sugar motif (5' to 3') selected from nnnnnnnnnnnnnnnnn and nnnnnnnnnnnnnnnnn, wherein 'e' represents a 2'-MOE sugar moiety and 'n' represents a 2'-NMA sugar moiety.

Embodiment 12. The oligomeric compound of any of embodiments 1-9, wherein the modified oligonucleotide has a sugar motif (5' to 3') of qqnnqqnnqqnnqqnn, wherein in each 'n' represents a 2'-NMA sugar moiety, and each 'q' is independently selected from a 2'-O-(N,N-dimethyl) acetamide sugar moiety, a 2'-O-(N-ethyl) acetamide sugar moiety, 10 a 2'-O-(N-propyl) acetamide sugar moiety, a 2'-O-(N-cyclopropyl) acetamide sugar moiety, and a 2'-O-(N-cyclopropylmethyl) acetamide sugar moiety.

Embodiment 13. The oligomeric compound of any of embodiments 1-9, wherein the modified oligonucleotide comprises at least one modified sugar moiety.

15 Embodiment 14. The oligomeric compound of embodiment 13, wherein the modified oligonucleotide comprises at least one bicyclic sugar moiety.

Embodiment 15. The oligomeric compound of embodiment 14, wherein the bicyclic sugar moiety has a 4'-2' bridge, wherein the 4'-2' bridge is selected from -CH₂-O-; and -CH(CH₃)-O-.

Embodiment 16. The oligomeric compound of embodiment 13, wherein the modified oligonucleotide comprises at least one non-bicyclic modified sugar moiety.

20 Embodiment 17. The oligomeric compound of embodiment 16, wherein the non-bicyclic modified sugar moiety is any of a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, or a 2'-F sugar moiety.

Embodiment 18. The oligomeric compound of embodiment 13, wherein the modified oligonucleotide comprises at least one sugar surrogate.

25 Embodiment 19. The oligomeric compound of embodiment 18, wherein the sugar surrogate is any of morpholino, modified morpholino, PNA, THP, and F-HNA.

Embodiment 20. The oligomeric compound of any of embodiments 1-6 and 10-19, wherein the modified oligonucleotide comprises at least one modified internucleoside linkage.

Embodiment 21. The oligomeric compound of embodiment 20, wherein each internucleoside linkage of the modified oligonucleotide is a modified internucleoside linkage.

30 Embodiment 22. The oligomeric compound of embodiment 20 or embodiment 21, wherein the modified internucleoside linkage is a phosphorothioate internucleoside linkage.

Embodiment 23. The oligomeric compound of any of embodiments 1-20 or 22, wherein the modified oligonucleotide comprises at least one phosphodiester internucleoside linkage.

Embodiment 24. The oligomeric compound of any of embodiments 20, 22, or 23, wherein each internucleoside linkage is independently selected from a phosphodiester internucleoside linkage and a phosphorothioate internucleoside linkage.

Embodiment 25. The oligomeric compound of any of embodiments 13-19, wherein the modified oligonucleotide has an internucleoside linkage motif (5' to 3') selected from: sososssssssssss, soosssssssssss, sossosssssssss, 5 sosssssssssss, sosssssssssss, ssoosssssssss, ssssoosssssss, ssssssoosssss, and ssssssssoosss; wherein 's' represents a phosphorothioate internucleoside linkage and 'o' represents a phosphodiester internucleoside linkage.

Embodiment 26. The oligomeric compound of any of embodiments 1-25, wherein the modified oligonucleotide comprises a modified nucleobase.

10 Embodiment 27. The oligomeric compound of embodiment 26, wherein the modified nucleobase is a 5-methyl cytosine.

Embodiment 28. The oligomeric compound of any of embodiments 1-27, wherein the modified oligonucleotide consists of 16, 17, 18, 19, or 20 linked nucleosides.

Embodiment 29. The oligomeric compound of any of embodiments 1-28, wherein the modified oligonucleotide comprises 1 or 2 non-complementary nucleobases.

15 Embodiment 30. The oligomeric compound of any of embodiments 1-29, wherein the modified oligonucleotide comprises 1 or 2 cleavable moieties.

Embodiment 31. The oligomeric compound of embodiment 30, wherein the cleavable moiety is a phosphodiester internucleoside linkage.

Embodiment 32. The oligomeric compound of any of embodiments 1-31, consisting of the modified oligonucleotide.

20 Embodiment 33. The oligomeric compound of any of embodiments 1-32, wherein the oligomeric compound is a singled-stranded oligomeric compound.

Embodiment 34. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: ${}^m\text{C}_{\text{es}} \text{A}_{\text{eo}} {}^m\text{C}_{\text{es}} \text{T}_{\text{eo}} \text{T}_{\text{es}} \text{T}_{\text{es}} {}^m\text{C}_{\text{es}} \text{A}_{\text{es}} \text{T}_{\text{es}} \text{A}_{\text{es}} \text{A}_{\text{es}} \text{T}_{\text{es}} \text{G}_{\text{es}} {}^m\text{C}_{\text{es}} \text{T}_{\text{es}} \text{G}_{\text{es}} \text{G}_{\text{es}} {}^m\text{C}_{\text{e}}$ (SEQ ID NO: 21), wherein:

- A = an adenine nucleobase,
- 25 ${}^m\text{C}$ = a 5-methyl cytosine nucleobase,
- G = a guanine nucleobase,
- T = a thymine nucleobase,
- e = a 2'-MOE sugar moiety,
- s = a phosphorothioate internucleoside linkage, and
- 30 o = a phosphodiester internucleoside linkage.

Embodiment 35. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $\text{T}_{\text{eo}} \text{T}_{\text{es}} {}^m\text{C}_{\text{es}} \text{A}_{\text{es}} {}^m\text{C}_{\text{es}} \text{T}_{\text{es}} \text{T}_{\text{es}} \text{T}_{\text{es}} {}^m\text{C}_{\text{es}} \text{A}_{\text{es}} \text{T}_{\text{es}} \text{A}_{\text{es}} \text{A}_{\text{es}} \text{T}_{\text{es}} \text{G}_{\text{es}} {}^m\text{C}_{\text{es}} \text{T}_{\text{es}} \text{G}_{\text{es}} \text{G}_{\text{eo}} {}^m\text{C}_{\text{e}}$ (SEQ ID NO: 22), wherein:

- A = an adenine nucleobase,
- ${}^m\text{C}$ = a 5-methyl cytosine nucleobase,
- 35 G = a guanine nucleobase,

T = a thymine nucleobase,
 e = a 2'-MOE sugar moiety,
 s = a phosphorothioate internucleoside linkage, and
 o = a phosphodiester internucleoside linkage.

- 5 Embodiment 36. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: $T_{eo} T_{ns} {}^mC_{ns} A_{ns} {}^mC_{ns} T_{ns} T_{ns} T_{ns} {}^mC_{ns} A_{ns} T_{ns} A_{ns} A_{ns} T_{ns} G_{ns} {}^mC_{ns} T_{ns} G_{ns} G_{no} {}^mC_e$ (SEQ ID NO: 22), wherein:

A = an adenine nucleobase,
 ${}^mC$ = a 5-methyl cytosine nucleobase,

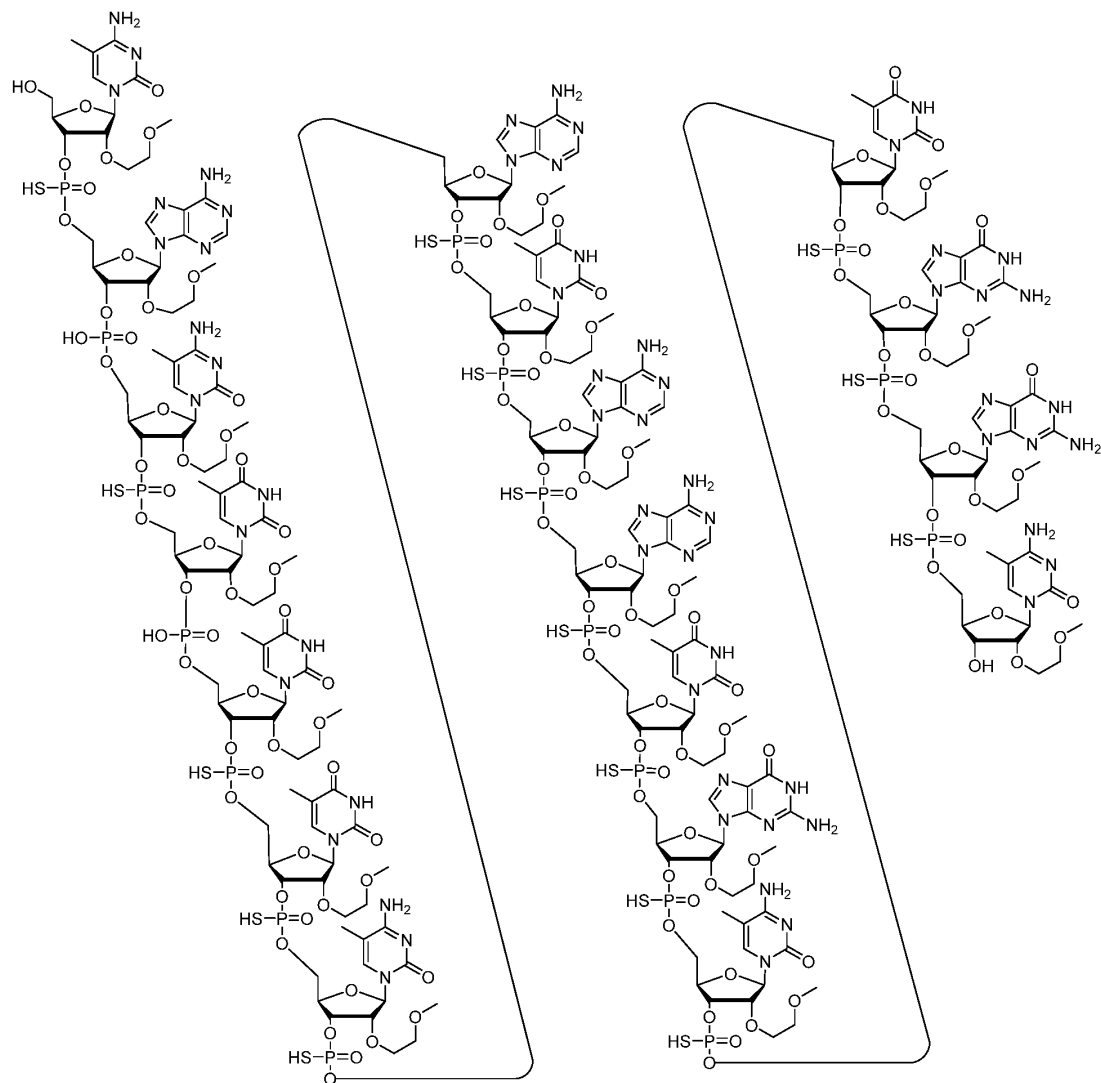
- 10 G = a guanine nucleobase,
 T = a thymine nucleobase,
 e = a 2'-MOE sugar moiety,
 n = a 2'-NMA sugar moiety,
 s = a phosphorothioate internucleoside linkage, and
 o = a phosphodiester internucleoside linkage.

- 15 Embodiment 37. An oligomeric compound comprising a modified oligonucleotide according to the following chemical notation: ${}^mC_{ns} A_{no} {}^mC_{ns} T_{no} T_{ns} T_{ns} {}^mC_{ns} A_{ns} T_{ns} A_{ns} A_{ns} T_{ns} G_{ns} {}^mC_{ns} T_{ns} G_{ns} G_{ns} {}^mC_n$ (SEQ ID NO: 21), wherein:

A = an adenine nucleobase,
 ${}^mC$ = a 5-methyl cytosine nucleobase,

- 20 G = a guanine nucleobase,
 T = a thymine nucleobase,
 n = a 2'-NMA sugar moiety,
 s = a phosphorothioate internucleoside linkage, and
 o = a phosphodiester internucleoside linkage.

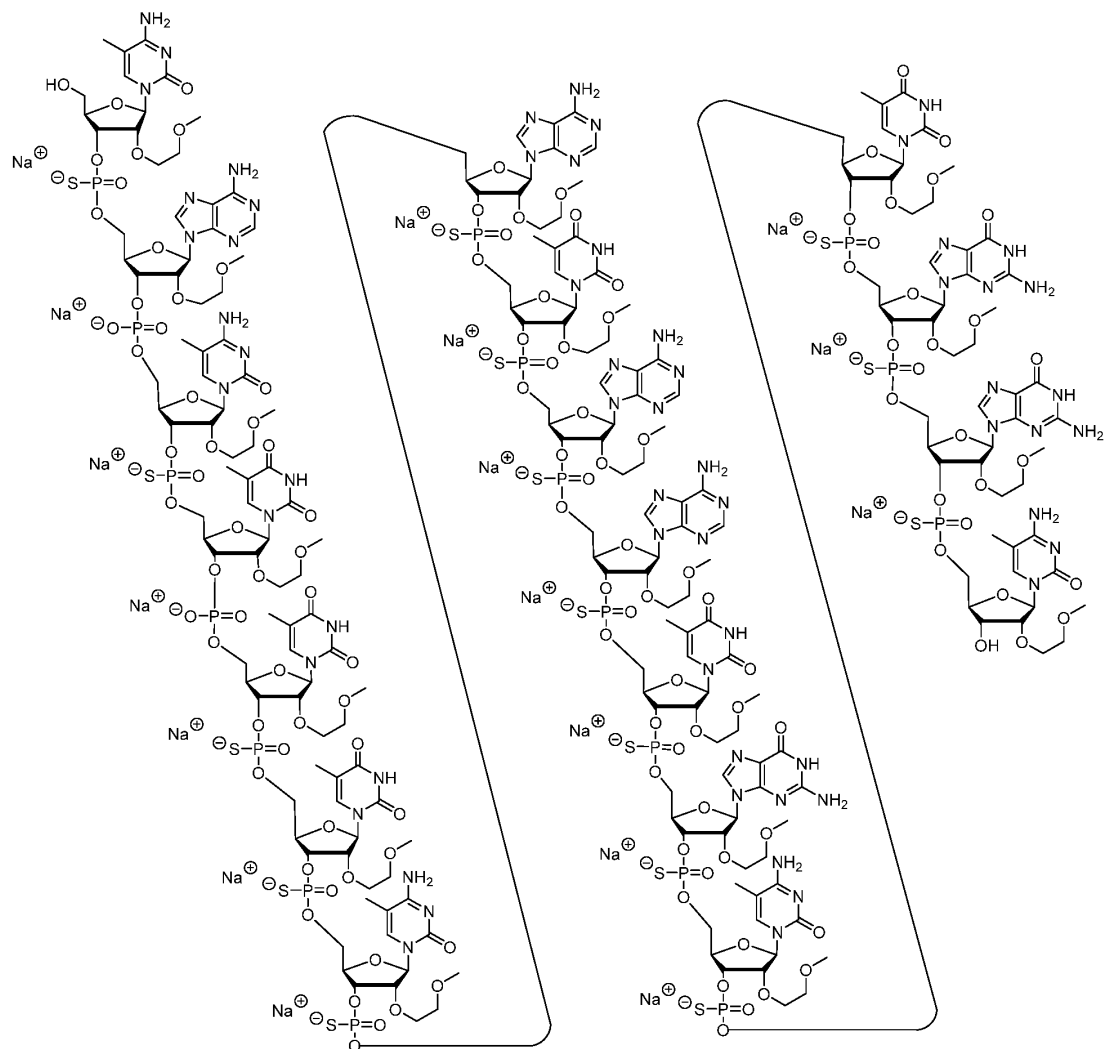
Embodiment 38. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 21), or a salt thereof.

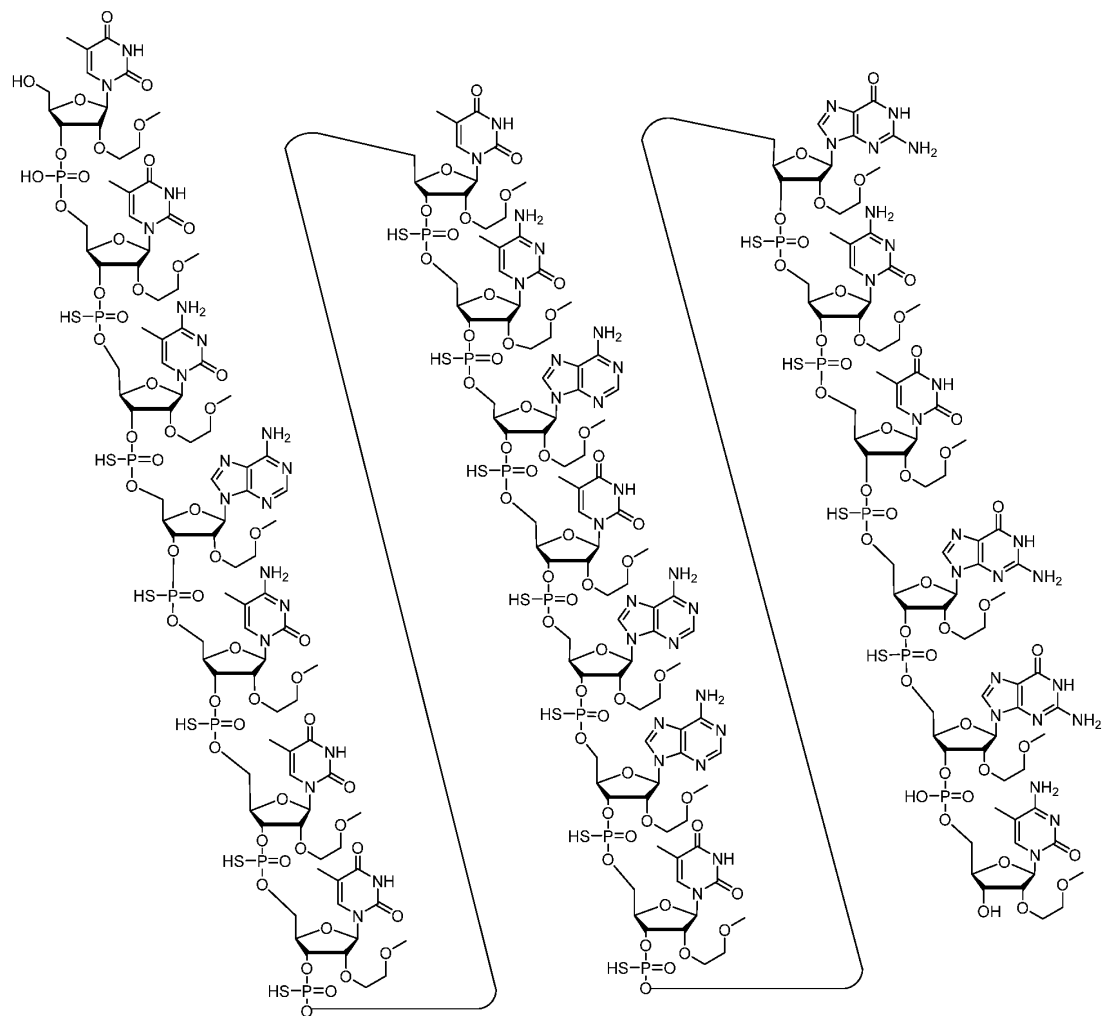
Embodiment 39. The modified oligonucleotide of embodiment 38, which is the sodium salt or the potassium salt.

Embodiment 40. A modified oligonucleotide according to the following chemical structure:



5 (SEQ ID NO: 21).

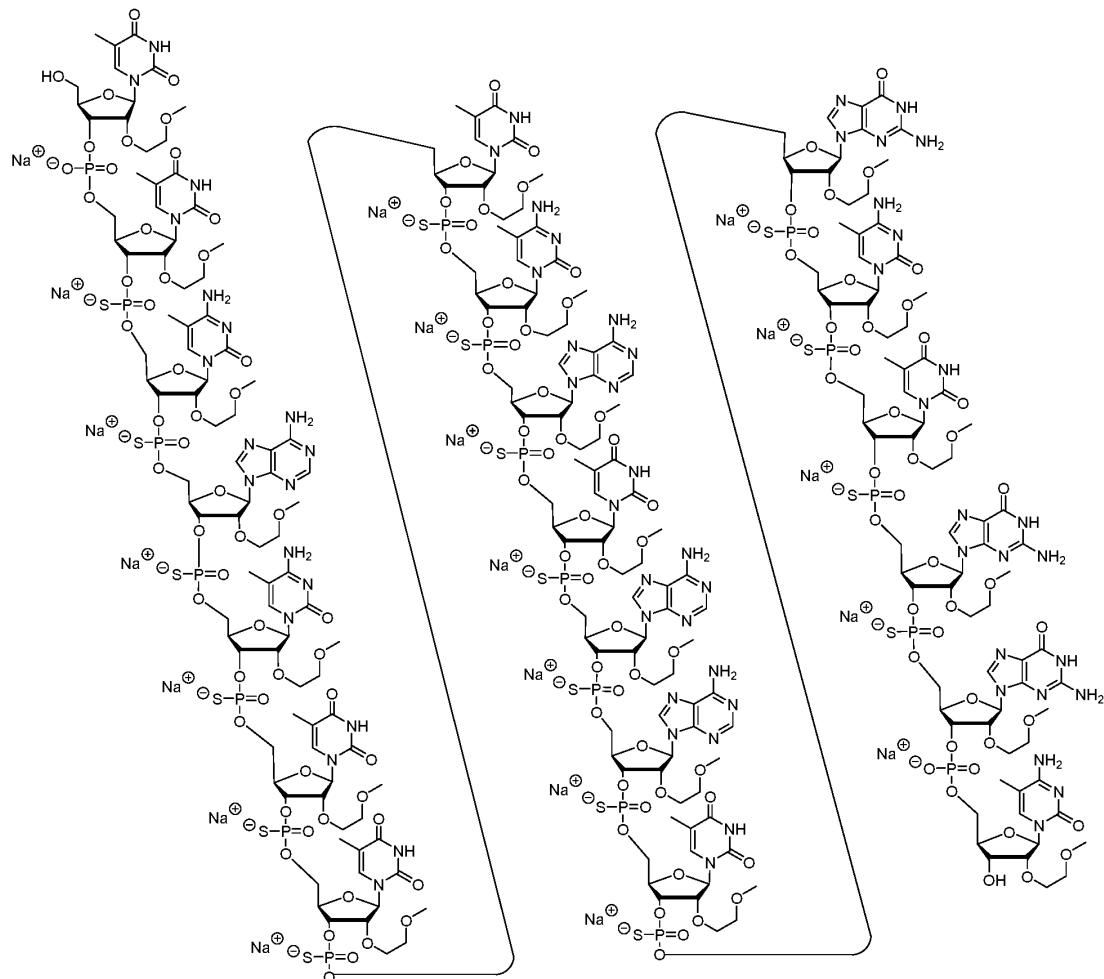
Embodiment 41. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 22), or a salt thereof.

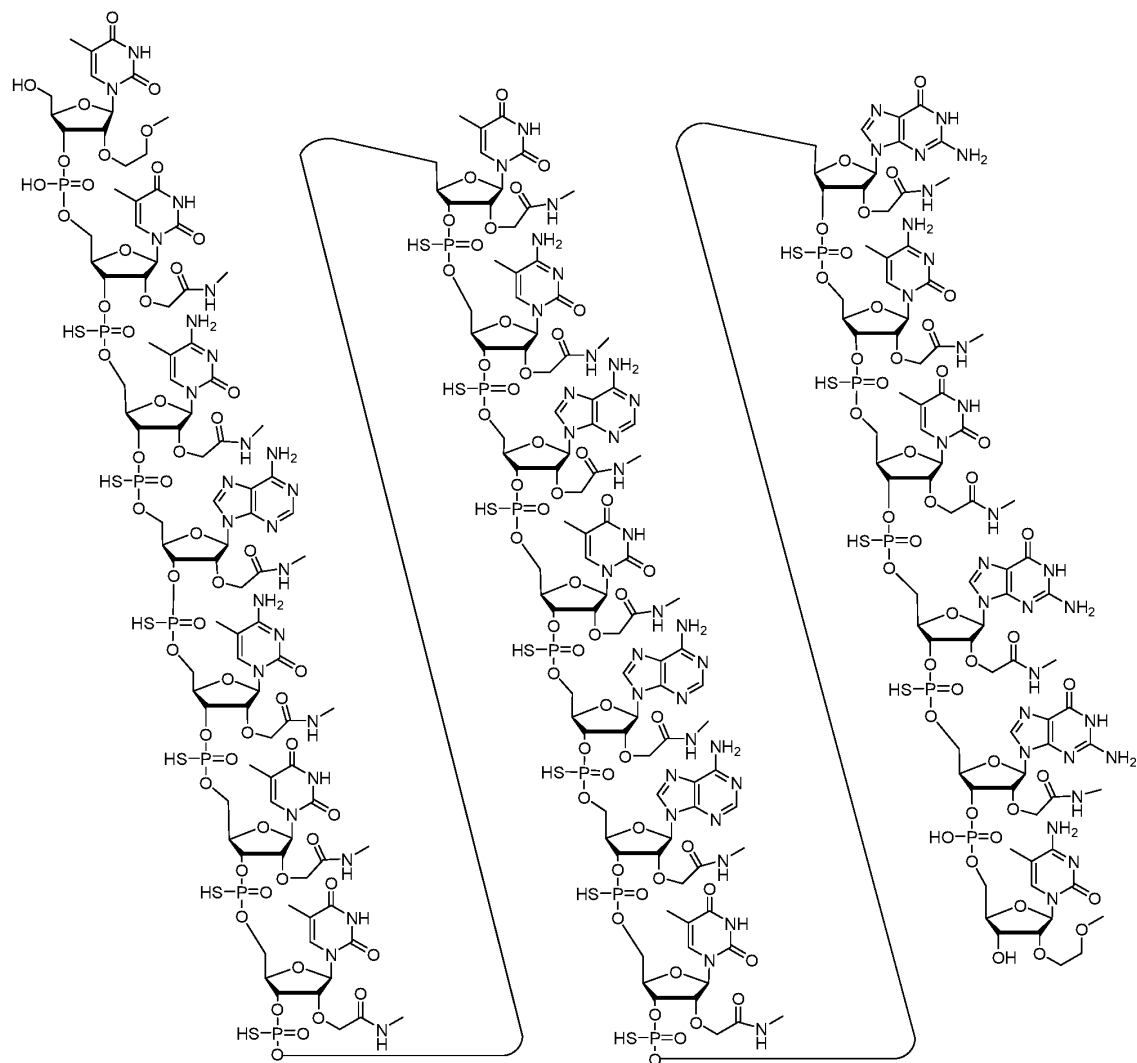
Embodiment 42. The modified oligonucleotide of embodiment 41, which is the sodium salt or the potassium salt.

Embodiment 43. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 22).

Embodiment 44. A modified oligonucleotide according to the following chemical structure:

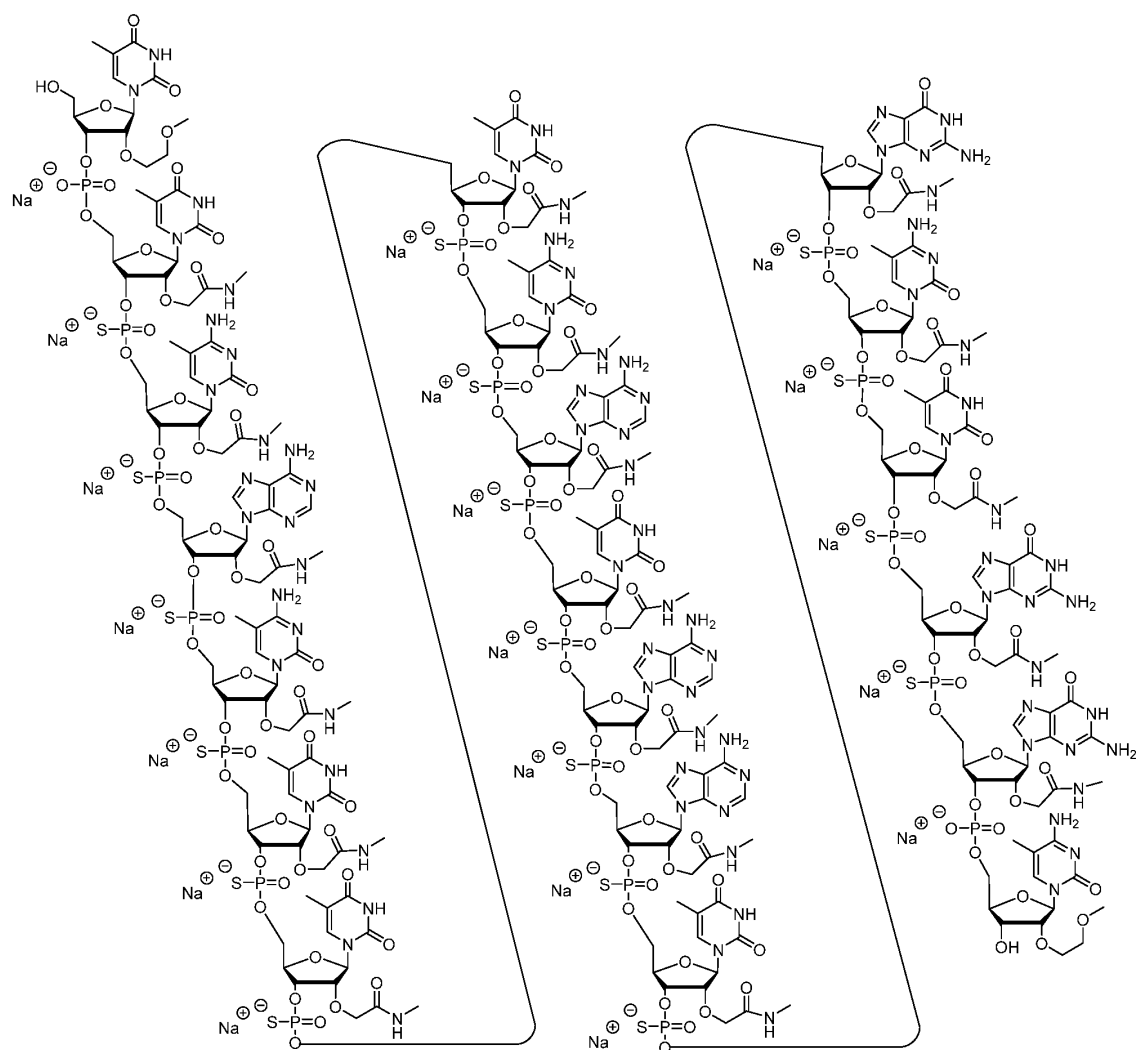


(SEQ ID NO: 22), or a salt thereof.

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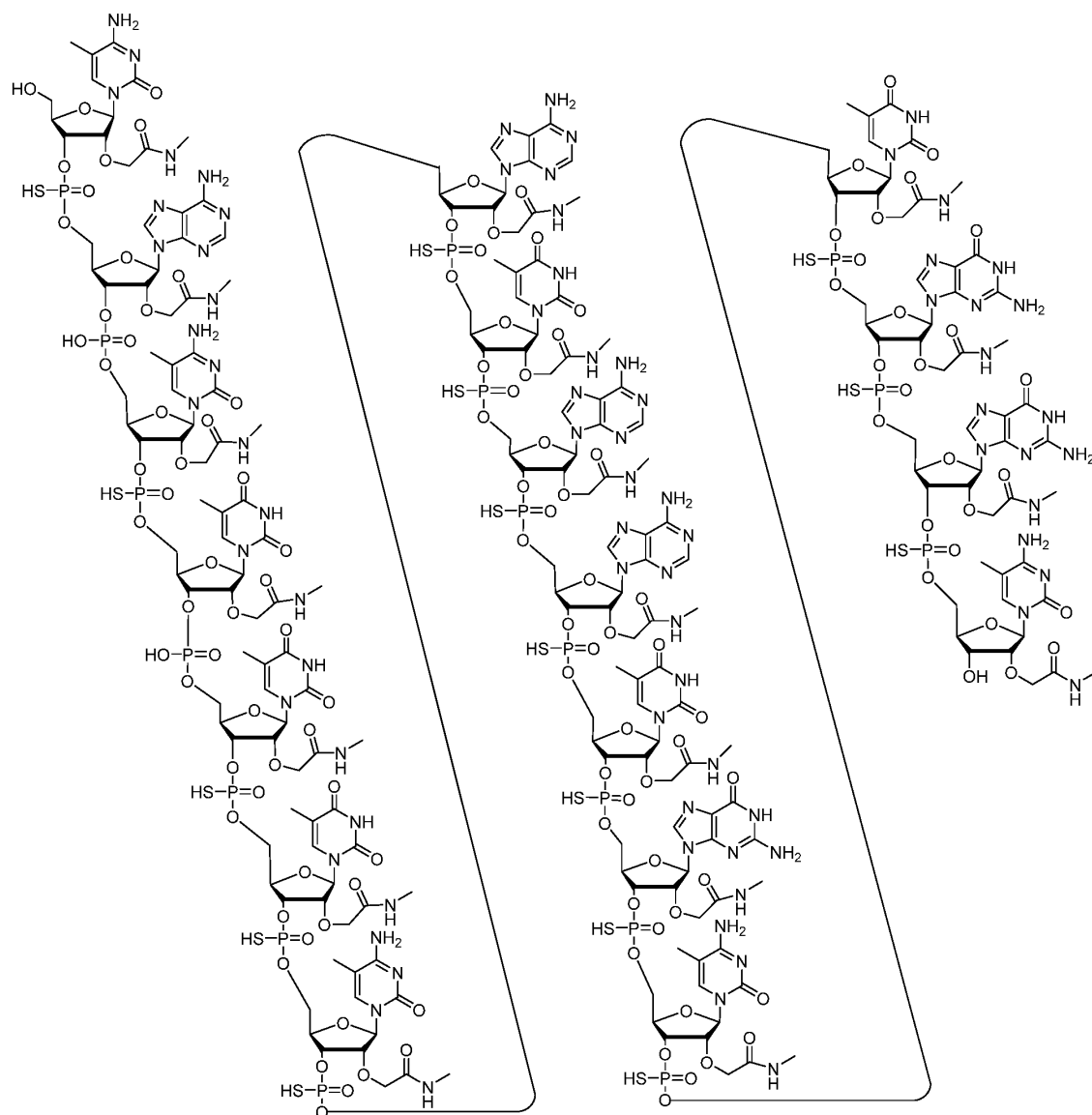
Embodiment 45. The modified oligonucleotide of embodiment 44, which is the sodium salt or the potassium salt.

Embodiment 46. A modified oligonucleotide corresponding to the following chemical structure:



(SEQ ID NO: 22).

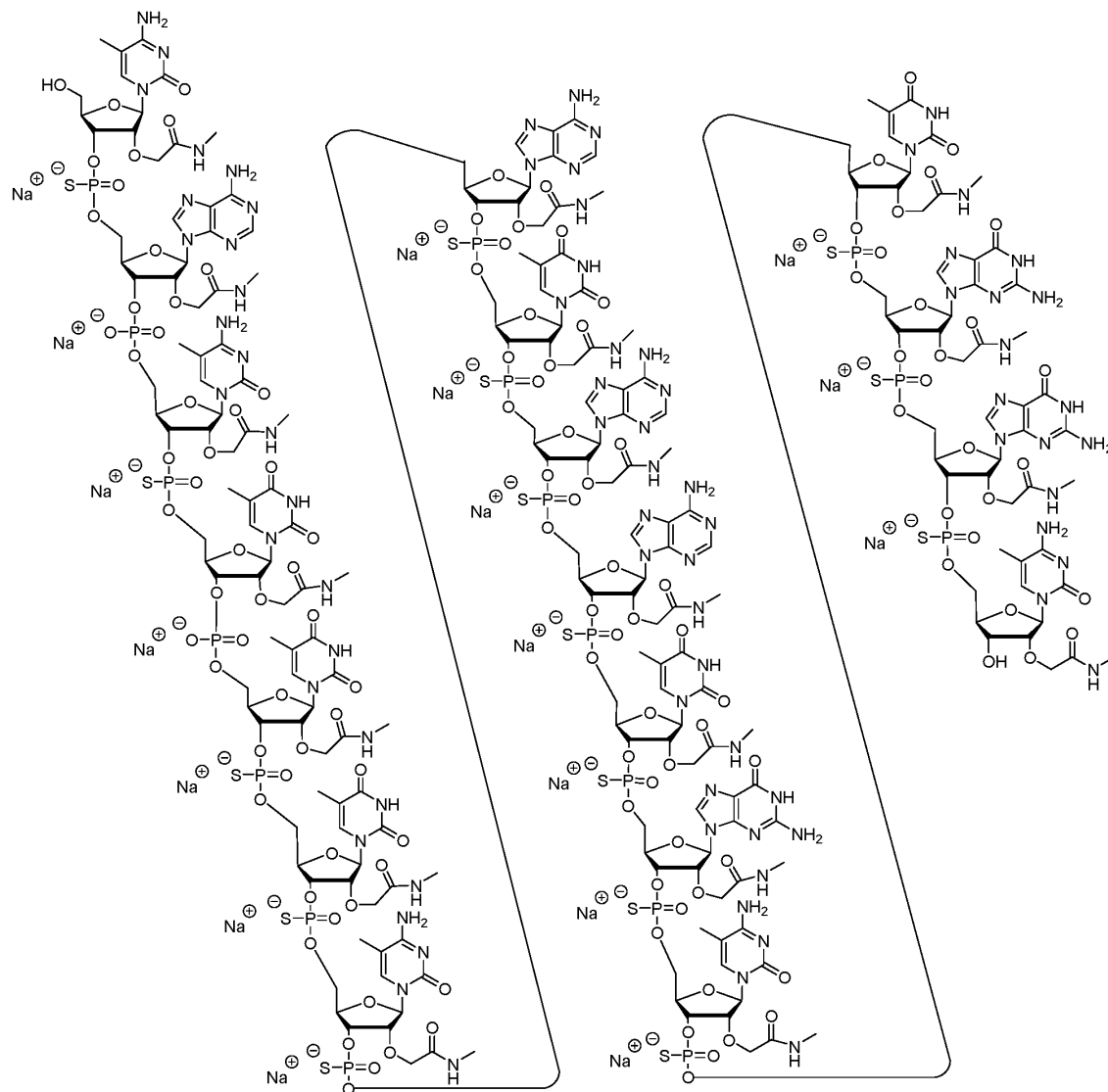
Embodiment 47. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 21), or a salt thereof.

Embodiment 48. The modified oligonucleotide of embodiment 47, which is the sodium salt or the potassium salt.

Embodiment 49. A modified oligonucleotide according to the following chemical structure:



(SEQ ID NO: 21).

- 5 Embodiment 50. A pharmaceutical composition comprising the oligomeric compound of any of embodiments 1-36 or the modified oligonucleotide of any of embodiments 38-49, and a pharmaceutically acceptable diluent or carrier.

Embodiment 51. The pharmaceutical composition of embodiment 50, comprising a pharmaceutically acceptable diluent and wherein the pharmaceutically acceptable diluent is artificial CSF (aCSF) or PBS.

- 10 Embodiment 52. The pharmaceutical composition of embodiment 51, wherein the pharmaceutical composition consists essentially of the modified oligonucleotide and artificial CSF (aCSF).

Embodiment 53. The pharmaceutical composition of embodiment 51, wherein the pharmaceutical composition consists essentially of the modified oligonucleotide and PBS.

Embodiment 54. A chirally enriched population of modified oligonucleotides of any of embodiments 38-49, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having a particular stereochemical configuration.

Embodiment 55. The chirally enriched population of embodiment 54, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having the (*Sp*) configuration.

Embodiment 56. The chirally enriched population of embodiment 54, wherein the population is enriched for modified oligonucleotides comprising at least one particular phosphorothioate internucleoside linkage having the (*Rp*) configuration.

Embodiment 57. The chirally enriched population of embodiment 54, wherein the population is enriched for modified oligonucleotides having a particular, independently selected stereochemical configuration at each phosphorothioate internucleoside linkage.

Embodiment 58. The chirally enriched population of embodiment 57, wherein the population is enriched for modified oligonucleotides having the (*Sp*) configuration at each phosphorothioate internucleoside linkage or for modified oligonucleotides having the (*Rp*) configuration at each phosphorothioate internucleoside linkage.

Embodiment 59. The chirally enriched population of embodiment 57, wherein the population is enriched for modified oligonucleotides having the (*Rp*) configuration at one particular phosphorothioate internucleoside linkage and the (*Sp*) configuration at each of the remaining phosphorothioate internucleoside linkages.

Embodiment 60. The chirally enriched population of embodiment 57, wherein the population is enriched for modified oligonucleotides having at least 3 contiguous phosphorothioate internucleoside linkages in the *Sp*, *Sp*, and *Rp* configurations, in the 5' to 3' direction.

Embodiment 61. A population of modified oligonucleotides of any of embodiments 38-49, wherein all of the phosphorothioate internucleoside linkages of the modified oligonucleotide are stereorandom.

Embodiment 62. A method of treating a disease associated with SMN1 or SMN2 comprising administering to a subject having or at risk for developing a disease associated with SMN1 or SMN2 a therapeutically effective amount of a pharmaceutical composition according to any of embodiments 50-53; and thereby treating the disease associated with SMN1 or SMN2.

Embodiment 63. The method of embodiment 62, wherein the disease associated with SMN1 or SMN2 is a neurodegenerative disease.

Embodiment 64. The method of embodiment 63, wherein the neurodegenerative disease is Spinal Muscular Atrophy (SMA).

Embodiment 65. The method of embodiment 64, wherein the SMA is any of Type I SMA, Type II SMA, Type III SMA, or Type IV SMA.

Embodiment 66. The method of embodiment 64 or embodiment 65, wherein at least one symptom of SMA is ameliorated.

Embodiment 67. The method of embodiment 66, wherein the symptom is any of reduced muscle strength; inability or reduced ability to sit upright, to stand, and/or walk; reduced neuromuscular activity; reduced electrical activity in one or more muscles; reduced respiration; inability or reduced ability to eat, drink, and/or breathe without assistance; loss of weight or reduced weight gain; and/or decreased survival.

Embodiment 68. The method of any of embodiments 62-67, wherein the pharmaceutical composition is administered to the central nervous system or systemically.

Embodiment 69. The method of embodiment 68, wherein the pharmaceutical composition is administered to the central nervous system and systemically.

Embodiment 70. The method of any of embodiment 62-67, wherein the pharmaceutical composition is administered any of intrathecally, systemically, subcutaneously, or intramuscularly.

Embodiment 71. A method of increasing SMN2 RNA including exon 7 comprising contacting a cell, tissue, or organ with an oligomeric compound of any of embodiments 1-37, a modified oligonucleotide of any of embodiments 38-49, or a pharmaceutical composition of any of embodiments 50-53.

Certain Oligonucleotides

In certain embodiments, provided herein are oligomeric compounds comprising oligonucleotides, which consist of linked nucleosides. Oligonucleotides may be unmodified oligonucleotides (RNA or DNA) or may be modified oligonucleotides. Modified oligonucleotides comprise at least one modification relative to unmodified RNA or DNA.

That is, modified oligonucleotides comprise at least one modified nucleoside (comprising a modified sugar moiety and/or a modified nucleobase) and/or at least one modified internucleoside linkage.

Certain Modified Nucleosides

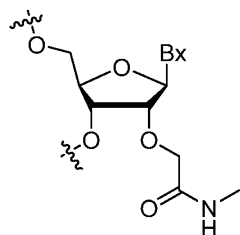
Modified nucleosides comprise a modified sugar moiety or a modified nucleobase or both a modified sugar moiety and a modified nucleobase.

Certain Sugar Moieties

In certain embodiments, modified sugar moieties are non-bicyclic modified sugar moieties. In certain embodiments, modified sugar moieties are bicyclic or tricyclic sugar moieties. In certain embodiments, modified sugar moieties are sugar surrogates. Such sugar surrogates may comprise one or more substitutions corresponding to those of other types of modified sugar moieties.

In certain embodiments, modified sugar moieties are non-bicyclic modified sugar moieties comprising a furanosyl ring with one or more substituent groups none of which bridges two atoms of the furanosyl ring to form a bicyclic structure. Such non bridging substituents may be at any position of the furanosyl, including but not limited to substituents at the 2', 4', and/or 5' positions. In certain embodiments one or more non-bridging substituent of non-bicyclic modified sugar moieties is branched. Examples of 2'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 2'-F, 2'-OCH₃ ("OMe" or "O-methyl"), and 2'-O(CH₂)₂OCH₃ ("MOE" or "O-methoxyethyl"), and 2'-O-N-alkyl acetamide, e.g., 2'-O-N-methyl acetamide ("NMA"), 2'-O-N-dimethyl acetamide, 2'-O-N-ethyl acetamide, or

2'-O-N-propyl acetamide. For example, see U.S. 6,147,200, Prakash et al., 2003, *Org. Lett.*, 5, 403-6. A "2'-O-N-methyl acetamide nucleoside" or "2'-NMA nucleoside" is shown below:



In certain embodiments, 2'-substituent groups are selected from among: halo, allyl, amino, azido, SH, CN, OCN, CF₃, OCF₃, O-C₁-C₁₀ alkoxy, O-C₁-C₁₀ substituted alkoxy, O-C₁-C₁₀ alkyl, O-C₁-C₁₀ substituted alkyl, S-alkyl, N(R_m)-alkyl, O-alkenyl, S-alkenyl, N(R_m)-alkenyl, O-alkynyl, S-alkynyl, N(R_m)-alkynyl, O-alkylenyl-O-alkyl, alkynyl, alkaryl, aralkyl, O-alkaryl, O-aralkyl, O(CH₂)₂SCH₃, O(CH₂)₂ON(R_m)(R_n) or OCH₂C(=O)-N(R_m)(R_n), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C₁-C₁₀ alkyl, and the 2'-substituent groups described in Cook et al., U.S. 6,531,584; Cook et al., U.S. 5,859,221; and Cook et al., U.S. 6,005,087. Certain
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 embodiments of these 2'-substituent groups can be further substituted with one or more substituent groups independently selected from among: hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro (NO₂), thiol, thioalkoxy, thioalkyl, halogen, alkyl, aryl, alkenyl and alkynyl. Examples of 4'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to alkoxy (*e.g.*, methoxy), alkyl, and those described in Manoharan et al., WO 2015/106128. Examples of 5'-substituent groups suitable for non-bicyclic modified sugar moieties include but are not limited to: 5'-methyl (*R* or *S*), 5'-vinyl, and 5'-methoxy. In certain embodiments, non-bicyclic modified sugar moieties comprise more than one non-bridging sugar substituent, for example, 2'-F-5'-methyl sugar moieties and the modified sugar moieties and modified nucleosides described in Migawa et al., WO 2008/101157 and Rajeev et al., US2013/0203836.

In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, NH₂, N₃, OCF₃, OCH₃, O(CH₂)₃NH₂, CH₂CH=CH₂, OCH₂CH=CH₂, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O(CH₂)₂ON(R_m)(R_n), O(CH₂)₂ON(CH₃)₂, O(CH₂)₂O(CH₂)₂N(CH₃)₂, and N-substituted acetamide (OCH₂C(=O)-N(R_m)(R_n)), where each R_m and R_n is, independently, H, an amino protecting group, or substituted or unsubstituted C₁-C₁₀ alkyl, *e.g.*, for example, OCH₂C(=O)-N(H)CH₃ ("NMA").

In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCF₃, OCH₃, OCH₂CH₂OCH₃, O(CH₂)₂SCH₃, O(CH₂)₂ON(CH₃)₂, O(CH₂)₂O(CH₂)₂N(CH₃)₂, and OCH₂C(=O)-N(H)CH₃ ("NMA").

In certain embodiments, a 2'-substituted non-bicyclic modified nucleoside comprises a sugar moiety comprising a non-bridging 2'-substituent group selected from: F, OCH₃, OCH₂CH₂OCH₃, and OCH₂C(=O)-N(H)CH₃.

Certain modified sugar moieties comprise a substituent that bridges two atoms of the furanosyl ring to form a second ring, resulting in a bicyclic sugar moiety. In certain such embodiments, the bicyclic sugar moiety comprises a bridge between the 4' and the 2' furanose ring atoms. Examples of such 4' to 2' bridging sugar substituents include but are not limited to: 4'-CH₂-2', 4'-(CH₂)₂-2', 4'-(CH₂)₃-2', 4'-CH₂-O-2' ("LNA"), 4'-CH₂-S-2', 4'-(CH₂)₂-O-2' ("ENA"), 4'-CH(CH₃)-O-2' (referred to as "constrained ethyl" or "cEt"), 4'-CH₂-O-CH₂-2', 4'-CH₂-N(R)-2', 4'-CH(CH₂OCH₃)-O-2' ("constrained MOE" or "cMOE") and analogs thereof (*see, e.g.*, Seth et al., U.S. 7,399,845, Bhat et al., U.S. 7,569,686,

Swayze et al., U.S. 7,741,457, and Swayze et al., U.S. 8,022,193), 4'-C(CH₃)(CH₃)-O-2' and analogs thereof (*see, e.g.*, Seth et al., U.S. 8,278,283), 4'-CH₂-N(OCH₃)-2' and analogs thereof (*see, e.g.*, Prakash et al., U.S. 8,278,425), 4'-CH₂-O-N(CH₃)-2' (*see, e.g.*, Allerson et al., U.S. 7,696,345 and Allerson et al., U.S. 8,124,745), 4'-CH₂-C(H)(CH₃)-2' (*see, e.g.*, Zhou, et al., *J. Org. Chem.*, 2009, 74, 118-134), 4'-CH₂-C(=CH₂)-2' and analogs thereof (*see e.g.*, Seth et al., U.S. 8,278,426), 4'-C(R_aR_b)-N(R)-O-2', 4'-C(R_aR_b)-O-N(R)-2', 4'-CH₂-O-N(R)-2', and 4'-CH₂-N(R)-O-2', wherein each R, R_a, and R_b is, independently, H, a protecting group, or C₁-C₁₂ alkyl (*see, e.g.* Imanishi et al., U.S. 7,427,672).

In certain embodiments, such 4' to 2' bridges independently comprise from 1 to 4 linked groups independently selected from: -[C(R_a)(R_b)]_n-, -[C(R_a)(R_b)]_n-O-, -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)-

10 wherein:

x is 0, 1, or 2;

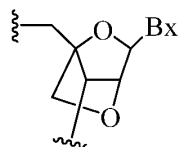
n is 1, 2, 3, or 4;

each R_a and R_b is, independently, H, a protecting group, hydroxyl, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, 15 heterocycle radical, substituted heterocycle radical, heteroaryl, substituted heteroaryl, C₅-C₇ alicyclic radical, substituted C₅-C₇ alicyclic radical, halogen, OJ₁, NJ₁J₂, SJ₁, N₃, COOJ₁, acyl (C(=O)-H), substituted acyl, CN, sulfonyl (S(=O)₂-J₁), or sulfoxyl (S(=O)-J₁); and

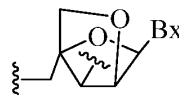
each J₁ and J₂ is, independently, H, C₁-C₁₂ alkyl, substituted C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, substituted C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, substituted C₂-C₁₂ alkynyl, C₅-C₂₀ aryl, substituted C₅-C₂₀ aryl, acyl (C(=O)-H), substituted 20 acyl, a heterocycle radical, a substituted heterocycle radical, C₁-C₁₂ aminoalkyl, substituted C₁-C₁₂ aminoalkyl, or a protecting group.

Additional bicyclic sugar moieties are known in the art, *see, for example*: Freier et al., *Nucleic Acids Research*, 1997, 25(22), 4429-4443; Alback et al., *J. Org. Chem.*, 2006, 71, 7731-7740; 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; Srivastava et al., *J. Am. Chem. Soc.*, 2007, 129, 8362-8379; Wengel et al., U.S. 7,053,207; Imanishi et al., U.S. 6,268,490; Imanishi et al. U.S. 6,770,748; Imanishi et al., U.S. RE44,779; Wengel et al., U.S. 6,794,499; Wengel et al., U.S. 6,670,461; Wengel et al., U.S. 7,034,133; Wengel et al., U.S. 8,080,644; Wengel et al., U.S. 8,034,909; Wengel et al., U.S. 8,153,365; Wengel et al., U.S. 7,572,582; and Ramasamy et al., U.S. 6,525,191; Torsten et al., WO 2004/106356; Wengel et al., WO 1999/014226; Seth et al., WO 2007/134181; Seth et al., U.S. 7,547,684; Seth et al., U.S. 7,666,854; Seth et al., U.S. 8,088,746; Seth et al., U.S. 7,750,131; Seth et al., U.S. 8,030,467; Seth et al., U.S. 8,268,980; Seth et al., U.S. 8,546,556; Seth et al., U.S. 8,530,640; Migawa et al., U.S. 9,012,421; Seth et al., U.S. 8,501,805; and U.S. Patent Publication Nos. Allerson et al., US2008/0039618 and Migawa et al., US2015/0191727.

In certain embodiments, bicyclic sugar moieties and nucleosides incorporating such bicyclic sugar moieties are 35 further defined by isomeric configuration. For example, an LNA nucleoside (described herein) may be in the α-L configuration or in the β-D configuration.



LNA (β -D-configuration)
bridge = 4'-CH₂-O-2'



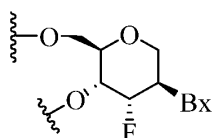
α -L-LNA (α -L-configuration)
bridge = 4'-CH₂-O-2'

α -L-methyleneoxy (4'-CH₂-O-2') or α -L-LNA bicyclic nucleosides have been incorporated into oligonucleotides that showed antisense activity (Frieden et al., *Nucleic Acids Research*, 2003, 21, 6365-6372). Herein, general descriptions of bicyclic nucleosides include both isomeric configurations. When the positions of specific bicyclic nucleosides (*e.g.*, LNA or cEt) are identified in exemplified embodiments herein, they are in the β -D configuration, unless otherwise specified.

In certain embodiments, modified sugar moieties comprise one or more non-bridging sugar substituent and one or more bridging sugar substituent (*e.g.*, 5'-substituted and 4'-2' bridged sugars).

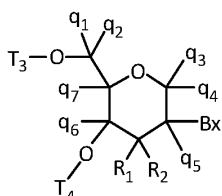
In certain embodiments, modified sugar moieties are sugar surrogates. In certain such embodiments, the oxygen atom of the sugar moiety is replaced, *e.g.*, with a sulfur, carbon or nitrogen atom. In certain such embodiments, such modified sugar moieties also comprise bridging and/or non-bridging substituents as described herein. For example, certain sugar surrogates comprise a 4'-sulfur atom and a substitution at the 2'-position (*see, e.g.*, Bhat et al., U.S. 7,875,733 and Bhat et al., U.S. 7,939,677) and/or the 5' position.

In certain embodiments, sugar surrogates comprise rings having other than 5 atoms. For example, in certain embodiments, a sugar surrogate comprises a six-membered tetrahydropyran ("THP"). Such tetrahydropyrans may be further modified or substituted. Nucleosides comprising such modified tetrahydropyrans include but are not limited to hexitol nucleic acid ("HNA"), anitol nucleic acid ("ANA"), manitol nucleic acid ("MNA") (*see, e.g.*, Leumann, CJ. *Bioorg. & Med. Chem.* 2002, 10, 841-854), fluoro HNA:



F-HNA

("F-HNA", *see e.g.* Swayze et al., U.S. 8,088,904; Swayze et al., U.S. 8,440,803; Swayze et al., U.S. 8,796,437; and Swayze et al., U.S. 9,005,906; F-HNA can also be referred to as a F-THP or 3'-fluoro tetrahydropyran), and nucleosides comprising additional modified THP compounds having the formula:



wherein, independently, for each of said modified THP nucleoside:

Bx is a nucleobase moiety;

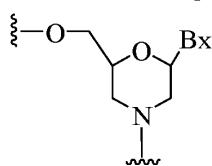
T₃ and T₄ are each, independently, an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide or one of T₃ and T₄ is an internucleoside linking group linking the modified THP nucleoside to the remainder of an oligonucleotide and the other of T₃ and T₄ is H, a hydroxyl protecting group, a linked conjugate group, or a 5' or 3'-terminal group;

- 5 q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each, independently, H, C₁-C₆ alkyl, substituted C₁-C₆ alkyl, C₂-C₆ alkenyl, substituted C₂-C₆ alkenyl, C₂-C₆ alkynyl, or substituted C₂-C₆ alkynyl; and

each of R₁ and R₂ is independently selected from among: hydrogen, halogen, substituted or unsubstituted alkoxy, NJ₁J₂, SJ₁, N₃, OC(=X)J₁, OC(=X)NJ₁J₂, NJ₃C(=X)NJ₁J₂, and CN, wherein X is O, S or NJ₁, and each J₁, J₂, and J₃ is, independently, H or C₁-C₆ alkyl.

- 10 In certain embodiments, modified THP nucleosides are provided wherein q₁, q₂, q₃, q₄, q₅, q₆ and q₇ are each H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is other than H. In certain embodiments, at least one of q₁, q₂, q₃, q₄, q₅, q₆ and q₇ is methyl. In certain embodiments, modified THP nucleosides are provided wherein one of R₁ and R₂ is F. In certain embodiments, R₁ is F and R₂ is H, in certain embodiments, R₁ is methoxy and R₂ is H, and in certain embodiments, R₁ is methoxyethoxy and R₂ is H.

- 15 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 oligonucleotides have been reported (*see, e.g.*, Braasch et al., *Biochemistry*, 2002, 41, 4503-4510 and Summerton et al., U.S. 5,698,685; Summerton et al., U.S. 5,166,315; Summerton et al., U.S. 5,185,444; and Summerton et al., U.S. 5,034,506). As used here, the term "morpholino" means a sugar surrogate having the following structure:



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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 "modified morpholinos."

- In certain embodiments, sugar surrogates comprise acyclic moieties. Examples of nucleosides and oligonucleotides comprising such acyclic sugar surrogates include but are not limited to: peptide nucleic acid ("PNA"), acyclic butyl nucleic acid (*see, e.g.*, Kumar et al., *Org. Biomol. Chem.*, 2013, 11, 5853-5865), and nucleosides and oligonucleotides described in Manoharan et al., WO2011/133876.

Many other bicyclic and tricyclic sugar and sugar surrogate ring systems are known in the art that can be used in modified nucleosides.

Certain Modified Nucleobases

- 30 In certain embodiments, modified oligonucleotides comprise one or more nucleosides comprising an unmodified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleosides comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more nucleosides that does not comprise a nucleobase, referred to as an abasic nucleoside.

- In certain embodiments, modified nucleobases are selected from: 5-substituted pyrimidines, 6-azapyrimidines, alkyl or alkynyl substituted pyrimidines, alkyl substituted purines, and N-2, N-6 and O-6 substituted purines. In certain

embodiments, modified nucleobases are selected from: 2-aminopropyladenine, 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-N-methylguanine, 6-N-methyladenine, 2-propyl adenine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-propynyl ($-C\equiv C-CH_3$) uracil, 5-propynylcytosine, 6-azouracil, 6-azocytosine, 6-azothymine, 5-ribosyluracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl, 8-aza and other 8-substituted purines, 5-halo, particularly 5-bromo, 5-trifluoromethyl, 5-halouracil, and 5-halocytosine, 7-methylguanine, 7-methyladenine, 2-F-adenine, 2-aminoadenine, 7-deazaguanine, 7-deazaadenine, 3-deazaguanine, 3-deazaadenine, 6-N-benzoyladenine, 2-N-isobutylguanine, 4-N-benzoylcytosine, 4-N-benzoyluracil, 5-methyl 4-N-benzoylcytosine, 5-methyl 4-N-benzoyluracil, universal bases, hydrophobic bases, promiscuous bases, size-expanded bases, and fluorinated bases. Further modified nucleobases include tricyclic pyrimidines, such as 1,3-diazaphenoxazine-2-one, 1,3-diazaphenothiazine-2-one and 9-(2-aminoethoxy)-1,3-diazaphenoxazine-2-one (G-clamp). Modified nucleobases may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deazaadenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in Merigan et al., U.S. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, Kroschwitz, J.I., Ed., John Wiley & Sons, 1990, 858-859; Englisch et al., *Angewandte Chemie*, International Edition, 1991, 30, 613; Sanghvi, Y.S., Chapter 15, *Antisense Research and Applications*, Crooke, S.T. and Lebleu, B., Eds., CRC Press, 1993, 273-288; and those disclosed in Chapters 6 and 15, *Antisense Drug Technology*, Crooke S.T., Ed., CRC Press, 2008, 163-166 and 442-443.

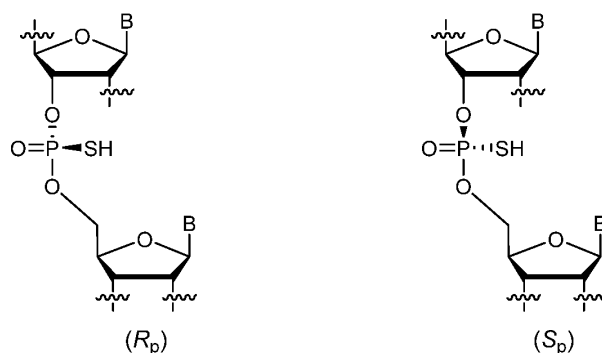
Publications that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include without limitation, Manoharan et al., US2003/0158403; Manoharan et al., US2003/0175906; Dinh et al., U.S. 4,845,205; Spielvogel et al., U.S. 5,130,302; Rogers et al., U.S. 5,134,066; Bischofberger et al., U.S. 5,175,273; Urdea et al., U.S. 5,367,066; Benner et al., U.S. 5,432,272; Matteucci et al., U.S. 5,434,257; Gmeiner et al., U.S. 5,457,187; Cook et al., U.S. 5,459,255; Froehler et al., U.S. 5,484,908; Matteucci et al., U.S. 5,502,177; Hawkins et al., U.S. 5,525,711; Haralambidis et al., U.S. 5,552,540; Cook et al., U.S. 5,587,469; Froehler et al., U.S. 5,594,121; Switzer et al., U.S. 5,596,091; Cook et al., U.S. 5,614,617; Froehler et al., U.S. 5,645,985; Cook et al., U.S. 5,681,941; Cook et al., U.S. 5,811,534; Cook et al., U.S. 5,750,692; Cook et al., U.S. 5,948,903; Cook et al., U.S. 5,587,470; Cook et al., U.S. 5,457,191; Matteucci et al., U.S. 5,763,588; Froehler et al., U.S. 5,830,653; Cook et al., U.S. 5,808,027; Cook et al., 6,166,199; and Matteucci et al., U.S. 6,005,096.

Certain Modified Internucleoside Linkages

In certain embodiments, nucleosides of modified oligonucleotides may be linked together using any internucleoside linkage. The two main classes of internucleoside linking groups are defined by the presence or absence of a phosphorus atom. Representative phosphorus-containing internucleoside linkages include but are not limited to phosphodiester, which contain a phosphodiester bond, $P(O_2)=O$, (also referred to as unmodified or naturally occurring linkages); phosphotriesters; methylphosphonates; methoxypropylphosphonates ("MOP"); phosphoramidates; mesyl phosphoramidates; phosphorothioates ($P(O_2)=S$); and phosphorodithioates ($HS-P=S$). Representative non-phosphorus containing internucleoside linking groups include but are not limited to methylenemethylimino ($-CH_2-N(CH_3)-O-CH_2-$); thiodiester, thionocarbamate ($-O-C(=O)(NH)-S-$); siloxane ($-O-SiH_2-O-$); and N,N'-dimethylhydrazine ($-CH_2-N(CH_3)-N(CH_3)-$). Modified internucleoside linkages, compared to naturally occurring phosphate linkages, can be used to alter, typically increase, nuclease resistance of the oligonucleotide. In certain embodiments, internucleoside linkages having a

chiral atom can be prepared as a racemic mixture, or as separate enantiomers. Methods of preparation of phosphorous-containing and non-phosphorous-containing internucleoside linkages are well known to those skilled in the art.

Representative internucleoside linkages having a chiral center include but are not limited to alkylphosphonates and phosphorothioates. Modified oligonucleotides comprising internucleoside linkages having a
 5 chiral center can be prepared as populations of modified oligonucleotides comprising stereorandom internucleoside linkages, or as populations of modified oligonucleotides comprising phosphorothioate internucleoside linkages in particular stereochemical configurations. In certain embodiments, populations of modified oligonucleotides comprise phosphorothioate internucleoside linkages wherein all of the phosphorothioate internucleoside linkages are stereorandom. Such modified oligonucleotides can be generated using synthetic methods that result in random selection
 10 of the stereochemical configuration of each phosphorothioate internucleoside linkage. Nonetheless, as is well understood by those of skill in the art, each individual phosphorothioate of each individual oligonucleotide molecule has a defined stereoconfiguration. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising one or more particular phosphorothioate internucleoside linkages in a particular, independently selected stereochemical configuration. In certain embodiments, the particular configuration of the
 15 particular phosphorothioate internucleoside linkage is present in at least 65% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate internucleoside linkage is present in at least 70% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate internucleoside linkage is present in at least 80% of the molecules in the population. In certain
 20 embodiments, the particular configuration of the particular phosphorothioate internucleoside linkage is present in at least 90% of the molecules in the population. In certain embodiments, the particular configuration of the particular phosphorothioate internucleoside linkage is present in at least 99% of the molecules in the population. Such chirally enriched populations of modified oligonucleotides can be generated using synthetic methods known in the art, *e.g.*, methods described in Oka et al., *JACS*, 2003, 125, 8307, Wan et al. *Nuc. Acid. Res.*, 2014, 42, 13456, and WO 2017/015555. In certain embodiments, a population of modified oligonucleotides is enriched for modified
 25 oligonucleotides having at least one indicated phosphorothioate in the (*Sp*) configuration. In certain embodiments, a population of modified oligonucleotides is enriched for modified oligonucleotides having at least one phosphorothioate in the (*Rp*) configuration. In certain embodiments, modified oligonucleotides comprising (*Rp*) and/or (*Sp*) phosphorothioates comprise one or more of the following formulas, respectively, wherein "B" indicates a nucleobase:



30 Unless otherwise indicated, chiral internucleoside linkages of modified oligonucleotides described herein can be stereorandom or in a particular stereochemical configuration.

In certain embodiments, modified oligonucleotides comprise an internucleoside motif of (5' to 3')
 sooooooooooooo. In certain embodiments, the particular stereochemical configuration of the modified
 oligonucleotides is (5' to 3') Sp-o-o-o-Sp-Sp-Sp-Rp-Sp-Sp-Rp-Sp-Sp-Sp-Sp-Sp-Sp-Sp or Sp-o-o-o-Sp-Sp-Sp-Rp-Sp-
 Sp-Sp-Sp-Sp-Sp-Sp-Sp-Sp-Sp-Sp; wherein each 'Sp' represents a phosphorothioate internucleoside linkage in the *S*
 5 configuration; Rp represents a phosphorothioate internucleoside linkage in the *R* configuration; and 'o' represents a
 phosphodiester internucleoside linkage.

Neutral internucleoside linkages include, without limitation, phosphotriesters, methylphosphonates, MMI (3'-
 CH₂-N(CH₃)-O-5'), amide-3 (3'-CH₂-C(=O)-N(H)-5'), amide-4 (3'-CH₂-N(H)-C(=O)-5'), formacetal (3'-O-CH₂-O-5'),
 methoxypropyl, and thioformacetal (3'-S-CH₂-O-5'). Further neutral internucleoside linkages include nonionic linkages
 10 comprising siloxane (dialkylsiloxane), carboxylate ester, carboxamide, sulfide, sulfonate ester and amides (*see e.g.*,
Carbohydrate Modifications in Antisense Research; Y.S. Sanghvi and P.D. Cook, Eds., ACS Symposium Series 580;
 Chapters 3 and 4, 40-65). Further neutral internucleoside linkages include nonionic linkages comprising mixed N, O, S
 and CH₂ component parts.

In certain embodiments, a modified internucleoside linkage is any of those described in WO 2021/030778,
 15 incorporated by reference herein.

Certain Motifs

In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides comprising a
 modified sugar moiety. In certain embodiments, modified oligonucleotides comprise one or more modified nucleosides
 20 comprising a modified nucleobase. In certain embodiments, modified oligonucleotides comprise one or more modified
 internucleoside linkages. In such embodiments, the modified, unmodified, and differently modified sugar moieties,
 nucleobases, and/or internucleoside linkages of a modified oligonucleotide define a pattern or motif. In certain
 embodiments, the patterns of sugar moieties, nucleobases, and internucleoside linkages are each independent of one
 another. Thus, a modified oligonucleotide may be described by its sugar motif, nucleobase motif and/or internucleoside
 25 linkage motif (as used herein, nucleobase motif describes the modifications to the nucleobases independent of the
 sequence of nucleobases).

Certain Sugar Motifs

In certain embodiments, oligonucleotides comprise one or more type of modified sugar and/or unmodified
 sugar moiety arranged along the oligonucleotide, or portion thereof, in a defined pattern or sugar motif. In certain
 30 instances, such sugar motifs include but are not limited to any of the sugar modifications discussed herein.

In certain embodiments, modified oligonucleotides have a gapmer motif, which is defined by two external
 regions or "wings" and a central or internal region or "gap." The three regions of a gapmer motif (the 5'-wing, the gap,
 and the 3'-wing) form a contiguous sequence of nucleosides wherein at least some of the sugar moieties of the
 nucleosides of each of the wings differ from at least some of the sugar moieties of the nucleosides of the gap.
 35 Specifically, at least the sugar moieties of the nucleosides of each wing that are closest to the gap (the 3'-most
 nucleoside of the 5'-wing and the 5'-most nucleoside of the 3'-wing) differ from the sugar moiety of the neighboring
 gap nucleosides, thus defining the boundary between the wings and the gap (i.e., the wing/gap junction). In certain
 embodiments, the sugar moieties within the gap are the same as one another. In certain embodiments, the gap includes
 one or more nucleoside having a sugar moiety that differs from the sugar moiety of one or more other nucleosides of the

gap. In certain embodiments, the sugar motifs of the two wings are the same as one another (symmetric gapmer). In certain embodiments, the sugar motif of the 5'-wing differs from the sugar motif of the 3'-wing (asymmetric gapmer).

In certain embodiments, the wings of a gapmer comprise 1-6 nucleosides. In certain embodiments, each nucleoside of each wing of a gapmer comprises a modified sugar moiety. In certain embodiments, at least one, at least two, at least three, at least four, at least five, or at least six nucleosides of each wing of a gapmer comprises a modified sugar moiety.

In certain embodiments, the gap of a gapmer comprises 7-12 nucleosides. In certain embodiments, each nucleoside of the gap of a gapmer comprises a 2'-deoxyribosyl sugar moiety. In certain embodiments, at least one nucleoside of the gap of a gapmer comprises a modified sugar moiety and each remaining nucleoside comprises a 2'-deoxyribosyl sugar moiety.

Herein, the lengths (number of nucleosides) of the three regions of a gapmer may be provided using the notation [# of nucleosides in the 5'-wing] – [# of nucleosides in the gap] – [# of nucleosides in the 3'-wing]. Thus, a 5-10-5 gapmer consists of 5 linked nucleosides in each wing and 10 linked nucleosides in the gap. Where such nomenclature is followed by a specific modification, that modification is the modification in each sugar moiety of each wing and the gap nucleosides comprise a 2'-deoxyribosyl sugar moiety. Thus, a 5-10-5 MOE gapmer consists of 5 linked 2'-MOE nucleosides in the 5'-wing, 10 linked 2'-deoxyribonucleosides in the gap, and 5 linked 2'-MOE nucleosides in the 3'-wing.

In certain embodiments, each nucleoside of a modified oligonucleotide, or portion thereof, comprises a 2'-substituted sugar moiety, a bicyclic sugar moiety, a sugar surrogate, or a 2'-deoxyribosyl sugar moiety. In certain embodiments, the 2'-substituted sugar moiety is selected from a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, and a 2'-F sugar moiety. In certain embodiments, the bicyclic sugar moiety is selected from a cEt sugar moiety and an LNA sugar moiety. In certain embodiments, the sugar surrogate is selected from morpholino, modified morpholino, PNA, THP, and F-HNA.

In certain embodiments, modified oligonucleotides comprise 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 nucleosides comprising a modified sugar moiety. In certain embodiments, the modified sugar moiety is selected independently from a 2'-substituted sugar moiety, a bicyclic sugar moiety, or a sugar surrogate. In certain embodiments, the 2'-substituted sugar moiety is selected from a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, and a 2'-F sugar moiety. In certain embodiments, the bicyclic sugar moiety is selected from a cEt sugar moiety and an LNA sugar moiety. In certain embodiments, the sugar surrogate is selected from morpholino, modified morpholino, THP, and F-HNA.

In certain embodiments, each nucleoside of a modified oligonucleotide comprises a modified sugar moiety ("fully modified oligonucleotide"). In certain embodiments, each nucleoside of a fully modified oligonucleotide comprises a 2'-substituted sugar moiety, a bicyclic sugar moiety, or a sugar surrogate. In certain embodiments, the 2'-substituted sugar moiety is selected from a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, and a 2'-F sugar moiety. In certain embodiments, the bicyclic sugar moiety is selected from a cEt sugar moiety and an LNA sugar moiety. In certain embodiments, the sugar surrogate is selected from morpholino, modified morpholino, THP, and F-HNA. In certain embodiments, each nucleoside of a fully modified oligonucleotide comprises the same modified sugar moiety ("uniformly modified sugar motif"). In certain embodiments, the uniformly modified sugar motif is 7 to 20 nucleosides in length. In certain embodiments, each nucleoside of the uniformly modified sugar motif comprises a

2'-substituted sugar moiety, a bicyclic sugar moiety, or a sugar surrogate. In certain embodiments, the 2'-substituted sugar moiety is selected from a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, and a 2'-F sugar moiety. In certain embodiments, the bicyclic sugar moiety is selected from a cEt sugar moiety and an LNA sugar moiety. In certain embodiments, the sugar surrogate is selected from morpholino, modified morpholino, THP, and F-HNA. In certain embodiments, modified oligonucleotides having at least one fully modified sugar motif may also comprise at least 1, at least 2, at least 3, or at least 4 2'-deoxyribonucleosides.

Certain Nucleobase Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified nucleobases arranged along the oligonucleotide, or portion thereof, in a defined pattern or motif. In certain embodiments, each nucleobase is modified.

In certain embodiments, none of the nucleobases are modified. In certain embodiments, each purine or each pyrimidine is modified. In certain embodiments, each adenine is modified. In certain embodiments, each guanine is modified. In certain embodiments, each thymine is modified. In certain embodiments, each uracil is modified. In certain embodiments, each cytosine is modified. In certain embodiments, some or all of the cytosine nucleobases in a modified oligonucleotide are 5-methyl cytosines. In certain embodiments, all of the cytosine nucleobases are 5-methyl cytosines and all of the other nucleobases of the modified oligonucleotide are unmodified nucleobases.

In certain embodiments, modified oligonucleotides comprise a block of modified nucleobases. In certain such embodiments, the block is at the 3'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 3'-end of the oligonucleotide. In certain embodiments, the block is at the 5'-end of the oligonucleotide. In certain embodiments the block is within 3 nucleosides of the 5'-end of the oligonucleotide.

In certain embodiments, oligonucleotides having a gapmer motif comprise a nucleoside comprising a modified nucleobase. In certain such embodiments, one nucleoside comprising a modified nucleobase is in the central gap of an oligonucleotide having a gapmer motif. In certain such embodiments, the sugar moiety of the nucleoside is a 2'-deoxyribose sugar moiety. In certain embodiments, the modified nucleobase is selected from: a 2-thiopyrimidine and a 5-propynepyrimidine.

Certain Internucleoside Linkage Motifs

In certain embodiments, oligonucleotides comprise modified and/or unmodified internucleoside linkages arranged along the oligonucleotide, or portion thereof, in a defined pattern or motif. In certain embodiments, each internucleoside linking group is a phosphodiester internucleoside linkage. In certain embodiments, each internucleoside linking group of a modified oligonucleotide is a phosphorothioate internucleoside linkage. In certain embodiments, each internucleoside linkage of a modified oligonucleotide is independently selected from a phosphorothioate internucleoside linkage and phosphodiester internucleoside linkage. In certain embodiments, each phosphorothioate internucleoside linkage is independently selected from a stereorandom phosphorothioate, a (*S*p) phosphorothioate, and a (*R*p) phosphorothioate. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer and the internucleoside linkages within the gap are all modified. In certain such embodiments, some or all of the internucleoside linkages in the wings are unmodified phosphodiester internucleoside linkages. In certain embodiments, the terminal internucleoside linkages are modified. In certain embodiments, the sugar motif of a modified oligonucleotide is a gapmer, and the internucleoside linkage motif comprises at least one phosphodiester internucleoside linkage in at least one wing, wherein the at least one phosphodiester internucleoside linkage is not a terminal internucleoside linkage, and the remaining internucleoside linkages are phosphorothioate internucleoside linkages. In certain such embodiments, all

of the phosphorothioate internucleoside linkages are stereorandom. In certain embodiments, all of the phosphorothioate internucleoside linkages in the wings are (Sp) phosphorothioates, and the gap comprises at least one Sp, Sp, Rp motif. In certain embodiments, populations of modified oligonucleotides are enriched for modified oligonucleotides comprising such internucleoside linkage motifs. In certain embodiments, one or more internucleoside linkage is a mesyl phosphoramidate internucleoside linkage. In certain embodiments, each internucleoside linkage is independently selected from a phosphodiester internucleoside linkage, a phosphorothioate internucleoside linkage, and a mesyl phosphoramidate internucleoside linkage. In certain embodiments, each internucleoside linkage is independently selected from a phosphorothioate internucleoside linkage and a mesyl phosphoramidate internucleoside linkage. In certain embodiments, one or more internucleoside linkage is a methoxypropylphosphonate internucleoside linkage. In certain embodiments, each internucleoside linkage is independently selected from a phosphodiester internucleoside linkage, a phosphorothioate internucleoside linkage, and a methoxypropylphosphonate internucleoside linkage. In certain embodiments, each internucleoside linkage is independently selected from a phosphorothioate internucleoside linkage and a methoxypropylphosphonate internucleoside linkage.

In certain embodiments, modified oligonucleotides comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, 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, or at least 19 phosphodiester internucleoside linkages. In certain embodiments, modified oligonucleotides comprise at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, 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, or at least 19 phosphorothioate internucleoside linkages. In certain embodiments, modified oligonucleotides comprise at least 1, at least 2, at least 3, at least 4, or at least 5 phosphodiester internucleoside linkages and the remainder of the internucleoside linkages are phosphorothioate internucleoside linkages.

Certain Lengths

It is possible to increase or decrease the length of an oligonucleotide without eliminating activity. For example, in Woolf et al., *Proc. Natl. Acad. Sci. USA*, 1992, 89, 7305-7309, 1992), a series of oligonucleotides 13-25 nucleobases in length were tested for their ability to induce cleavage of a target nucleic acid in an oocyte injection model. Oligonucleotides 25 nucleobases in length with 8 or 11 mismatch bases near the ends of the oligonucleotides were able to direct specific cleavage of the target nucleic acid, albeit to a lesser extent than the oligonucleotides that contained no mismatches. Similarly, target specific cleavage was achieved using 13 nucleobase oligonucleotides, including those with 1 or 3 mismatches.

In certain embodiments, oligonucleotides (including modified oligonucleotides) can have any of a variety of ranges of lengths. In certain embodiments, oligonucleotides consist of X to Y linked nucleosides, where X represents the fewest number of nucleosides in the range and Y represents the largest number nucleosides in the range. In certain such embodiments, X and Y are each independently selected from 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, and 50; provided that $X \leq Y$. For example, in certain embodiments, oligonucleotides consist of 12 to 13, 12 to 14, 12 to 15, 12 to 16, 12 to 17, 12 to 18, 12 to 19, 12 to 20, 12 to 21, 12 to 22, 12 to 23, 12 to 24, 12 to 25, 12 to 26, 12 to 27, 12 to 28, 12 to 29, 12 to 30, 13 to 14, 13 to 15, 13 to 16, 13 to 17, 13 to 18, 13 to 19, 13 to 20, 13 to 21, 13 to 22, 13 to 23, 13 to 24, 13 to 25, 13 to 26, 13 to 27, 13 to 28, 13 to 29, 13 to 30, 14 to 15, 14 to 16, 14 to 17, 14 to 18, 14 to 19, 14 to 20, 14 to 21, 14 to 22, 14 to 23, 14 to 24, 14 to 25, 14 to 26, 14 to 27, 14 to 28, 14 to 29, 14 to 30, 15 to 16, 15 to 17, 15 to 18, 15 to 19, 15

to 20, 15 to 21, 15 to 22, 15 to 23, 15 to 24, 15 to 25, 15 to 26, 15 to 27, 15 to 28, 15 to 29, 15 to 30, 16 to 17, 16 to 18, 16 to 19, 16 to 20, 16 to 21, 16 to 22, 16 to 23, 16 to 24, 16 to 25, 16 to 26, 16 to 27, 16 to 28, 16 to 29, 16 to 30, 17 to 18, 17 to 19, 17 to 20, 17 to 21, 17 to 22, 17 to 23, 17 to 24, 17 to 25, 17 to 26, 17 to 27, 17 to 28, 17 to 29, 17 to 30, 18 to 19, 18 to 20, 18 to 21, 18 to 22, 18 to 23, 18 to 24, 18 to 25, 18 to 26, 18 to 27, 18 to 28, 18 to 29, 18 to 30, 19 to 20, 19 to 21, 19 to 22, 19 to 23, 19 to 24, 19 to 25, 19 to 26, 19 to 27, 19 to 28, 19 to 29, 19 to 30, 20 to 21, 20 to 22, 20 to 23, 20 to 24, 20 to 25, 20 to 26, 20 to 27, 20 to 28, 20 to 29, 20 to 30, 21 to 22, 21 to 23, 21 to 24, 21 to 25, 21 to 26, 21 to 27, 21 to 28, 21 to 29, 21 to 30, 22 to 23, 22 to 24, 22 to 25, 22 to 26, 22 to 27, 22 to 28, 22 to 29, 22 to 30, 23 to 24, 23 to 25, 23 to 26, 23 to 27, 23 to 28, 23 to 29, 23 to 30, 24 to 25, 24 to 26, 24 to 27, 24 to 28, 24 to 29, 24 to 30, 25 to 26, 25 to 27, 25 to 28, 25 to 29, 25 to 30, 26 to 27, 26 to 28, 26 to 29, 26 to 30, 27 to 28, 27 to 29, 27 to 30, 28 to 29, 28 to 30, or 29 to 30 linked nucleosides.

In certain embodiments, oligonucleotides consist of 16 linked nucleosides. In certain embodiments, oligonucleotides consist of 17 linked nucleosides. In certain embodiments, oligonucleotides consist of 18 linked nucleosides. In certain embodiments, oligonucleotides consist of 19 linked nucleosides. In certain embodiments, oligonucleotides consist of 20 linked nucleosides.

15 **Certain Modified Oligonucleotides**

In certain embodiments, the above modifications (sugar, nucleobase, internucleoside linkage) are incorporated into a modified oligonucleotide. In certain embodiments, modified oligonucleotides are characterized by their modification motifs and overall lengths. In certain embodiments, such parameters are each independent of one another. Thus, unless otherwise indicated, each internucleoside linkage of an oligonucleotide having a gapmer sugar motif may be modified or unmodified and may or may not follow the gapmer modification pattern of the sugar modifications. For example, the internucleoside linkages within the wing regions of a sugar gapmer may be the same or different from one another and may be the same or different from the internucleoside linkages of the gap region of the sugar motif. Likewise, such sugar gapmer oligonucleotides may comprise one or more modified nucleobase independent of the gapmer pattern of the sugar modifications. Unless otherwise indicated, all modifications are independent of nucleobase sequence.

20 **Certain Populations of Modified Oligonucleotides**

Populations of modified oligonucleotides in which all of the modified oligonucleotides of the population have the same molecular formula can be stereorandom populations or chirally enriched populations. All of the chiral centers of all of the modified oligonucleotides are stereorandom in a stereorandom population. In a chirally enriched population, at least one particular chiral center is not stereorandom in the modified oligonucleotides of the population. In certain embodiments, the modified oligonucleotides of a chirally enriched population are enriched for β -D ribosyl sugar moieties, and all of the phosphorothioate internucleoside linkages are stereorandom. In certain embodiments, the modified oligonucleotides of a chirally enriched population are enriched for both β -D ribosyl sugar moieties and at least one, particular phosphorothioate internucleoside linkage in a particular stereochemical configuration.

30 **Nucleobase Sequence**

In certain embodiments, oligonucleotides (unmodified or modified oligonucleotides) are further described by their nucleobase sequence. In certain embodiments oligonucleotides have a nucleobase sequence that is complementary to a second oligonucleotide or an identified reference nucleic acid, such as a target nucleic acid. In certain such embodiments, a portion of an oligonucleotide has a nucleobase sequence that is complementary to a second

oligonucleotide or an identified reference nucleic acid, such as a target nucleic acid. In certain embodiments, the nucleobase sequence of a portion or entire length of an oligonucleotide is at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% complementary to the second oligonucleotide or nucleic acid, such as a target nucleic acid.

5

Certain Oligomeric Compounds

In certain embodiments, provided herein are oligomeric compounds, which consist of an oligonucleotide (modified or unmodified) and optionally one or more conjugate groups and/or terminal groups. Conjugate groups consist of one or more conjugate moiety and a conjugate linker which links the conjugate moiety to the oligonucleotide.

10 Conjugate groups may be attached to either or both ends of an oligonucleotide and/or at any internal position. In certain embodiments, conjugate groups are attached to the 2'-position of a nucleoside of a modified oligonucleotide. In certain embodiments, conjugate groups that are attached to either or both ends of an oligonucleotide are terminal groups. In certain such embodiments, conjugate groups or terminal groups are attached at the 3' and/or 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 3'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached at the 5'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached near the 3'-end of oligonucleotides. In certain such embodiments, conjugate groups (or terminal groups) are attached near the 5'-end of oligonucleotides.

Examples of terminal groups include but are not limited to conjugate groups, capping groups, phosphate moieties, protecting groups, abasic nucleosides, modified or unmodified nucleosides, and two or more nucleosides that are independently modified or unmodified.

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Certain Conjugate Groups

In certain embodiments, oligonucleotides are covalently attached to one or more conjugate groups. In certain embodiments, conjugate groups modify one or more properties of the attached oligonucleotide, including but not limited to pharmacodynamics, pharmacokinetics, stability, binding, absorption, tissue distribution, cellular distribution, cellular uptake, charge, and clearance. In certain embodiments, conjugate groups impart a new property on the attached oligonucleotide, *e.g.*, fluorophores or reporter groups that enable detection of the oligonucleotide. Certain conjugate groups and conjugate moieties have been described previously, for example: cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053-1060), a thioether, *e.g.*, hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, *e.g.*, do-decan-diol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, *e.g.*, di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid a palmitoyl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923-937), a tocopherol group (Nishina et al., *Molecular Therapy Nucleic Acids*, 2015, 4, e220; and Nishina et al., *Molecular Therapy*, 2008, 16, 734-740), or a GalNAc cluster (*e.g.*, WO2014/179620).

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Conjugate Moieties

Conjugate moieties include, without limitation, intercalators, reporter molecules, polyamines, polyamides, peptides, carbohydrates, vitamin moieties, polyethylene glycols, thioethers, polyethers, cholesterol, thiocholesterol, cholic acid moieties, folate, lipids, lipophilic groups, phospholipids, biotin, phenazine, phenanthridine, anthraquinone, adamantane, acridine, fluoresceins, rhodamines, coumarins, fluorophores, and dyes.

In certain embodiments, a conjugate moiety comprises an active drug substance, for example, aspirin, warfarin, phenylbutazone, ibuprofen, suprofen, fen-bufen, ketoprofen, (S)-(+)-pranoprofen, carprofen, dansylsarcosine, 2,3,5-triiodobenzoic acid, fingolimod, flufenamic acid, folic acid, a benzothiadiazide, chlorothiazide, a diazepam, indo-methicin, a barbiturate, a cephalosporin, a sulfa drug, an antidiabetic, an antibacterial, or an antibiotic.

Conjugate Linkers

Conjugate moieties are attached to oligonucleotides through conjugate linkers. In certain oligomeric compounds, the conjugate linker is a single chemical bond (i.e., the conjugate moiety is attached directly to an oligonucleotide through a single bond). In certain oligomeric compounds, a conjugate moiety is attached to an oligonucleotide via a more complex conjugate linker comprising one or more conjugate linker moieties, which are sub-units making up a conjugate linker. In certain embodiments, the conjugate linker comprises a chain structure, such as a hydrocarbyl chain, or an oligomer of repeating units such as ethylene glycol, nucleosides, or amino acid units.

In certain embodiments, a conjugate linker comprises one or more groups selected from alkyl, amino, oxo, amide, disulfide, polyethylene glycol, ether, thioether, and hydroxylamino. In certain such embodiments, the conjugate linker comprises groups selected from alkyl, amino, oxo, amide and ether groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and amide groups. In certain embodiments, the conjugate linker comprises groups selected from alkyl and ether groups. In certain embodiments, the conjugate linker comprises at least one phosphorus moiety. In certain embodiments, the conjugate linker comprises at least one phosphate group. In certain embodiments, the conjugate linker includes at least one neutral linking group.

In certain embodiments, conjugate linkers, including the conjugate linkers described above, are bifunctional linking moieties, *e.g.*, those known in the art to be useful for attaching conjugate groups to parent compounds, such as the oligonucleotides provided herein. In general, a bifunctional linking moiety comprises at least two functional groups. One of the functional groups is selected to bind to a particular site on a parent compound and the other is selected to bind to a conjugate group. Examples of functional groups used in a bifunctional linking moiety include but are not limited to electrophiles for reacting with nucleophilic groups and nucleophiles for reacting with electrophilic groups. In certain embodiments, bifunctional linking moieties comprise one or more groups selected from amino, hydroxyl, carboxylic acid, thiol, alkyl, alkenyl, and alkynyl.

Examples of conjugate linkers include but are not limited to pyrrolidine, 8-amino-3,6-dioxaoctanoic acid (ADO), succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) and 6-aminohexanoic acid (AHX or AHA). Other conjugate linkers include but are not limited to substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl or substituted or unsubstituted C₂-C₁₀ alkynyl, wherein a nonlimiting list of preferred substituent groups includes hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl.

In certain embodiments, conjugate linkers comprise 1-10 linker-nucleosides. In certain embodiments, conjugate linkers comprise 2-5 linker-nucleosides. In certain embodiments, conjugate linkers comprise exactly 3 linker-

nucleosides. In certain embodiments, conjugate linkers comprise the TCA motif. In certain embodiments, such linker-nucleosides are modified nucleosides. In certain embodiments such linker-nucleosides comprise a modified sugar moiety. In certain embodiments, linker-nucleosides are unmodified. In certain embodiments, linker-nucleosides comprise an optionally protected heterocyclic base selected from a purine, substituted purine, pyrimidine or substituted pyrimidine. In certain embodiments, a cleavable moiety is a nucleoside selected from uracil, thymine, cytosine, 4-N-benzoylcytosine, 5-methyl cytosine, 4-N-benzoyl-5-methyl cytosine, adenine, 6-N-benzoyladenine, guanine and 2-N-isobutyrylguanine. It is typically desirable for linker-nucleosides to be cleaved from the oligomeric compound after it reaches a target tissue. Accordingly, linker-nucleosides are typically linked to one another and to the remainder of the oligomeric compound through cleavable bonds. In certain embodiments, such cleavable bonds are phosphodiester bonds.

Herein, linker-nucleosides are not considered to be part of the oligonucleotide. Accordingly, in embodiments in which an oligomeric compound comprises an oligonucleotide consisting of a specified number or range of linked nucleosides and/or a specified percent complementarity to a reference nucleic acid and the oligomeric compound also comprises a conjugate group comprising a conjugate linker comprising linker-nucleosides, those linker-nucleosides are not counted toward the length of the oligonucleotide and are not used in determining the percent complementarity of the oligonucleotide for the reference nucleic acid. For example, an oligomeric compound may comprise (1) a modified oligonucleotide consisting of 8-30 nucleosides and (2) a conjugate group comprising 1-10 linker-nucleosides that are contiguous with the nucleosides of the modified oligonucleotide. The total number of contiguous linked nucleosides in such an oligomeric compound is more than 30. Alternatively, an oligomeric compound may comprise a modified oligonucleotide consisting of 8-30 nucleosides and no conjugate group. The total number of contiguous linked nucleosides in such an oligomeric compound is no more than 30. Unless otherwise indicated conjugate linkers comprise no more than 10 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 5 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 3 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 2 linker-nucleosides. In certain embodiments, conjugate linkers comprise no more than 1 linker-nucleoside.

In certain embodiments, it is desirable for a conjugate group to be cleaved from the oligonucleotide. For example, in certain circumstances oligomeric compounds comprising a particular conjugate moiety are better taken up by a particular cell type, but once the oligomeric compound has been taken up, it is desirable that the conjugate group be cleaved to release the unconjugated or parent oligonucleotide. Thus, certain conjugate linkers may comprise one or more cleavable moieties. In certain embodiments, a cleavable moiety is a cleavable bond. In certain embodiments, a cleavable moiety is a group of atoms comprising at least one cleavable bond. In certain embodiments, a cleavable moiety comprises a group of atoms having one, two, three, four, or more than four cleavable bonds. In certain embodiments, a cleavable moiety is selectively cleaved inside a cell or subcellular compartment, such as a lysosome. In certain embodiments, a cleavable moiety is selectively cleaved by endogenous enzymes, such as nucleases.

In certain embodiments, a cleavable bond is selected from among: an amide, an ester, an ether, one or both esters of a phosphodiester, a phosphate ester, a carbamate, or a disulfide. In certain embodiments, a cleavable bond is one or both of the esters of a phosphodiester. In certain embodiments, a cleavable moiety comprises a phosphate or phosphodiester. In certain embodiments, the cleavable moiety is a phosphate linkage between an oligonucleotide and a conjugate moiety or conjugate group.

In certain embodiments, a cleavable moiety comprises or consists of one or more linker-nucleosides. In certain such embodiments, the one or more linker-nucleosides are linked to one another and/or to the remainder of the oligomeric compound through cleavable bonds. In certain embodiments, such cleavable bonds are unmodified phosphodiester bonds. In certain embodiments, a cleavable moiety is 2'-deoxyribonucleoside that is attached to either the 3' or 5'-terminal nucleoside of an oligonucleotide by a phosphate internucleoside linkage and covalently attached to the remainder of the conjugate linker or conjugate moiety by a phosphate or phosphorothioate internucleoside linkage. In certain such embodiments, the cleavable moiety is 2'-deoxyadenosine.

Certain Terminal Groups

In certain embodiments, oligomeric compounds comprise one or more terminal groups. In certain such embodiments, oligomeric compounds comprise a stabilized 5'-phosphate. Stabilized 5'-phosphates include, but are not limited to 5'-phosphanates, including, but not limited to 5'-vinylphosphonates. In certain embodiments, terminal groups comprise one or more abasic nucleosides and/or inverted nucleosides. In certain embodiments, terminal groups comprise one or more 2'-linked nucleosides. In certain such embodiments, the 2'-linked nucleoside is an abasic nucleoside.

Oligomeric Duplexes

In certain embodiments, oligomeric compounds described herein comprise an oligonucleotide, having a nucleobase sequence complementary to that of a target nucleic acid. In certain embodiments, an oligomeric compound is paired with a second oligomeric compound to form an oligomeric duplex. Such oligomeric duplexes comprise a first oligomeric compound having a portion complementary to a target nucleic acid and a second oligomeric compound having a portion complementary to the first oligomeric compound. In certain embodiments, the first oligomeric compound of an oligomeric duplex comprises or consists of (1) a modified or unmodified oligonucleotide and optionally a conjugate group and (2) a second modified or unmodified oligonucleotide and optionally a conjugate group. Either or both oligomeric compounds of an oligomeric duplex may comprise a conjugate group. The oligonucleotides of each oligomeric compound of an oligomeric duplex may include non-complementary overhanging nucleosides.

Antisense Activity

In certain embodiments, oligomeric compounds and oligomeric duplexes are capable of hybridizing to a target nucleic acid, resulting in at least one antisense activity; such oligomeric compounds and oligomeric duplexes are antisense compounds. In certain embodiments, antisense compounds have antisense activity when they reduce, modulate, or increase the amount or activity of a target nucleic acid by 25% or more in the standard cell assay. In certain embodiments, antisense compounds selectively affect one or more target nucleic acid. Such antisense compounds comprise a nucleobase sequence that hybridizes to one or more target nucleic acid, resulting in one or more desired antisense activity and does not hybridize to one or more non-target nucleic acid or does not hybridize to one or more non-target nucleic acid in such a way that results in significant undesired antisense activity.

In certain antisense activities, hybridization of an antisense compound to a target nucleic acid results in recruitment of a protein that cleaves the target nucleic acid. For example, certain antisense compounds result in RNase H mediated cleavage of the target nucleic acid. RNase H is a cellular endonuclease that cleaves the RNA strand of an RNA:DNA duplex. The DNA in such an RNA:DNA duplex need not be unmodified DNA. In certain embodiments, provided herein are antisense compounds that are sufficiently "DNA-like" to elicit RNase H activity. In certain embodiments, one or more non-DNA-like nucleoside in the gap of a gapmer is tolerated.

In certain antisense activities, an antisense compound or a portion of an antisense compound is loaded into an RNA-induced silencing complex (RISC), ultimately resulting in cleavage of the target nucleic acid. For example, certain antisense compounds result in cleavage of the target nucleic acid by Argonaute. Antisense compounds that are loaded into RISC are RNAi compounds. RNAi compounds may be double-stranded (siRNA) or single-stranded (ssRNA).

In certain embodiments, hybridization of an antisense compound to a target nucleic acid does not result in recruitment of a protein that cleaves that target nucleic acid. In certain embodiments, hybridization of the antisense compound to the target nucleic acid results in alteration of splicing of the target nucleic acid. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in inhibition of a binding interaction between the target nucleic acid and a protein or other nucleic acid. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in alteration of translation of the target nucleic acid. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in exon inclusion. In certain embodiments, hybridization of an antisense compound to a target nucleic acid results in an increase in the amount or activity of a target nucleic acid. In certain embodiments, hybridization of an antisense compound complementary to a target nucleic acid results in alteration of splicing, leading to the inclusion of an exon in the mRNA.

Antisense activities may be observed directly or indirectly. In certain embodiments, observation or detection of an antisense activity involves observation or detection of a change in an amount of a target nucleic acid or protein encoded by such target nucleic acid, a change in the ratio of splice variants of a nucleic acid or protein and/or a phenotypic change in a cell or subject.

Certain Target Nucleic Acids

In certain embodiments, oligomeric compounds comprise or consist of an oligonucleotide comprising a portion that is complementary to a target nucleic acid. In certain embodiments, the target nucleic acid is an endogenous RNA molecule. In certain embodiments, the target nucleic acid encodes a protein. In certain such embodiments, the target nucleic acid is selected from: a mature mRNA and a pre-mRNA, including intronic, exonic and untranslated regions. In certain embodiments, the target nucleic acid is a mature mRNA. In certain embodiments, the target nucleic acid is a pre-mRNA. In certain embodiments, the target region is entirely within an intron. In certain embodiments, the target region spans an intron/exon junction. In certain embodiments, the target region is at least 50% within an intron.

Complementarity/Mismatches to the Target Nucleic Acid

It is possible to introduce mismatch bases without eliminating activity. For example, 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 oligonucleotides, and a 28 and 42 nucleobase oligonucleotides comprised of the sequence of two or three of the tandem oligonucleotides, respectively, for their ability to arrest translation of human DHFR in a rabbit reticulocyte assay. Each of the three 14 nucleobase oligonucleotides alone was able to inhibit translation, albeit at a more modest level than the 28 or 42 nucleobase oligonucleotides.

In certain embodiments, oligonucleotides are complementary to the target nucleic acid over the entire length of the oligonucleotide. In certain embodiments, oligonucleotides are 99%, 95%, 90%, 85%, or 80% complementary to the

target nucleic acid. In certain embodiments, oligonucleotides are at least 80% complementary to the target nucleic acid over the entire length of the oligonucleotide and comprise a portion that is 100% or fully complementary to a target nucleic acid. In certain embodiments, the portion of full complementarity is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleobases in length.

5 In certain embodiments, oligonucleotides comprise one or more mismatched nucleobases relative to the target nucleic acid. In certain embodiments, the mismatch is at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 from the 5'-end of the oligonucleotide.

SMN2

10 In certain embodiments, oligomeric compounds comprise or consist of a modified oligonucleotide that is complementary to a target nucleic acid encoding SMN2, or a portion thereof. In certain embodiments, SMN2 has the sequence set forth in SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777).

15 In certain embodiments, contacting a cell with the oligomeric compound complementary to SEQ ID NO: 1 modulates the splicing of SMN2 RNA in a cell. In certain embodiments, contacting a cell with the oligomeric compound complementary to SEQ ID NO: 1 increases the amount of SMN2 RNA including exon 7. In certain embodiments, contacting a cell with the oligomeric compound complementary to SEQ ID NO: 1 increases full-length SMN2 protein expression. In certain embodiments, the oligomeric compound consists of a modified oligonucleotide.

20 In certain embodiments, contacting a cell in a subject with an oligomeric compound complementary to SEQ ID NO: 1 ameliorates one or more symptom of a neurodegenerative disease. In certain embodiments, the neurodegenerative disease is SMA, including Type I SMA, Type II SMA, Type III SMA, and Type IV SMA. In certain embodiments, the symptom is any of reduced muscle strength; inability or reduced ability to sit upright, to stand, and/or walk; reduced neuromuscular activity; reduced electrical activity in one or more muscles; reduced respiration; inability or reduced ability to eat, drink, and/or breathe without assistance; loss of weight or reduced weight gain; and/or decreased survival.

25 In certain embodiments, an oligomeric compound complementary to SEQ ID NO: 1 is capable of increasing SMN2 RNA including exon 7 *in vivo* by at least 1 fold, 2 fold, or 3 fold when administered according to the standard *in vivo* assay. In certain embodiments, an oligomeric compound complementary to SEQ ID NO: 1 is capable of increasing full-length SMN2 protein *in vivo* by at least 1 fold, 2 fold, or 3 fold when administered according to the standard *in vivo* assay.

Certain Target Nucleic Acids in Certain Tissues

30 In certain embodiments, oligomeric compounds comprise or consist of an oligonucleotide comprising a portion that is complementary to a target nucleic acid, wherein the target nucleic acid is expressed in a pharmacologically relevant tissue. In certain embodiments, the pharmacologically relevant tissues are the cells and tissues that comprise the central nervous system (CNS). Such tissues include brain tissues, such as, spinal cord, cortex, and coronal brain tissue.

Certain Pharmaceutical Compositions

In certain embodiments, described herein are pharmaceutical compositions comprising one or more oligomeric compounds. In certain embodiments, the one or more oligomeric compounds each consists of a modified

oligonucleotide. In certain embodiments, the pharmaceutical composition comprises a pharmaceutically acceptable diluent or carrier. In certain embodiments, a pharmaceutical composition comprises or consists of a sterile saline solution and one or more oligomeric compound. In certain embodiments, the sterile saline is pharmaceutical grade saline. In certain embodiments, a pharmaceutical composition comprises or consists of one or more oligomeric compound and sterile water. In certain embodiments, the sterile water is pharmaceutical grade water. In certain
5 embodiments, a pharmaceutical composition comprises or consists of one or more oligomeric compound and phosphate-buffered saline (PBS). In certain embodiments, the sterile PBS is pharmaceutical grade PBS. In certain embodiments, a pharmaceutical composition comprises or consists of one or more oligomeric compound and artificial cerebrospinal fluid ("artificial CSF" or "aCSF"). In certain embodiments, the artificial cerebrospinal fluid is pharmaceutical grade.

10 In certain embodiments, a pharmaceutical composition comprises a modified oligonucleotide and artificial cerebrospinal fluid. In certain embodiments, a pharmaceutical composition consists of a modified oligonucleotide and artificial cerebrospinal fluid. In certain embodiments, a pharmaceutical composition consists essentially of a modified oligonucleotide and artificial cerebrospinal fluid. In certain embodiments, the artificial cerebrospinal fluid is pharmaceutical grade.

15 In certain embodiments, pharmaceutical compositions comprise one or more oligomeric compound and one or more excipients. In certain embodiments, excipients are selected from water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylase, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose and polyvinylpyrrolidone.

20 In certain embodiments, oligomeric compounds may be admixed with pharmaceutically acceptable active and/or inert substances for the preparation of pharmaceutical compositions or formulations. Compositions and methods for the formulation of pharmaceutical compositions depend on a number of criteria, including, but not limited to, route of administration, extent of disease, or dose to be administered.

25 In certain embodiments, pharmaceutical compositions comprising an oligomeric compound encompass any pharmaceutically acceptable salts of the oligomeric compound, esters of the oligomeric compound, or salts of such esters. In certain embodiments, pharmaceutical compositions comprising oligomeric compounds comprising one or more oligonucleotide, upon administration to a subject, including a human, are 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 oligomeric compounds, prodrugs, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents. Suitable pharmaceutically acceptable salts include, but are not limited to, sodium
30 and potassium salts. In certain embodiments, prodrugs comprise one or more conjugate group attached to an oligonucleotide, wherein the conjugate group is cleaved by endogenous nucleases within the body. In certain embodiments, prodrugs comprise one or more conjugate group attached to an oligonucleotide, wherein the conjugate group is cleaved by endogenous nucleases within the body.

35 Lipid moieties have been used in nucleic acid therapies in a variety of methods. In certain such methods, the nucleic acid, such as an oligomeric compound, is introduced into preformed liposomes or lipoplexes made of mixtures of cationic lipids and neutral lipids. In certain methods, DNA complexes with mono- or poly-cationic lipids are formed without the presence of a neutral lipid. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to a particular cell or tissue. In certain embodiments, a lipid moiety is selected to increase

distribution of a pharmaceutical agent to fat tissue. In certain embodiments, a lipid moiety is selected to increase distribution of a pharmaceutical agent to muscle tissue.

In certain embodiments, pharmaceutical compositions comprise a delivery system. Examples of delivery systems include, but are not limited to, liposomes and emulsions. Certain delivery systems are useful for preparing certain pharmaceutical compositions including those comprising hydrophobic compounds. In certain embodiments, 5 certain organic solvents such as dimethylsulfoxide are used.

In certain embodiments, pharmaceutical compositions comprise one or more tissue-specific delivery molecules designed to deliver the one or more pharmaceutical agents comprising an oligomeric compound provided herein to specific tissues or cell types. For example, in certain embodiments, pharmaceutical compositions include liposomes 10 coated with a tissue-specific antibody.

In certain embodiments, pharmaceutical compositions comprise a co-solvent system. Certain of such co-solvent systems comprise, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. In certain embodiments, such co-solvent systems are used for hydrophobic compounds. A non-limiting example of such a co-solvent system is the VPD co-solvent system, which is a solution of absolute ethanol comprising 3% w/v 15 benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™ and 65% w/v polyethylene glycol 300. The proportions of such co-solvent systems may be varied considerably without significantly altering their solubility and toxicity characteristics. Furthermore, the identity of co-solvent components may be varied: for example, other surfactants may be used instead of Polysorbate 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g., polyvinyl pyrrolidone; and other sugars or 20 polysaccharides may substitute for dextrose.

In certain embodiments, pharmaceutical compositions are prepared for oral administration. In certain embodiments, pharmaceutical compositions are prepared for buccal administration. In certain embodiments, a pharmaceutical composition is prepared for administration by injection (e.g., intravenous, subcutaneous, intramuscular, intrathecal (IT), intracerebroventricular (ICV), etc.). In certain of such embodiments, a pharmaceutical composition 25 comprises a carrier and is formulated in aqueous solution, such as water or physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. In certain embodiments, other ingredients are included (e.g., ingredients that aid in solubility or serve as preservatives). In certain embodiments, injectable suspensions are prepared using appropriate liquid carriers, suspending agents and the like. Certain pharmaceutical compositions for injection are presented in unit dosage form, e.g., in ampoules or in multi-dose containers. Certain pharmaceutical 30 compositions for injection are suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Certain solvents suitable for use in pharmaceutical compositions for injection include, but are not limited to, lipophilic solvents and fatty oils, such as sesame oil, synthetic fatty acid esters, such as ethyl oleate or triglycerides, and liposomes.

Under certain conditions, certain compounds disclosed herein act as acids. Although such compounds may be 35 drawn or described in protonated (free acid) form, or ionized and in association with a cation (salt) form, aqueous solutions of such compounds exist in equilibrium among such forms. For example, a phosphate linkage of an oligonucleotide in aqueous solution exists in equilibrium among free acid, anion and salt forms. Unless otherwise indicated, compounds described herein are intended to include all such forms. Moreover, certain oligonucleotides have several such linkages, each of which is in equilibrium. Thus, oligonucleotides in solution exist in an ensemble of forms

at multiple positions all at equilibrium. The term “oligonucleotide” is intended to include all such forms. Drawn structures necessarily depict a single form. Nevertheless, unless otherwise indicated, such drawings are likewise intended to include corresponding forms. Herein, a structure depicting the free acid of a compound followed by the term “or a salt thereof” expressly includes all such forms that may be fully or partially protonated/de-protonated/in association with a cation. In certain instances, one or more specific cation is identified.

In certain embodiments, modified oligonucleotides or oligomeric compounds are in aqueous solution with sodium. In certain embodiments, modified oligonucleotides or oligomeric compounds are in aqueous solution with potassium. In certain embodiments, modified oligonucleotides or oligomeric compounds are in PBS. In certain embodiments, modified oligonucleotides or oligomeric compounds are in water. In certain such embodiments, the pH of the solution is adjusted with NaOH and/or HCl to achieve a desired pH.

Herein, certain specific doses are described. A dose may be in the form of a dosage unit. For clarity, a dose (or dosage unit) of a modified oligonucleotide or an oligomeric compound in milligrams indicates the mass of the free acid form of the modified oligonucleotide or oligomeric compound. As described above, in aqueous solution, the free acid is in equilibrium with anionic and salt forms. However, for the purpose of calculating dose, it is assumed that the modified oligonucleotide or oligomeric compound exists as a solvent-free, sodium-acetate free, anhydrous, free acid. For example, where a modified oligonucleotide or an oligomeric compound is in solution comprising sodium (e.g., saline), the modified oligonucleotide or oligomeric compound may be partially or fully de-protonated and in association with Na⁺ ions. However, the mass of the protons are nevertheless counted toward the weight of the dose, and the mass of the Na⁺ ions are not counted toward the weight of the dose. Thus, for example, a dose, or dosage unit, of 10 mg of Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 equals the number of fully protonated molecules that weighs 10 mg. This would be equivalent to 10.53 mg of solvent-free, sodium acetate-free, anhydrous sodiated Compound No. 1263789, 10.53 mg of solvent-free, sodium acetate-free, anhydrous sodiated Compound No. 1287717, 10.52 mg of solvent-free, sodium acetate-free, anhydrous sodiated Compound No. 1287745, and 10.51 mg of solvent-free, sodium acetate-free, anhydrous sodiated Compound No. 1358996. When an oligomeric compound comprises a conjugate group, the mass of the conjugate group is included in calculating the dose of such oligomeric compound. If the conjugate group also has an acid, the conjugate group is likewise assumed to be fully protonated for the purpose of calculating dose.

Certain Compositions

Compound No. 1263789

In certain embodiments, Compound No. 1263789 is characterized as a modified oligonucleotide having a sequence of (from 5' to 3') CACTTTCATAATGCTGGC (SEQ ID NO: 21), wherein each nucleoside comprises a 2'-MOE sugar moiety, wherein the internucleoside linkages between nucleosides 2 to 3 and 4 to 5 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 1 to 2, 3 to 4, 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, 14 to 15, 15 to 16, 16 to 17, and 17 to 18 are phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine.

In certain embodiments, Compound No. 1263789 is represented by the following chemical notation (5' to 3'): ^mC_{es} A_{eo} ^mC_{es} T_{eo} T_{es} T_{es} ^mC_{es} A_{es} T_{es} A_{es} A_{es} T_{es} G_{es} ^mC_{es} T_{es} G_{es} G_{es} ^mC_e (SEQ ID NO: 21), wherein,

A = an adenine nucleobase,

^mC = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

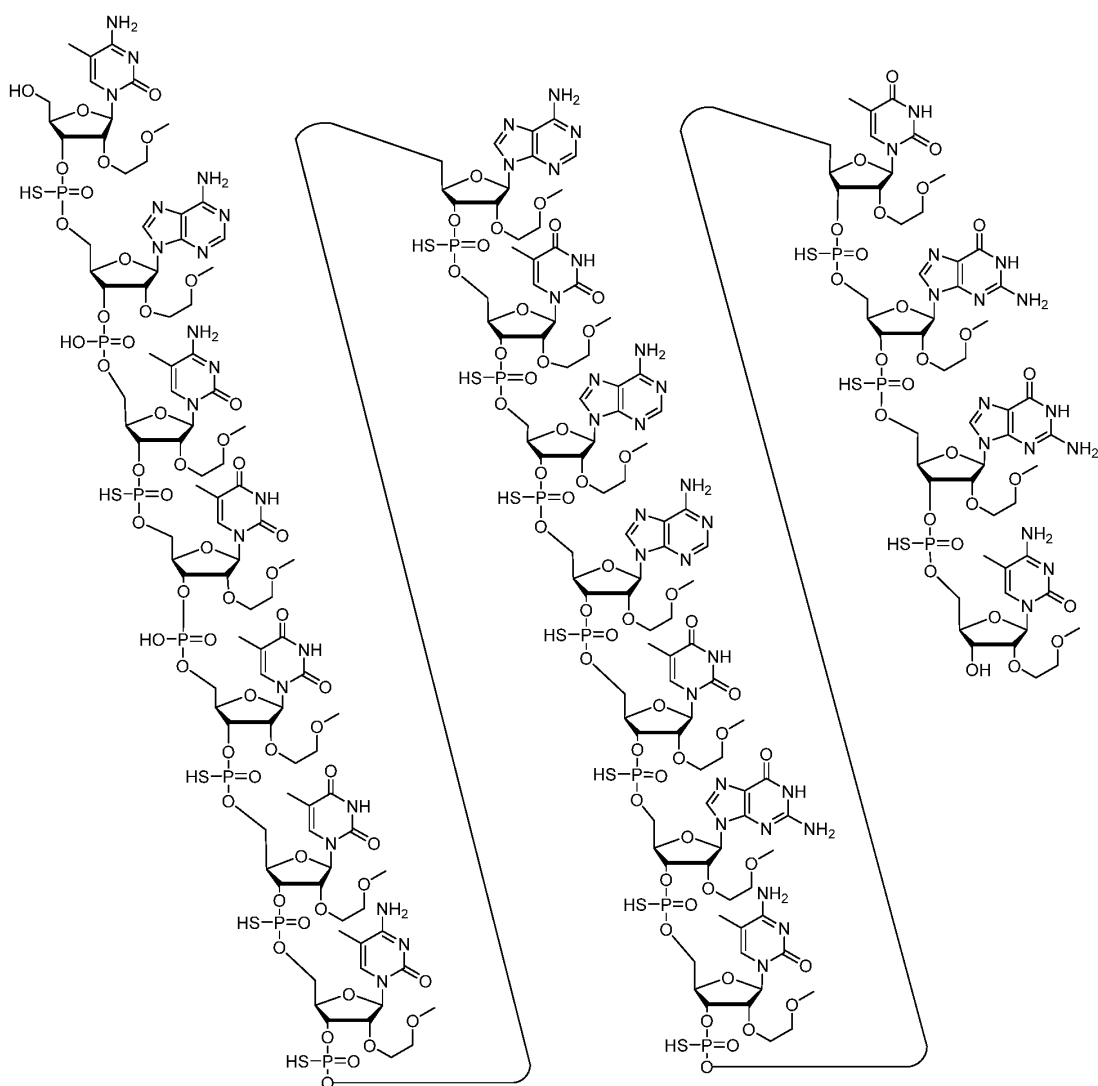
T = a thymine nucleobase,

5 e = a 2'-MOE sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1263789 is represented by the following chemical structure:

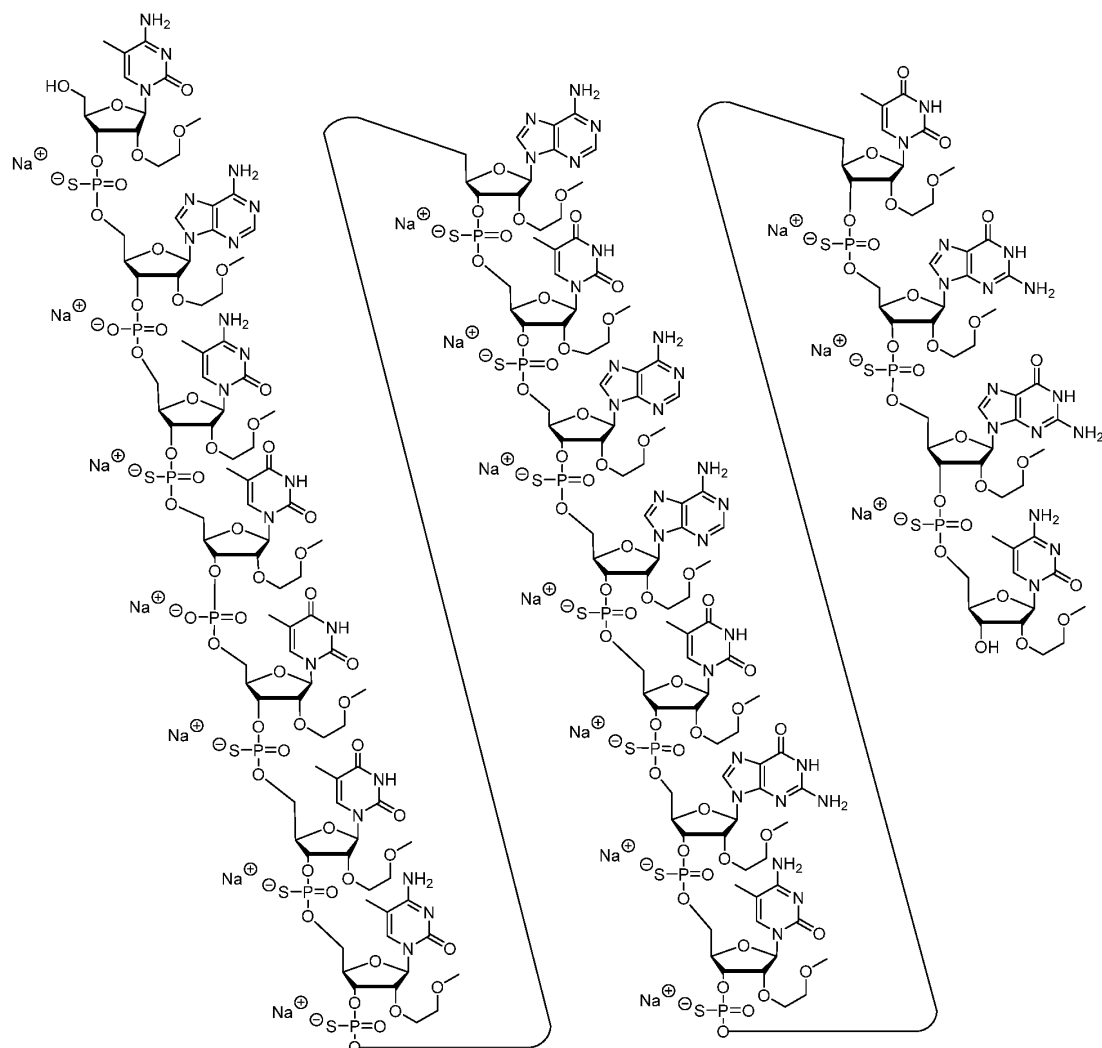


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(SEQ ID NO: 21).

Structure 1. Compound No. 1263789

In certain embodiments, the sodium salt of Compound No. 1263789 is represented by the following chemical structure:



(SEQ ID NO: 21).

5 Structure 2. The sodium salt of Compound No. 1263789

Compound No: 1287717

In certain embodiments, Compound No. 1287717 is characterized as a modified oligonucleotide having a sequence of (from 5' to 3') TTCACCTTTCATAATGCTGGC (SEQ ID NO: 22), wherein each nucleoside comprises a 2'-MOE sugar moiety, wherein the internucleoside linkages between nucleosides 1 to 2 and 19 to 20 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 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, 14 to 15, 15 to 16, 16 to 17, 17 to 18, and 18 to 19 are phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine.

In certain embodiments, Compound No. 1287717 is represented by the following chemical notation (5' to 3'): T_{eo} T_{es} ^mC_{es} A_{es} ^mC_{es} T_{es} T_{es} T_{es} ^mC_{es} A_{es} T_{es} A_{es} A_{es} T_{es} G_{es} ^mC_{es} T_{es} G_{es} G_{eo} ^mC_e (SEQ ID NO: 22)

wherein,

A = an adenine nucleobase,

^mC = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

5 T = a thymine nucleobase,

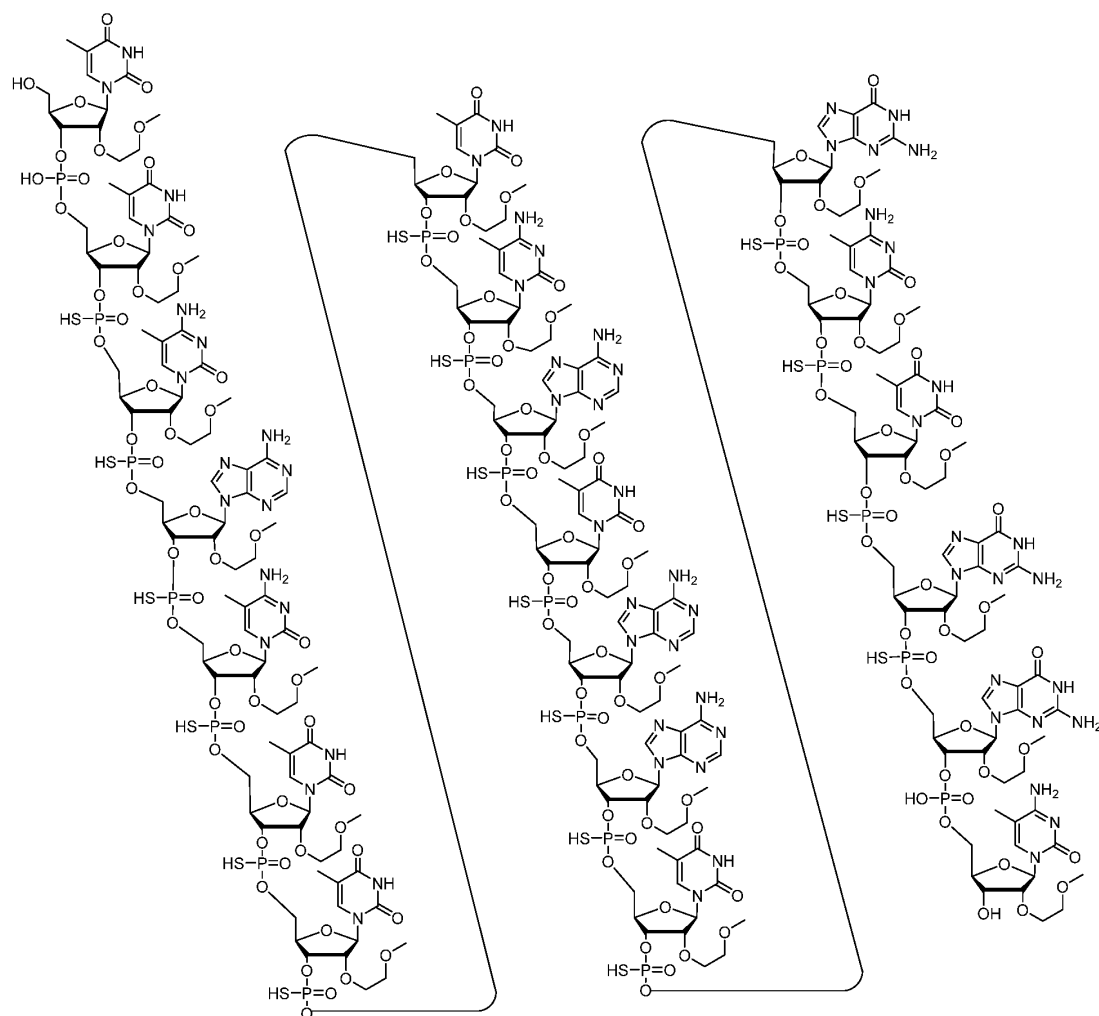
e = a 2'-MOE sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1287717 is represented by the following chemical structure:

10



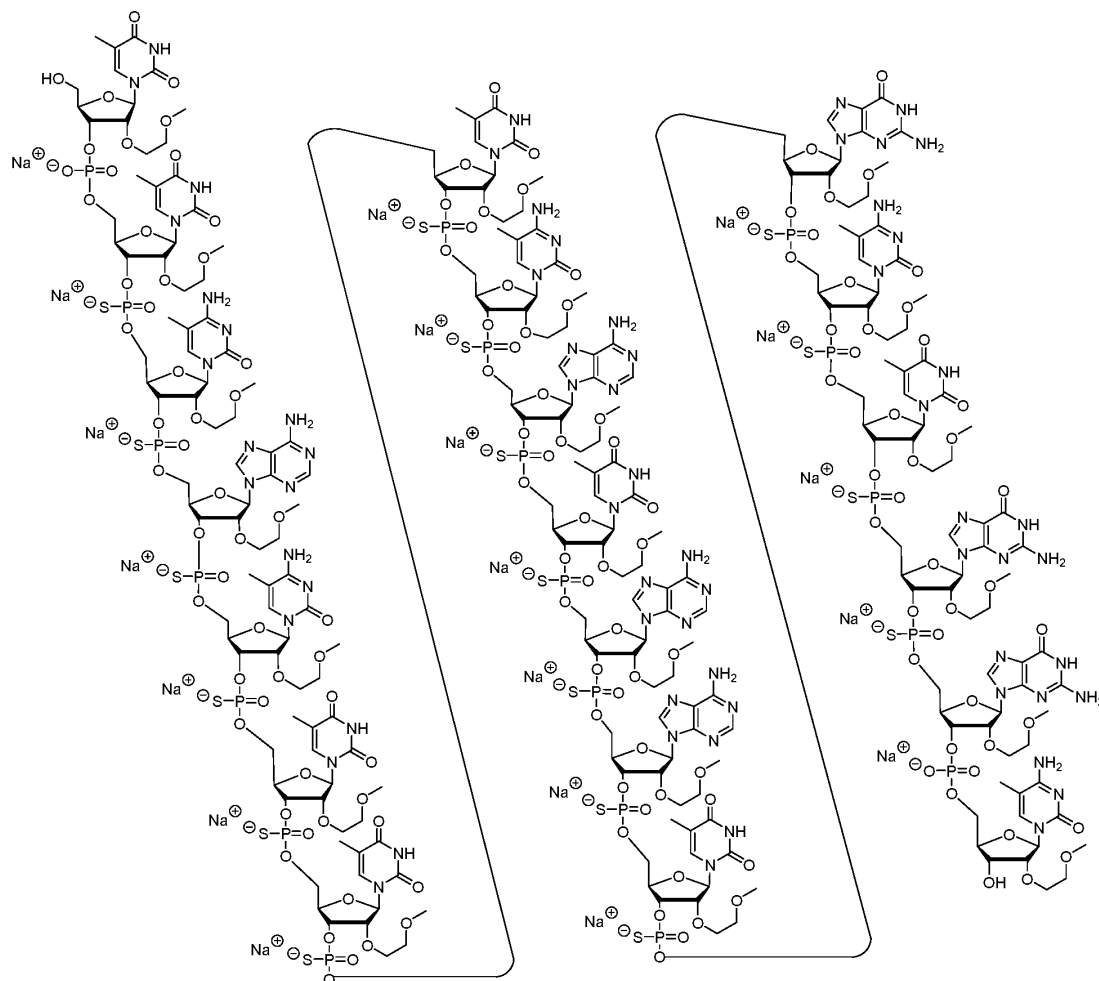
(SEQ ID NO: 22).

Structure 3. Compound No. 1287717

15

In certain embodiments, the sodium salt of Compound No. 1287717 is represented by the following chemical

structure:



(SEQ ID NO: 22).

Structure 4. The sodium salt of Compound No. 1287717

5 Compound No: 1287745

In certain embodiments, Compound No. 1287745 is characterized as a modified oligonucleotide having a sequence of (from 5' to 3') TTCACTTTCATAATGCTGGC (SEQ ID NO: 22), wherein each of nucleosides 1 and 20 comprises a 2'-MOE sugar moiety, each of nucleosides 2-19 comprises a 2'-NMA sugar moiety, wherein the internucleoside linkages between nucleosides 1 to 2 and 19 to 20 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 2 to 3, 3 to 4, 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, 14 to 15, 15 to 16, 16 to 17, 17 to 18, and 18 to 19 are phosphorothioate internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine.

In certain embodiments, Compound No. 1287745 is represented by the following chemical notation (5' to 3'):

$T_{eo} T_{ns} {}^mC_{ns} A_{ns} {}^mC_{ns} T_{ns} T_{ns} T_{ns} {}^mC_{ns} A_{ns} T_{ns} A_{ns} A_{ns} T_{ns} G_{ns} {}^mC_{ns} T_{ns} G_{ns} G_{no} {}^mC_e$ (SEQ ID NO: 22)

15 wherein,

A = an adenine nucleobase,

^mC = a 5-methyl cytosine nucleobase ,

G = a guanine nucleobase,

T = a thymine nucleobase,

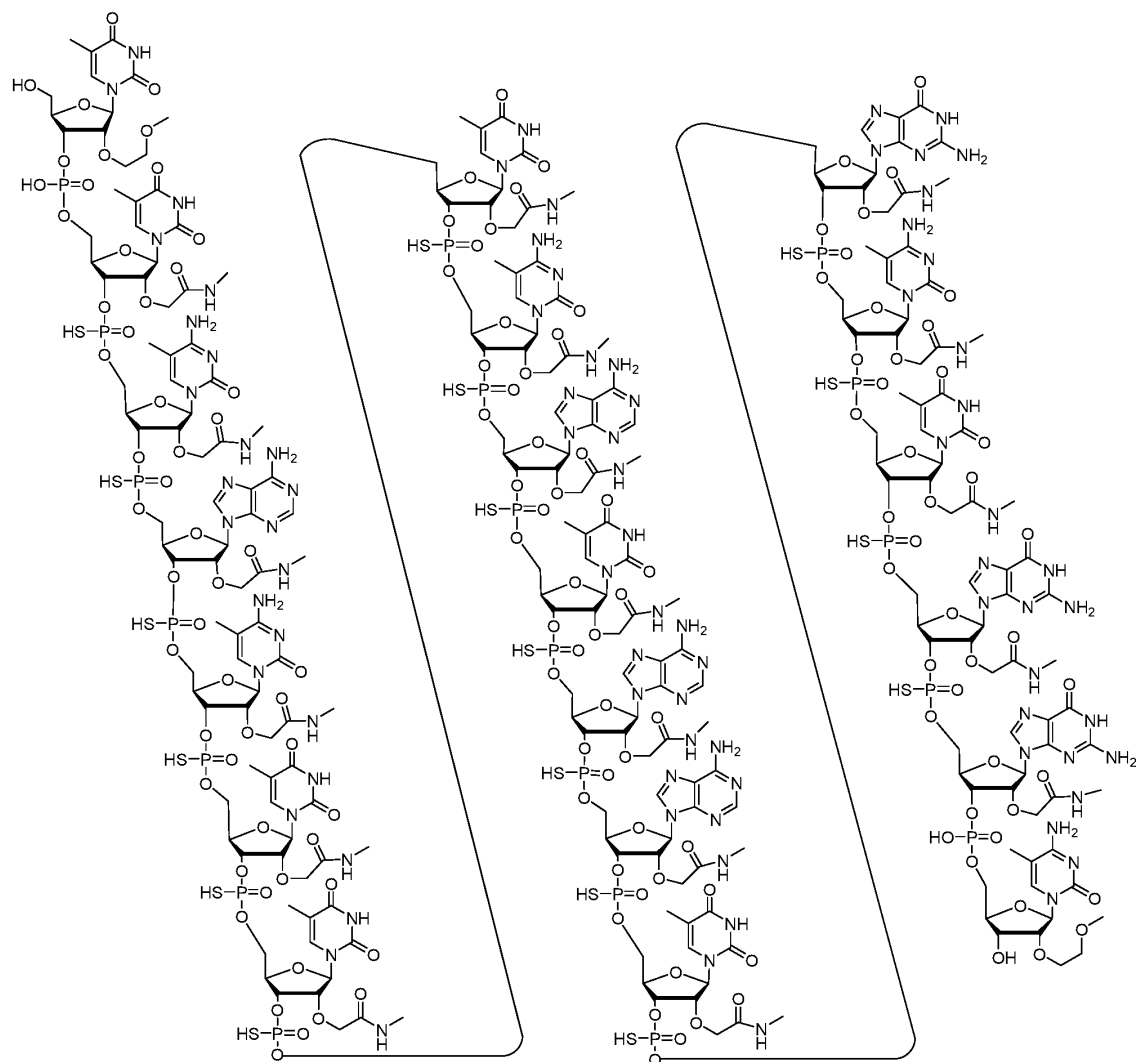
e = a 2'-MOE sugar moiety,

5 n = a 2'-NMA sugar moiety,

s = a phosphorothioate internucleoside linkage, and

o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1287745 is represented by the following chemical structure:

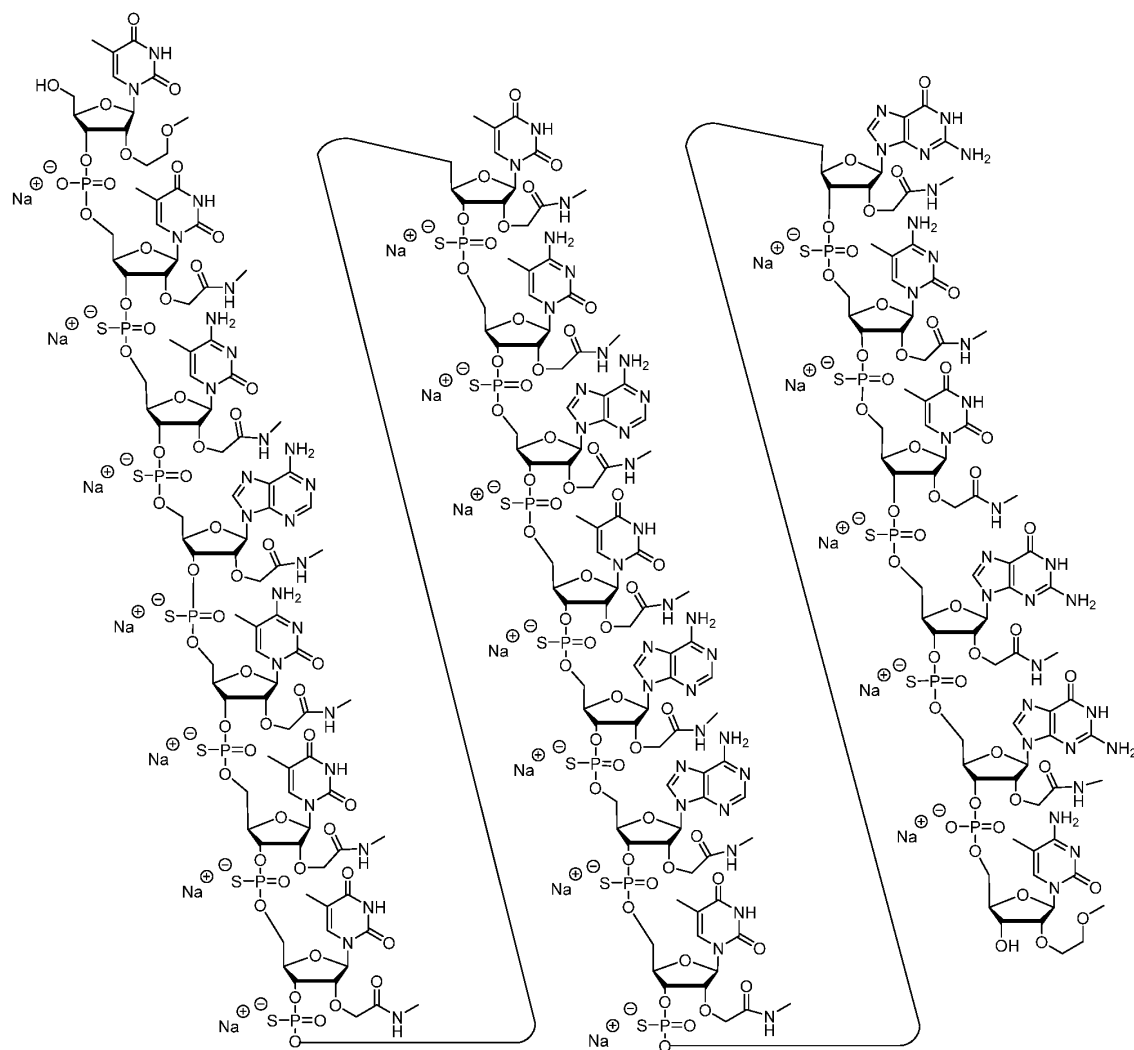


10

(SEQ ID NO: 22).

Structure 5. Compound No. 1287745

In certain embodiments, the sodium salt of Compound No. 1287745 is represented by the following chemical structure:



(SEQ ID NO: 22).

Structure 6. The sodium salt of Compound No. 1287745Compound No: 1358996

5 In certain embodiments, Compound No. 1358996 is characterized as modified oligonucleotide having a sequence of (from 5' to 3') CACTTTCATAATGCTGGC (SEQ ID NO: 21), wherein each nucleoside comprises a 2'-NMA sugar moiety, wherein the internucleoside linkages between nucleosides 2 to 3 and 4 to 5 are phosphodiester internucleoside linkages and the internucleoside linkages between nucleosides 1 to 2, 3 to 4, 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, 14 to 15, 15 to 16, 16 to 17, and 17 to 18 are phosphorothioate

10 internucleoside linkages, and wherein each cytosine is a 5-methyl cytosine.

In certain embodiments, Compound No. 1358996 is represented by the following chemical notation (5' to 3'):

^mC_{ns} A_{no} ^mC_{ns} T_{no} T_{ns} T_{ns} ^mC_{ns} A_{ns} T_{ns} A_{ns} A_{ns} T_{ns} G_{ns} ^mC_{ns} T_{ns} G_{ns} G_{ns} ^mC_n (SEQ ID NO: 21)

wherein,

A = an adenine nucleobase,

^mC = a 5-methyl cytosine nucleobase,

G = a guanine nucleobase,

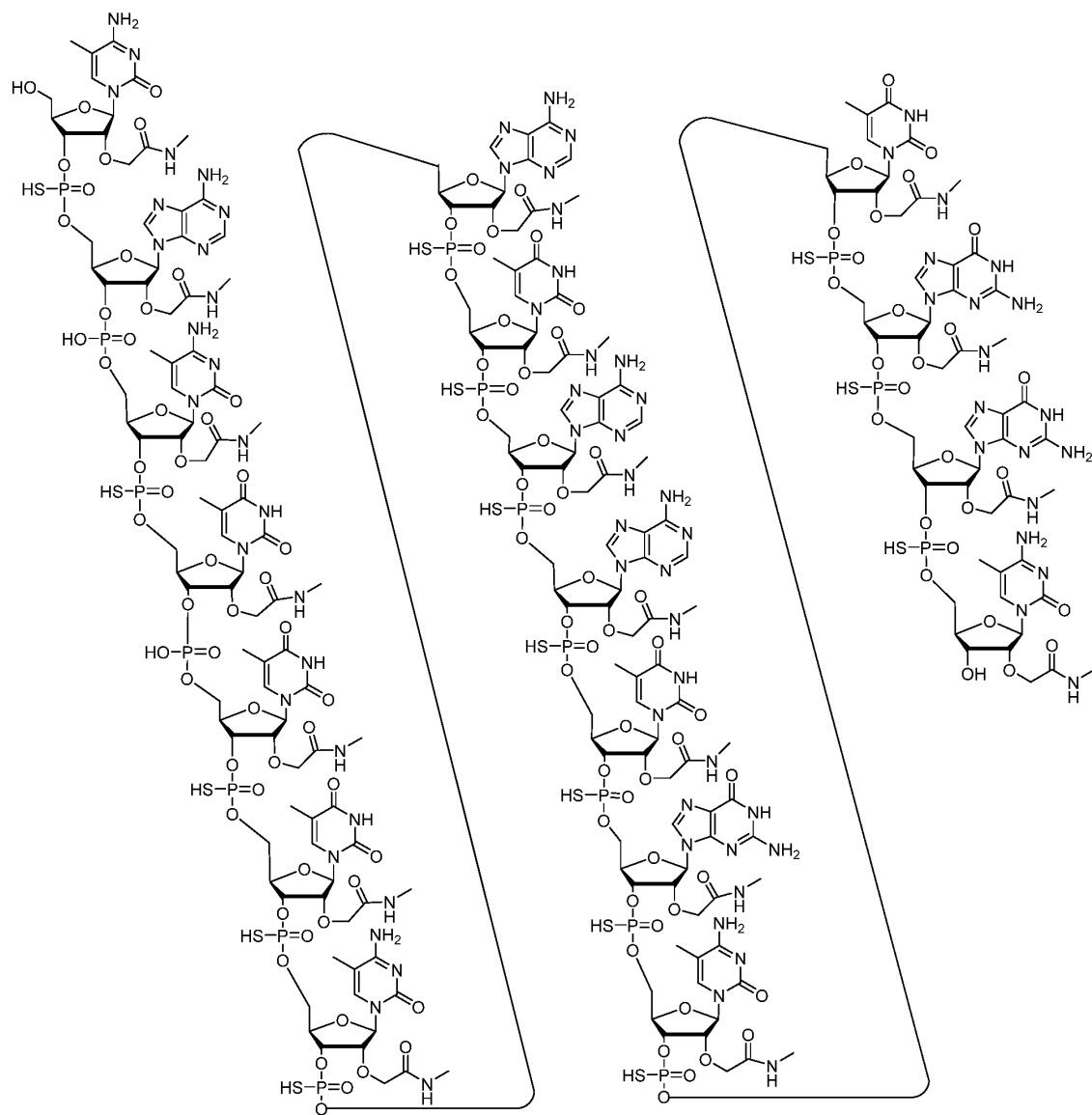
T = a thymine nucleobase,

n = a 2'-NMA sugar moiety,

5 s = a phosphorothioate internucleoside linkage, and

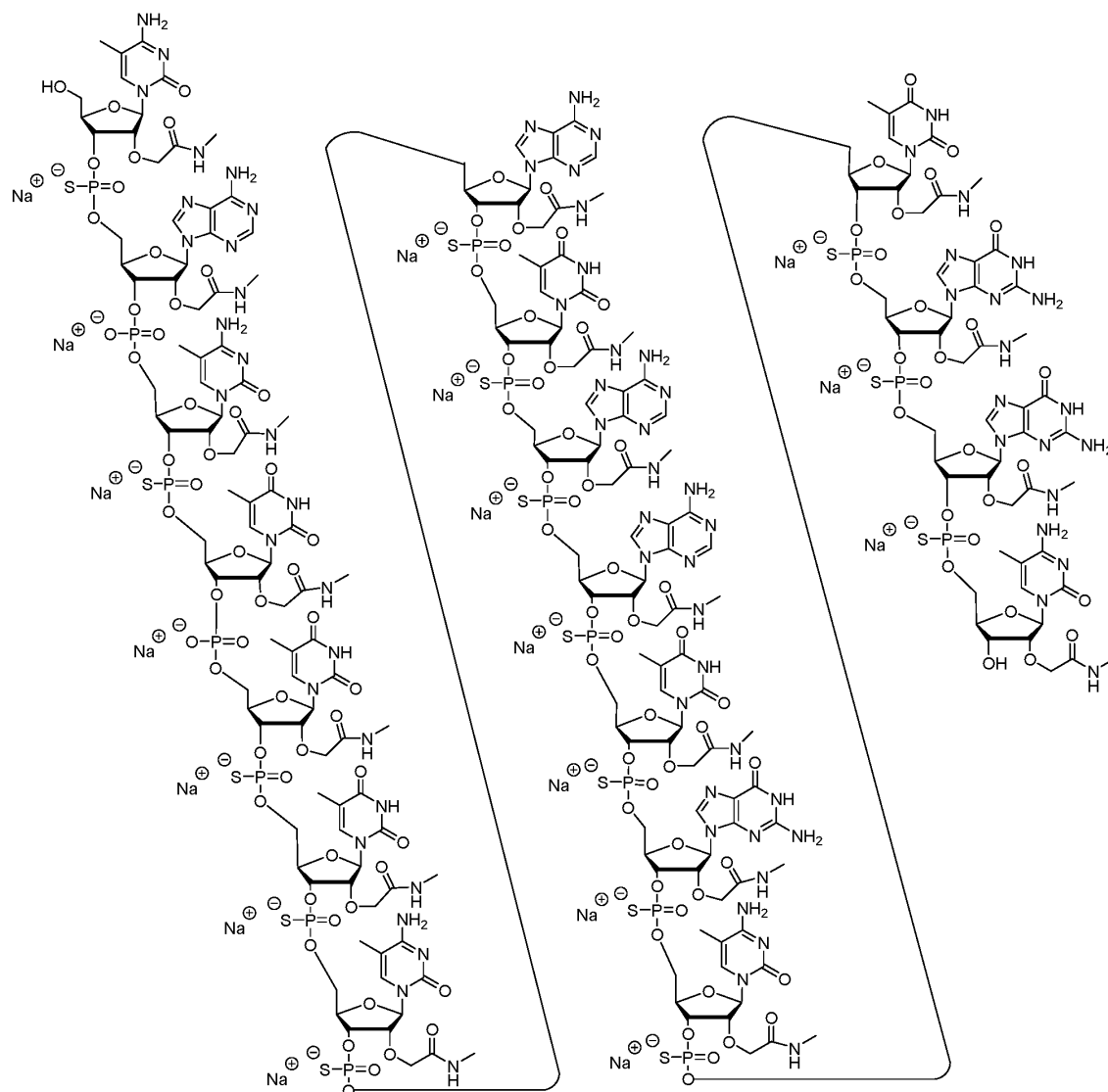
o = a phosphodiester internucleoside linkage.

In certain embodiments, Compound No. 1358996 is represented by the following chemical structure:



(SEQ ID NO: 21).

In certain embodiments, the sodium salt of Compound No. 1358996 is represented by the following chemical structure:



(SEQ ID NO: 21).

5 Structure 8. The sodium salt of Compound No. 1358996

Certain Comparator Compositions

- In certain embodiments, Spinraza® (generic name nusinersen; Compound No. 396443), approved for treatment of SMA, is a comparator compound (See, e.g., Chiroboga, et al., *Neurology*, 86(10): 890-897, 2016; Finkel, et al., *Lancet*, 338(10063): 3017-3026, 2016; Finkel, et al., *N. Engl. J. Med.*, 377(18):1723-1732 2017; Mercuri, et al., *N. Engl. J. Med.*, 378(7):625-635, 2018; Montes, et al., *Muscle Nerve*. 60(4): 409-414, 2019; Darras, et al., *Neurology*, 92(21):e2492-e2506, 2019). Spinraza® was previously described in WO2010120820, incorporated herein by reference,

and has a sequence (from 5' to 3') of TCACTTTCATAATGCTGG (SEQ ID NO: 23), wherein each nucleoside comprises a 2'-MOE sugar moiety, each internucleoside linkage is a phosphorothioate internucleoside linkage, and each cytosine is a 5-methyl cytosine.

In certain embodiments, although not approved for human therapy, other previously described compounds, including Compound Nos. 387954, 396442, 443305, and 819735, are comparator compounds.

Compound No. 387954 was previously described in WO 2014/179620, incorporated herein by reference. Compound No. 387954 has a sequence (from 5' to 3') of ATTCACTTTCATAATGCTGG (SEQ ID NO: 20), wherein each nucleoside comprises a 2'-MOE sugar moiety, each internucleoside linkage is a phosphorothioate internucleoside linkage, and each cytosine is a 5-methyl cytosine.

Compound No. 396442 was previously described in WO 2010/120820, incorporated herein by reference. Compound No. 396442 has a sequence (from 5' to 3') of CACTTTCATAATGCTGGC (SEQ ID NO: 21), wherein each nucleoside comprises a 2'-MOE sugar moiety, each internucleoside linkage is a phosphorothioate internucleoside linkage, and each cytosine is a 5-methyl cytosine.

Compound No. 443305 was previously described in WO 2018/014041, incorporated herein by reference. Compound No. 443305 has a sequence (from 5' to 3') of TCACTTTCATAATGCTGG (SEQ ID NO: 23), wherein each nucleoside comprises a 2'-NMA sugar moiety, each internucleoside linkage is a phosphorothioate internucleoside linkage, and each cytosine is a 5-methyl cytosine.

Compound No. 819735 was previously described in WO 2018/014041, incorporated herein by reference. Compound No. 819735 has a sequence (from 5' to 3') of CACTTTCATAATGCTGGC (SEQ ID NO: 21), wherein each nucleoside comprises a 2'-NMA sugar moiety, each internucleoside linkage is a phosphorothioate internucleoside linkage, and each cytosine is a 5-methyl cytosine.

Table 1
Certain Comparator Compositions

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif	Internucleoside Linkage Motif	SEQ ID NO:	Reference Number
396443	TCACTTTCATAATGCTGG	Full 2'-MOE	Full PS	23	WO 2010/120820
387954	ATTCACTTTCATAATGCTGG	Full 2'-MOE	Full PS	20	WO 2014/179620
396442	CACTTTCATAATGCTGGC	Full 2'-MOE	Full PS	21	WO 2010/120820
443305	TCACTTTCATAATGCTGG	Full 2'-NMA	Full PS	23	WO 2018/014041
819735	CACTTTCATAATGCTGGC	Full 2'-NMA	Full PS	21	WO 2018/014041

In certain embodiments, compounds described herein are superior relative to compounds described in WO 2007/002390, WO2010/120820, WO 2015/161170, and WO 2018/014041, because they demonstrate one or more improved properties, such as, potency, efficacy, and tolerability.

For example, Compound No. 1263789, Compound No. 1287745, and Compound No. 1358996 each

demonstrated improved potency *in vivo* as compared to Compound No. 396443. As shown in Example 5, Compound No. 1263789, Compound No. 1287745, and Compound No. 1358996 achieved an ED₅₀ in spinal cord of 13.3, 8.8, and 7.4, respectively. In comparison, Compound No. 396443 achieved an ED₅₀ in spinal cord of 22.0. Therefore, each of Compound No. 1263789, Compound No. 1287745, and Compound No. 1358996 are more potent than Compound No. 396443 in this assay.

For example, Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 each demonstrated improved 3 hour FOB scores as compared to Compound No. 396443, Compound No. 387954, and Compound No. 443305. As shown in Example 6, at 700 µg, Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 achieved 3 hour FOB scores of 0, 3.25, 1, and 0, respectively. In comparison, at half the dose (350 µg) Compound No. 396443 achieved a 3 hour FOB score of 4.0; and at the same dose (700 µg) Compound No. 387954 and Compound No. 443305 achieved a 3 hour FOB score of 4.0 and 4.75, respectively. Therefore, each of Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 are more tolerable than Compound No. 396443, Compound No. 387954, and Compound No. 443305 in this assay.

For example, Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 each demonstrated improved long-term tolerability as compared to Compound No. 396442 and Compound No. 819735. As shown in Example 7, Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 demonstrated no adverse events, no Purkinje cell loss, and cortex GFAP mRNA less than 2-fold of control. In comparison, 396442 and 819735 each demonstrated adverse events, Purkinje cell loss, and cortex GFAP mRNA greater than 2-fold of control in certain treated animals. Therefore, each of Compound No. 1263789, Compound No. 1287717, Compound No. 1287745, and Compound No. 1358996 are more tolerable than Compound No. 396442 and Compound No. 819735 in this assay.

Nonlimiting disclosure and incorporation by reference

Each of the literature and patent publications listed herein is incorporated by reference in its entirety. 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, GenBank accession numbers, and the like recited in the present application is incorporated herein by reference in its entirety.

Although the sequence listing accompanying this filing identifies each sequence as either “RNA” or “DNA” as required, in reality, those sequences may be modified with any combination of chemical modifications. One of skill in the art will readily appreciate that such designation as “RNA” or “DNA” to describe modified oligonucleotides is, in certain instances, arbitrary. For example, an oligonucleotide comprising a nucleoside comprising a 2'-OH sugar moiety and a thymine base could be described as a DNA having a modified sugar moiety (2'-OH in place of one 2'-H of DNA) or as an RNA having a modified base (thymine (methylated uracil) in place of a uracil of RNA). Accordingly, nucleic acid sequences provided herein, including, but not limited to those in the sequence listing, are intended to encompass nucleic acids containing any combination of natural or modified RNA and/or DNA, including, but not limited to such nucleic acids having modified nucleobases. By way of further example and without limitation, an oligomeric compound having the nucleobase sequence “ATCGATCG” encompasses any oligomeric compounds having such nucleobase

sequence, whether modified or unmodified, including, but not limited to, such compounds comprising RNA bases, such as those having sequence “AUCGAUCG” and those having some DNA bases and some RNA bases such as “AUCGATCG” and oligomeric compounds having other modified nucleobases, such as “AT^mCGAUCG,” wherein ^mC indicates a cytosine base comprising a methyl group at the 5-position.

5 Certain compounds described herein (e.g., modified oligonucleotides) have one or more asymmetric center and thus give rise to enantiomers, diastereomers, and other stereoisomeric configurations that may be defined, in terms of absolute stereochemistry, as (*R*) or (*S*), as α or β such as for sugar anomers, or as (*D*) or (*L*), such as for amino acids, etc. Compounds provided herein that are drawn or described as having certain stereoisomeric configurations include only the indicated compounds. Compounds provided herein that are drawn or described with undefined stereochemistry include
10 all such possible isomers, including their stereorandom and optically pure forms, unless specified otherwise. Likewise, all *cis*- and *trans*-isomers and tautomeric forms of the compounds herein are also included unless otherwise indicated. Oligomeric compounds described herein include chirally pure or enriched mixtures as well as racemic mixtures. For example, oligomeric compounds having a plurality of phosphorothioate internucleoside linkages include such compounds in which chirality of the phosphorothioate internucleoside linkages is controlled or is random. Unless
15 otherwise indicated, compounds described herein are intended to include corresponding salt forms.

The compounds described herein include variations in which one or more atoms are replaced with a non-radioactive isotope or radioactive isotope of the indicated element. For example, compounds herein that comprise hydrogen atoms encompass all possible deuterium substitutions for each of the ¹H hydrogen atoms. Isotopic substitutions encompassed by the compounds herein include but are not limited to: ²H or ³H in place of ¹H, ¹³C or ¹⁴C in
20 place of ¹²C, ¹⁵N in place of ¹⁴N, ¹⁷O or ¹⁸O in place of ¹⁶O, and ³³S, ³⁴S, ³⁵S, or ³⁶S in place of ³²S. In certain embodiments, non-radioactive isotopic substitutions may impart new properties on the oligomeric compound that are beneficial for use as a therapeutic or research tool. In certain embodiments, radioactive isotopic substitutions may make the compound suitable for research or diagnostic purposes such as imaging.

25 EXAMPLES

The following examples illustrate certain embodiments of the present disclosure and are not limiting. Moreover, where specific embodiments are provided, the inventors have contemplated generic application of those specific embodiments.

30 Example 1: Design of modified oligonucleotides complementary to a human SMN2 nucleic acid

Modified oligonucleotides complementary to a human SMN2 nucleic acid were designed and synthesized as indicated in the tables below.

The modified oligonucleotides in the tables below are 16, 17, 18, 19, or 20 nucleosides in length, as specified. The modified oligonucleotides comprise 2'-MOE sugar moieties, 2'-NMA sugar moieties, cEt sugar moieties, 2'-OMe
35 sugar moieties, and/or 2'- β -D-deoxyribsyl sugar moieties, as specified. Each internucleoside linkage throughout the modified oligonucleotides is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage, as specified. Cytosines are either non-methylated cytosines or 5-methyl cytosines, as specified.

Each modified oligonucleotide listed in the tables below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically

stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. Each modified oligonucleotide listed in the tables below targets an active site on the SMN2 transcript for inclusion of exon 7. “Start site” indicates the 5’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. “Stop site” indicates the 3’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 2

The modified oligonucleotides in Table 2 below are 16, 17, 18, 19 or 20 nucleosides in length. Each nucleoside comprises a 2’-MOE sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each ‘e’ represents a 2’-MOE sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide is provided in the Internucleoside Linkage Motif column, wherein each ‘s’ represents a phosphorothioate internucleoside linkage, and each ‘o’ represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each modified oligonucleotide listed in Table 2 below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. “Start site” indicates the 5’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. “Stop site” indicates the 3’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 2**2’-MOE modified oligonucleotides with PS or mixed PS/PO internucleoside linkages**

Compound Number	Nucleobase Sequence (5’ to 3’)	Sugar Motif (5’ to 3’)	Internucleoside Linkage Motif (5’ to 3’)	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1287063	ACTTTCATAATGCTGGCAG	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27059	27077	24
1287048	CACTTTCATAATGCTGGCAG	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27059	27078	25
1287064	CACTTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27060	27078	26
1287049	TCACTTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27060	27079	27
1210340	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27061	27076	28
1212868	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssssssooooo	27061	27076	28
1212867	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssssssooooo	27061	27076	28
1212863	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssooooo	27061	27076	28
1212866	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssooooo	27061	27076	28
1212861	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssooooo	27061	27076	28
1212860	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	sssssooooo	27061	27076	28

1212865	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSS0000SSSSSS	27061	27076	28
1212859	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSS000000SSSS	27061	27076	28
1212851	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212850	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212852	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212853	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212854	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212864	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SS0000SSSSSSSS	27061	27076	28
1212855	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212856	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212857	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1212858	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27076	28
1210339	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212849	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212848	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212845	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212844	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212843	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212842	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212841	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212847	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212832	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212833	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212834	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212835	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212836	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212846	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212837	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212838	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212839	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1212840	ACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27077	29
1263814	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27078	21
1263816	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeee	SSSSSSSSSSSSSS	27061	27078	21

[illegible]

1212831	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssssssss0000ss	27061	27078	21
1212818	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	s000ssssssss00ss	27061	27078	21
1212823	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssss00000sssss	27061	27078	21
1212819	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	s000ssssssss000ss	27061	27078	21
1212822	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssss000000sssss	27061	27078	21
1212820	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	s0000ssssssss000ss	27061	27078	21
1212821	CACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssss0000000sssss	27061	27078	21
1287065	TCACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27061	27079	30
1210341	ACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27077	31
524403	CACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27078	32
1287121	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287120	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287113	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287110	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287119	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1364782	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1364777	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287118	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1364783	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287109	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1364784	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287117	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27079	23
1287112	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssss00ssssssss	27062	27079	23
1287116	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27079	23
1287115	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27079	23
1287114	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	s0ssssssssssssss	27062	27079	23
1287106	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	s0ssssssssssssss	27062	27079	23
1287107	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	s0ssssssssssssss	27062	27079	23
1287108	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	s0s0ssssssssssss	27062	27079	23
1287111	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	s00ssssssssssssss	27062	27079	23
1287066	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27080	33
1287074	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27080	33
1287071	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssss00ss	27062	27080	33

1287073	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27062	27080	33
1287070	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssssss	27062	27080	33
1287072	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27080	33
1287067	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27080	33
1287068	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27080	33
1287069	TTCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sosoooooooooooooooo	27062	27080	33
1287060	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssso	27062	27081	20
1287057	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sssssssssssssoos	27062	27081	20
1287054	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sssssssssssssoos	27062	27081	20
1287059	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27081	20
1287053	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sssssssssssssoos	27062	27081	20
1287056	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sssssssssooooooooo	27062	27081	20
1287058	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27081	20
1287050	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27081	20
1287051	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27081	20
1287052	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sosoooooooooooooooo	27062	27081	20
1287055	ATTCACCTTCATAATGCTGG	eeeeeeeeeeeeeeeeee	sooooooooooooooooo	27062	27081	20
1287075	ATTCACCTTCATAATGCTG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27063	27081	34
1287062	AGATTCACCTTCATAATGCT	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27064	27083	35
1287061	GATTCACCTTCATAATGCTG	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27063	27082	49
1287076	GATTCACCTTCATAATGCT	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27064	27082	50
1287701	TCACCTTCATAATGCTGG <u>I</u>	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27079	36
1287702	TCACCTTCATAATGCTGG <u>A</u>	eeeeeeeeeeeeeeeeee	ssssssssssssssss	27062	27079	37

Table 3

The modified oligonucleotides in Table 3 below are 16, 17, 18, 19 or 20 nucleosides in length. Each nucleoside comprises a 2'-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'n' represents a 2'-NMA sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide is provided in the Internucleoside Linkage Motif column, wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each modified oligonucleotide listed in Table 3 below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777). "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site"

indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 3
2'-NMA modified oligonucleotides with PS or mixed PS/PO internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1287127	CACTTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssssss	27060	27078	26
1287122	TCACTTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssssss	27060	27079	27
1212871	CTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssss	27061	27076	28
1212869	ACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssss	27061	27077	29
1358996	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	sosssssssssssssss	27061	27078	21
1212873	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssosssssssssssoss	27061	27078	21
1212874	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssosssssosssssoss	27061	27078	21
1212875	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssosssosssosssoss	27061	27078	21
1212879	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	soossssssssssooss	27061	27078	21
1212880	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	soossssssssssooss	27061	27078	21
1212881	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	soossssssssooss	27061	27078	21
1212885	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	sssssoooooosssss	27061	27078	21
1212887	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	sssssoooooosssss	27061	27078	21
1287128	TCACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssss	27061	27079	30
1212870	CACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	ssssssssssssssss	27062	27078	32
1287132	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sosssssssssssoss	27062	27079	23
1287133	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	ssssssosssssssss	27062	27079	23
1332246	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	ssssssosssssoss	27062	27079	23
1332265	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sssssssssssooss	27062	27079	23
1364778	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sssssssssooss	27062	27079	23
1364779	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sssssssssooss	27062	27079	23
1364780	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sssssssssooss	27062	27079	23
1364781	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnn	sssssssssooss	27062	27079	23

1287129	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssssssss	27062	27080	33
1287130	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	sosssssssssssssoss	27062	27080	33
1287131	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	ssssssssosssssssss	27062	27080	33
1332263	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	ssssssssosssssssoss	27062	27080	33
1332264	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssssssoss	27062	27080	33
1332266	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssososs	27062	27080	33
1332270	TTCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnnnnnnnn	sssssssssssssooss	27062	27080	33
1287124	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssssssss	27062	27081	20
1287125	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	sosssssssssssssoss	27062	27081	20
1287126	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	ssssssssosssssssss	27062	27081	20
1332267	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssssssoss	27062	27081	20
1332268	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	sssssssssssssooss	27062	27081	20
1332269	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssososs	27062	27081	20
1332271	ATTCAC T T T C A T A A T G C T G G	nnnnnnnnnnnnnnnnnnnnnn	ssssssssosssssssoss	27062	27081	20
1287123	TTCACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnnnn	ssssssssssssssssss	27061	27080	22

Table 4

The modified oligonucleotides in Table 4 below are 18 or 19 nucleosides in length. Each nucleoside comprises either a 2'-MOE sugar moiety or a 2'-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, and each 'n' represents a 2'-NMA sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide is provided in the Internucleoside Linkage Motif column, wherein each 's' represents a phosphorothioate internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each modified oligonucleotide listed in Table 4 below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 4
Mixed 2'-MOE/2'-NMA modified oligonucleotides with PS internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1212931	CACTTTCATAATGCTGGC	nennnnnneneennnnnnn	ssssssssssssssssss	27061	27078	21
1212936	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnenneen	ssssssssssssssssss	27061	27078	21
1212941	CACTTTCATAATGCTGGC	nennnnnneneennnnnnn	ssssssssssssssssss	27061	27078	21
1287728	TCACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnne	ssssssssssssssssss	27061	27079	30
1287729	TCACTTTCATAATGCTGG <u>T</u>	nnnnnnnnnnnnnnnnnnne	ssssssssssssssssss	27062	27079	36
1287730	TCACTTTCATAATGCTGG <u>A</u>	nnnnnnnnnnnnnnnnnnne	ssssssssssssssssss	27062	27079	37

Table 5

5 The modified oligonucleotides in Table 5 below are 16, 17, or 18 nucleosides in length. Each nucleoside comprises either a 2'-MOE sugar moiety or a cEt sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, and each 'k' represents a cEt sugar moiety. Each internucleoside linkage is a phosphorothioate internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide is provided in the Internucleoside Linkage Motif column, wherein each 's' represents a phosphorothioate internucleoside linkage. Each cytosine is a 5-methyl cytosine.

10 Each modified oligonucleotide listed in Table 5 below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777). "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 5
Mixed 2'-MOE/cEt modified oligonucleotides with PS internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1212961	CACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27078	21
1212962	CACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27078	21
1212963	CACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27078	21
1212964	CACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27078	21
1212965	CACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27078	21
1212966	CACTTTCATAATGCTGGC	eekeeekeeekeeekeek	ssssssssssssssssss	27061	27078	21
1212967	CACTTTCATAATGCTGGC	eekeeekeeekeeekeek	ssssssssssssssssss	27061	27078	21

1212968	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeee	ssssssssssssssssss	27061	27078	21
1212969	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeeee	ssssssssssssssssss	27061	27078	21
1212970	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeeee	ssssssssssssssssss	27061	27078	21
1212971	CACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27078	21
1212972	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27078	21
1212973	CACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27078	21
1212974	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27078	21
1212975	CACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27078	21
1212976	CACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27078	21
1212977	ACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27077	29
1212978	ACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27077	29
1212979	ACTTTCATAATGCTGGC	keeekeeekeeekeeeek	ssssssssssssssssss	27061	27077	29
1212980	ACTTTCATAATGCTGGC	keeeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212981	ACTTTCATAATGCTGGC	keeeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212982	ACTTTCATAATGCTGGC	eekeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212983	ACTTTCATAATGCTGGC	eekeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212984	ACTTTCATAATGCTGGC	eeeekeeekeeekeeeke	ssssssssssssssssss	27061	27077	29
1212985	ACTTTCATAATGCTGGC	eeeekeeekeeekeeeeee	ssssssssssssssssss	27061	27077	29
1212986	ACTTTCATAATGCTGGC	eeeekeeekeeekeeeeee	ssssssssssssssssss	27061	27077	29
1212987	ACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27077	29
1212988	ACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212989	ACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27077	29
1212990	ACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212991	ACTTTCATAATGCTGGC	keeekeeekeeekeeeeee	ssssssssssssssssss	27061	27077	29
1212992	ACTTTCATAATGCTGGC	eeeeeekeeekeeekeek	ssssssssssssssssss	27061	27077	29
1212993	CTTTCATAATGCTGGC	keeekeeekeeekeek	ssssssssssssssssss	27061	27076	28
1212994	CTTTCATAATGCTGGC	keeekeeekeeekeek	ssssssssssssssssss	27061	27076	28
1212995	CTTTCATAATGCTGGC	keeekeeekeeekeek	ssssssssssssssssss	27061	27076	28
1212996	CTTTCATAATGCTGGC	keeeeekeeekeeekeek	ssssssssssssssssss	27061	27076	28
1212997	CTTTCATAATGCTGGC	keeeeekeeekeeekeek	ssssssssssssssssss	27061	27076	28
1212998	CTTTCATAATGCTGGC	keeekeeekeeekeeeke	ssssssssssssssssss	27061	27076	28
1212999	CTTTCATAATGCTGGC	eekeeekeeekeeekeke	ssssssssssssssssss	27061	27076	28
1213000	CTTTCATAATGCTGGC	eeeekeeekeeekeeeke	ssssssssssssssssss	27061	27076	28

1213001	CTTTCATAATGCTGGC	eeeeeeeeeeeeee	ssssssssssssss	27061	27076	28
1213002	CTTTCATAATGCTGGC	eeeeeeeeeeeeee	ssssssssssssss	27061	27076	28
1213003	CTTTCATAATGCTGGC	keeeeeeeeeeeeee	ssssssssssssss	27061	27076	28
1213004	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeek	ssssssssssssss	27061	27076	28
1213005	CTTTCATAATGCTGGC	keeeeeeeeeeeeee	ssssssssssssss	27061	27076	28
1213006	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeek	ssssssssssssss	27061	27076	28
1213007	CTTTCATAATGCTGGC	keeeeeeeeeeeeee	ssssssssssssss	27061	27076	28
1213008	CTTTCATAATGCTGGC	eeeeeeeeeeeeeeek	ssssssssssssss	27061	27076	28

Table 6

The modified oligonucleotides in Table 6 below are 19 or 20 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, a 2'-OMe sugar moiety, or a 2'-β-D-deoxyribosyl sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, each 'n' represents a 2'-NMA sugar moiety, each 'y' represents a 2'-OMe sugar moiety, and each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): sssssssssssssso; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Cytosines are either non-methylated cytosines or 5-methyl cytosines, wherein each lowercase 'c' in the Nucleobase Sequence column represents a non-methylated cytosine, and each uppercase 'C' in the Nucleobase Sequence column represents a 5-methyl cytosine.

Each nucleobase in the modified oligonucleotides listed in Table 6 below is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 6**Modified oligonucleotides with mixed PS/PO internucleoside linkages**

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1287707	TCACTTTCATAATGCTGGC	eeeeeeeeeeeeeeeee	ssssssssssssssso	27061	27079	30
1287708	TCACTTTCATAATGCTGGc	eeeeeeeeeeeeeeeee	ssssssssssssssso	27061	27079	30
1287709	TCACTTTCATAATGCTGG <u>T</u>	eeeeeeeeeeeeeeeee	ssssssssssssssso	27062	27079	36
1287710	TCACTTTCATAATGCTGG <u>A</u>	eeeeeeeeeeeeeeeee	ssssssssssssssso	27062	27079	37

1287711	TCAC ^T TTTCATAATGCTGG ^c	eeeeeeeeeeeeeeeeey	ssssssssssssso	27061	27079	30
1287712	TCAC ^T TTTCATAATGCTGG ^{<u>U</u>}	eeeeeeeeeeeeeeeeey	ssssssssssssso	27062	27079	38
1287713	TCAC ^T TTTCATAATGCTGG ^{<u>A</u>}	eeeeeeeeeeeeeeeeey	ssssssssssssso	27062	27079	37
1287731	TCAC ^T TTTCATAATGCTGG ^C	nnnnnnnnnnnnnnnnne	ssssssssssssso	27061	27079	30
1287732	TCAC ^T TTTCATAATGCTGG ^c	nnnnnnnnnnnnnnnnne	ssssssssssssso	27061	27079	30
1287733	TCAC ^T TTTCATAATGCTGG ^{<u>T</u>}	nnnnnnnnnnnnnnnnne	ssssssssssssso	27062	27079	36
1287734	TCAC ^T TTTCATAATGCTGG ^{<u>A</u>}	nnnnnnnnnnnnnnnnne	ssssssssssssso	27062	27079	37
1287735	TCAC ^T TTTCATAATGCTGG ^C	nnnnnnnnnnnnnnnnnd	ssssssssssssso	27061	27079	30
1287736	TCAC ^T TTTCATAATGCTGG ^c	nnnnnnnnnnnnnnnnnd	ssssssssssssso	27061	27079	30
1287737	TCAC ^T TTTCATAATGCTGG ^{<u>T</u>}	nnnnnnnnnnnnnnnnnd	ssssssssssssso	27062	27079	36
1287738	TCAC ^T TTTCATAATGCTGG ^{<u>A</u>}	nnnnnnnnnnnnnnnnnd	ssssssssssssso	27062	27079	37
1287739	TCAC ^T TTTCATAATGCTGG ^c	nnnnnnnnnnnnnnnnny	ssssssssssssso	27061	27079	30
1287740	TCAC ^T TTTCATAATGCTGG ^{<u>U</u>}	nnnnnnnnnnnnnnnnny	ssssssssssssso	27062	27079	38
1287741	TCAC ^T TTTCATAATGCTGG ^{<u>A</u>}	nnnnnnnnnnnnnnnnny	ssssssssssssso	27062	27079	37
1287705	TCAC ^T TTTCATAATGCTGG ^{<u>T</u>}	eeeeeeeeeeeeeeeeee	ssssssssssssso	27062	27079	36
1287706	TCAC ^T TTTCATAATGCTGG ^{<u>A</u>}	eeeeeeeeeeeeeeeeee	ssssssssssssso	27062	27079	37
1287704	TCAC ^T TTTCATAATGCTGG ^c	eeeeeeeeeeeeeeeeee	ssssssssssssso	27061	27079	30
1287703	TCAC ^T TTTCATAATGCTGG ^C	eeeeeeeeeeeeeeeeee	ssssssssssssso	27061	27079	30

Table 7

The modified oligonucleotides in Table 7 below are 19 or 20 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, or a 2'-β-D-deoxyribosyl sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, each 'n' represents a 2'-NMA sugar moiety, and each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): ssssssssssssoo; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each nucleobase in the modified oligonucleotide listed in Table 6 below is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 7

Modified oligonucleotides with mixed PS/PO internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1318749	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	nnnnnnnnnnnnnnnnnnndd	sssssssssssssssssoo	27062	27079	39
1318750	TCAC ^T TTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnned	sssssssssssssssssoo	27060	27079	27
1318751	TCAC ^T TTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnndd	sssssssssssssssssoo	27060	27079	27
1318752	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	nnnnnnnnnnnnnnnnnnned	sssssssssssssssssoo	27062	27079	39
1318753	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	nnnnnnnnnnnnnnnnnnnde	sssssssssssssssssoo	27062	27079	39
1318754	TCAC ^T TTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnnde	sssssssssssssssssoo	27060	27079	27
1318755	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	nnnnnnnnnnnnnnnnnnnee	sssssssssssssssssoo	27062	27079	39
1318756	TCAC ^T TTTCATAATGCTGGCA	nnnnnnnnnnnnnnnnnnnee	sssssssssssssssssoo	27060	27079	27
1318757	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	40
1318758	TCAC ^T TTTCATAATGCTGG ^A <u>C</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	41
1318759	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	42
1318760	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	39
1318761	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	43
1318762	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27062	27079	44
1318763	TCAC ^T TTTCATAATGCTGG ^C <u>C</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27061	27079	45
1318764	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	eeeeeeeeeeeeeeeeeed	sssssssssssssssssoo	27062	27079	39
1318765	TCAC ^T TTTCATAATGCTGG ^A <u>C</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	41
1318766	TCAC ^T TTTCATAATGCTGG ^C <u>T</u>	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27061	27079	46
1318767	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	43
1318768	TCAC ^T TTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeedd	sssssssssssssssssoo	27060	27079	27
1318769	TCAC ^T TTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeeed	sssssssssssssssssoo	27060	27079	27
1318770	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	40
1318771	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	44
1318772	TCAC ^T TTTCATAATGCTGG ^A <u>A</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	39
1318773	TCAC ^T TTTCATAATGCTGG ^A <u>T</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27062	27079	42
1318774	TCAC ^T TTTCATAATGCTGG ^C <u>C</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27061	27079	45
1318775	TCAC ^T TTTCATAATGCTGG ^C <u>T</u>	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27061	27079	46
1318776	TCAC ^T TTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeede	sssssssssssssssssoo	27060	27079	27

1333508	TCAC <u>T</u> TTTCATAATGCTGGC <u>T</u>	nnnnnnnnnnnnnnnnnnnnnnnee	sssssssssssssssssssssoo	27061	27079	46
1318777	TCAC <u>T</u> TTTCATAATGCTGG <u>AC</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	41
1318778	TCAC <u>T</u> TTTCATAATGCTGG <u>TT</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	43
1318779	TCAC <u>T</u> TTTCATAATGCTGG <u>AA</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	39
1318780	TCAC <u>T</u> TTTCATAATGCTGG <u>TC</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	42
1318781	TCAC <u>T</u> TTTCATAATGCTGG <u>AT</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	40
1318782	TCAC <u>T</u> TTTCATAATGCTGGC <u>T</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27061	27079	46
1318783	TCAC <u>T</u> TTTCATAATGCTGG <u>TA</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27062	27079	44
1318784	TCAC <u>T</u> TTTCATAATGCTGGC <u>C</u>	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27061	27079	45
1318748	TCAC <u>T</u> TTTCATAATGCTGGCA	eeeeeeeeeeeeeeeeeeeeeee	sssssssssssssssssssssoo	27060	27079	27

Table 8

The modified oligonucleotides in Table 8 below are each 19 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, or a 2'-β-D-deoxyribsyl sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, each 'n' represents a 2'-NMA sugar moiety, and each 'd' represents a 2'-β-D-deoxyribsyl sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): sssssssssssososso; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine. Each nucleobase in the modified oligonucleotide listed in Table 8 below is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 8
Modified oligonucleotides with mixed PS/PO internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1332247	TCACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnnnd	ssssssssssssososso	27061	27079	30
1332248	TCACTTTCATAATGCTGG <u>A</u>	nnnnnnnnnnnnnnnnnnnnnd	ssssssssssssososso	27062	27079	37
1332249	TCACTTTCATAATGCTGG <u>A</u>	nnnnnnnnnnnnnnnnnnnne	ssssssssssssososso	27062	27079	37
1332251	TCACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnnnnnne	ssssssssssssososso	27061	27079	30

1332255	TCAC TTT CATAATGCTGGC	eeeeeeeeeeeeeeeeed	ssssssssssssosso	27061	27079	30
1332257	TCAC TTT CATAATGCTGG A	eeeeeeeeeeeeeeeeed	ssssssssssssosso	27062	27079	37
1332256	TCAC TTT CATAATGCTGG A	eeeeeeeeeeeeeeeeee	ssssssssssssosso	27062	27079	37
1332258	TCAC TTT CATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssssssssssosso	27061	27079	30

Table 9

The modified oligonucleotides in Table 9 below are each 19 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety, a 2'-NMA sugar moiety, or a 2'-β-D-deoxyribosyl sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, each 'n' represents a 2'-NMA sugar moiety, and each 'd' represents a 2'-β-D-deoxyribosyl sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): sssssssssssosso; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine. Each nucleobase in the modified oligonucleotide listed in Table 9 below is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 9
Modified oligonucleotides with mixed PS/PO internucleoside linkages

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1332250	TCAC TTT CATAATGCTGG A	nnnnnnnnnnnnnnnnnd	ssssssssssssosso	27062	27079	37
1332252	TCAC TTT CATAATGCTGGC	nnnnnnnnnnnnnnnnnd	ssssssssssssosso	27061	27079	30
1332253	TCAC TTT CATAATGCTGG A	nnnnnnnnnnnnnnnnne	ssssssssssssosso	27062	27079	37
1332254	TCAC TTT CATAATGCTGGC	nnnnnnnnnnnnnnnnne	ssssssssssssosso	27061	27079	30
1332259	TCAC TTT CATAATGCTGG A	eeeeeeeeeeeeeeeeed	ssssssssssssosso	27062	27079	37
1332260	TCAC TTT CATAATGCTGGC	eeeeeeeeeeeeeeeeed	ssssssssssssosso	27061	27079	30
1332261	TCAC TTT CATAATGCTGG A	eeeeeeeeeeeeeeeeee	ssssssssssssosso	27062	27079	37
1332262	TCAC TTT CATAATGCTGGC	eeeeeeeeeeeeeeeeee	ssssssssssssosso	27061	27079	30

Table 10

The modified oligonucleotides in Table 10 below are each 19 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety or a 2'-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, and each 'n' represents a 2'-NMA sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): ossssssssssssssss; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each nucleobase in the modified oligonucleotide listed in Table 10 below is complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777), unless specifically stated otherwise. Non-complementary nucleobases are specified in the Nucleobase Sequence column in **underlined, bold, italicized font**. "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 10**Modified oligonucleotides with mixed PS/PO internucleoside linkages**

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1287742	<u>C</u> TCACTTTCATAATGCTGG	ennnnnnnnnnnnnnnnnnnn	osssssssssssssssss	27062	27079	47
1287743	TTCAC <u>T</u> TTTCATAATGCTGG	ennnnnnnnnnnnnnnnnnnn	osssssssssssssssss	27062	27080	33
1287744	<u>A</u> TCACTTTCATAATGCTGG	ennnnnnnnnnnnnnnnnnnn	osssssssssssssssss	27062	27079	48
1287714	<u>C</u> TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeeeee	osssssssssssssssss	27062	27079	47
1287716	<u>A</u> TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeeeeeee	osssssssssssssssss	27062	27079	48
1287715	TTCAC <u>T</u> TTTCATAATGCTGG	eeeeeeeeeeeeeeeeeeee	osssssssssssssssss	27062	27080	33

Table 11

The modified oligonucleotides in Table 11 below are each 20 nucleosides in length. Each nucleoside comprises a 2'-MOE sugar moiety or a 2'-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, and each 'n' represents a 2'-NMA sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage or a phosphodiester internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, provided in the Internucleoside Linkage Motif column, is (from 5' to 3'): ossssssssssssssso; wherein each 's' represents a phosphorothioate internucleoside linkage, and each 'o' represents a phosphodiester internucleoside linkage. Each cytosine is a 5-methyl cytosine.

Each modified oligonucleotide listed in Table 11 below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777). "Start site" indicates

the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

Table 11

5 **Modified oligonucleotides with mixed PS/PO internucleoside linkages**

Compound Number	Nucleobase Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1287745	TTCACCTTCATAATGCTGGC	ennnnnnnnnnnnnnnnnne	osssssssssssssso	27061	27080	22
1287717	TTCACCTTCATAATGCTGGC	ccccccccccccccccccc	osssssssssssssso	27061	27080	22

Example 2: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, single dose (35 µg)

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice.

- 10 Taiwan strain of SMA Type III mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). These mice lack mouse SMN and are homozygous for human SMN2 (mSMN -/-; hSMN2 +/-; FVB.Cg-Tg(SMN2)2HungSMN1tm1Hung/J, stock number 005058; Bar Harbor, Maine), or are heterozygous for mouse SMN and heterozygous for human SMN2 (mSMN +/-; hSMN2 +/-; FVB.Cg-Tg(SMN2)2HungSMN1tm1Hung/J) obtained by breeding HOM/HOM (stock# 00005058) to FVB/NJ (Stock #001800).

15 *Treatment*

Homozygous or heterozygous transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of 35 µg of modified oligonucleotide. Comparator Compound Nos. 387954, 396442, and 396443 were also tested in this assay. A group of 4 mice received PBS as a negative control.

20 *RNA analysis*

- Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Primer probe set hSMN2vd#4_LTS00216_MGB (forward sequence: GCTGATGCTTTGGGAAGTATGTTA (SEQ ID NO: 11); reverse sequence CACCTTCCTTCTTTTGATTTTGTC, designated herein as SEQ ID NO: 12; probe sequence TACATGAGTGGCTATCATACT (SEQ ID NO: 13)) was used to determine the amount of SMN2 RNA including exon 7 (exon 7⁺). Primer probe set hSMN2_Sumner68_PPS50481 (forward sequence: CATGGTACATGAGTGGCTATCATACTG (SEQ ID NO: 14); reverse sequence: TGCTGTCATTTAGTGCTGCTCTATG (SEQ ID NO: 15); probe sequence CCAGCATTTCATATAATAGC (SEQ ID NO: 16) was used to determine the amount of SMN2 RNA excluding exon 7 (exon 7⁻). Total SMN2 RNA levels were measured using primer probe set hSMN2_LTS00935 (forward sequence: CAGGAGGATTCCGTGCTGTT (SEQ ID NO: 17); reverse sequence CAGTGCTGTATCATCCCAAATGTC, (SEQ ID NO: 18); probe sequence: ACAGGCCAGAGCGAT (SEQ ID NO: 19)).
- 25
- 30

Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. Each of Tables 12-18 represents a different experiment.

Table 12

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
396442	35	3.3	0.3	3.4	0.3
396443	35	3.0	0.5	2.3	0.5
524403	35	3.3	0.4	2.5	0.5
1210339	35	2.5	0.5	3.0	0.3
1210340	35	2.1	0.6	2.6	0.4
1210341	35	1.8	0.7	2.0	0.6
1210342	35	2.5	0.5	2.9	0.3
1210343	35	3.0	0.4	2.4	0.5
1212817	35	2.4	0.6	2.2	0.6
1212818	35	2.4	0.5	2.1	0.6
1212823	35	2.0	0.6	2.0	0.6
1212824	35	2.1	0.6	2.1	0.6
1212825	35	2.9	0.4	2.5	0.5
1212826	35	2.5	0.6	2.2	0.7
1212827	35	2.5	0.6	2.6	0.5
1212828	35	2.9	0.5	2.4	0.6
1212830	35	2.8	0.7	2.1	0.8
1212831	35	2.5	0.7	2.3	0.7
1212832	35	2.9	0.6	2.9	0.5
1212833	35	2.4	0.7	2.7	0.5
1212837	35	2.5	0.6	2.7	0.5
1212838	35	2.1	0.7	2.5	0.6
1212844	35	2.6	0.6	2.4	0.7
1212845	35	2.3	0.7	2.5	0.7
1212846	35	2.8	0.6	2.6	0.6
1212849	35	2.1	0.7	2.3	0.6
1212850	35	1.8	0.8	2.2	0.7
1212855	35	2.0	0.7	2.1	0.8

5

Table 13

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1.0	1.0	1.0	1.0
396443	35	2.7	0.3	1.9	0.5
1210342	35	2.4	0.5	2.5	0.4
1212961	35	1.8	0.7	1.7	0.6

1212962	35	2.0	0.6	1.9	0.5
1212963	35	2.3	0.5	2.5	0.3
1212966	35	1.6	0.8	2.0	0.5
1212967	35	1.9	0.6	1.9	0.4
1212971	35	1.6	0.5	2.0	0.4
1212972	35	1.8	0.6	2.2	0.5
1212977	35	2.1	0.5	2.2	0.4
1212978	35	2.1	0.6	2.2	0.4
1212979	35	2.1	0.5	2.6	0.3
1212982	35	2.0	0.7	1.8	0.6
1212983	35	1.9	0.6	1.7	0.5
1212984	35	1.9	0.6	1.9	0.5
1212987	35	2.4	0.4	2.5	0.4
1212988	35	1.8	0.7	1.8	0.5
1212995	35	2.5	0.5	2.5	0.4
1212998	35	1.8	0.6	1.8	0.7
1212999	35	2.0	0.6	2.0	0.5
1213003	35	1.9	0.7	2.3	0.5
1213004	35	1.8	0.7	2.3	0.6

Table 14

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1.0	1.0	1.0	1.0
396443	35	2.6	0.5	3.1	0.5
1212964	35	2.5	0.6	3.6	0.4
1212965	35	2.9	0.5	3.3	0.4
1212968	35	2.2	0.6	2.3	0.6
1212973	35	2.6	0.5	3.2	0.4
1212974	35	2.3	0.6	2.8	0.5
1212975	35	2.9	0.3	3.1	0.4
1212976	35	2.5	0.5	2.8	0.5
1212980	35	2.6	0.5	3.2	0.4
1212981	35	2.9	0.4	3.6	0.3
1212985	35	2.4	0.6	2.9	0.5
1212986	35	2.8	0.4	3.3	0.4
1212989	35	3.3	0.3	3.6	0.2
1212990	35	1.8	0.8	2.1	0.7
1212991	35	3.2	0.3	3.8	0.3
1212992	35	2.4	0.5	2.2	0.6
1212996	35	2.2	0.6	3.2	0.5
1212997	35	2.9	0.4	3.9	0.4

1213001	35	2.1	0.5	2.8	0.6
1213002	35	2.0	0.6	2.9	0.6
1213005	35	2.8	0.5	3.2	0.3
1213006	35	1.9	0.9	2.0	0.8
1213007	35	3.3	0.2	2.9	0.5
1213008	35	2.3	0.7	2.2	0.7

Table 15

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1.0	1.0	1.0	1.0
387954	35	2.3	0.6	2.2	0.5
396443	35	2.5	0.5	2.4	0.5
1287048	35	2.2	0.5	2.2	0.5
1287049	35	2.3	0.6	2.5	0.4
1287061	35	2.4	0.5	2.2	0.4
1287062	35	3.0	0.3	2.3	0.4
1287050	35	2.8	0.5	2.3	0.4
1287054	35	2.2	0.5	2.3	0.4
1287063	35	1.8	0.7	1.7	0.6
1287064	35	2.6	0.3	2.4	0.4
1287065	35	2.5	0.4	2.3	0.4
1287066	35	2.2	0.5	2	0.5
1287075	35	2.3	0.6	1.8	0.7
1287076	35	2.6	0.4	1.9	0.6
1287067	35	2.7	0.4	1.9	0.6
1287070	35	2.5	0.5	1.8	0.7
1287071	35	2.6	0.4	1.8	0.7
1287074	35	2.6	0.5	2	0.6
1287109	35	2.7	0.6	2.4	0.5
1287110	35	2.6	0.5	2.3	0.5
1287701	35	2.6	0.6	2.8	0.3
1287702	35	3	0.5	2.8	0.4
1287703	35	2.3	0.6	2.4	0.4
1287704	35	2.7	0.5	2.3	0.4
1287717	35	3.3	0.3	2.4	0.6

Table 16

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
396442	35	2.5	0.6	3.2	0.3
396443	35	3	0.5	2.8	0.5
1263783	35	3	0.3	2.6	0.5
1263785	35	3.1	0.4	2.9	0.5
1263787	35	2.4	0.6	2.9	0.4
1263789	35	3.8	0.2	2.6	0.5
1263800	35	3.6	0.2	2.6	0.5
1263802	35	3.4	0.3	2.9	0.4
1263806	35	3.5	0.2	2.7	0.5
1263808	35	3.2	0.4	2.7	0.5
1263810	35	2.8	0.5	2.4	0.5

5

Table 17

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
396443	35	2.5	0.6	2.9	0.4
1364784	35	2.3	0.7	2.8	0.5
1364783	35	2.9	0.5	2.4	0.5
1364777	35	2.7	0.6	2.3	0.5
1364782	35	2.7	0.6	2.6	0.5

Table 18

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
396443	35	2	0.7	2.7	0.5
1318748	35	2.1	0.7	2.5	0.6
1318782	35	2.2	0.8	2.5	0.6
1332262	35	3.3	0.4	2.9	0.5
1332258	35	2.4	0.7	2.3	0.6

10

Example 3: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, single dose (15 µg)

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice essentially as described above in Example 2. Comparator Compound Nos. 396443 and 819735 were also tested in this assay. The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of 15µg of modified oligonucleotide. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. Each of Tables 19-23 represents a different experiment.

Table 19

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1.0	1.0	1.0	1.0
819735	15	2.4	0.4	3.3	0.3
1212869	15	2.4	0.4	3.2	0.4
1212870	15	2.1	0.5	2.8	0.4
1212873	15	2.2	0.4	2.0	0.6
1212874	15	2.1	0.5	2.4	0.6
1212875	15	2.1	0.5	2.3	0.5
1212880	15	1.7	0.6	2.0	0.6
1212881	15	1.8	0.6	2.3	0.6
1212885	15	2.3	0.4	2.4	0.5
1212887	15	2.0	0.5	2.2	0.5
1212931	15	2.9	0.2	2.9	0.3
1212936	15	2.9	0.3	3.3	0.3
1212941	15	3.0	0.1	3.4	0.2

Table 20

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1.0	1.0	1.0	1.0
396443	15	2.2	0.5	2.2	0.7
819735	15	2.7	0.5	3.1	0.5
1287122	15	2.9	0.5	2.3	0.6
1287123	15	3.0	0.4	3.0	0.4
1287124	15	3.0	0.4	3.2	0.3
1287125	15	3.0	0.4	3.0	0.4
1287126	15	2.8	0.4	2.8	0.4
1287127	15	2.7	0.5	3.0	0.5

1287128	15	2.6	0.5	3.2	0.5
1287129	15	2.9	0.4	2.9	0.5
1287130	15	3.7	0.1	3.1	0.5
1287131	15	2.2	0.6	2.7	0.4
1287132	15	3.2	0.3	2.2	0.6
1287133	15	2.9	0.4	2.8	0.4
1287728	15	2.8	0.6	3.4	0.3
1287729	15	3.1	0.4	3.0	0.3
1287730	15	3.1	0.3	2.7	0.4
1287731	15	3.3	0.3	2.8	0.5
1287735	15	2.9	0.5	2.6	0.5
1287738	15	3.7	0.2	3.2	0.3
1287739	15	3.3	0.4	3.2	0.4
1287743	15	3.6	0.4	3.8	0.4
1287745	15	3.1	0.5	3.8	0.5

Table 21

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1.0
396443	15	1.9	0.6	1.7	0.7
819735	15	2.3	0.5	1.9	0.6

5

Table 22

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
1364781	15	2.5	0.6	2.7	0.4
1364780	15	2.8	0.5	2.6	0.5
1364779	15	2.7	0.5	2.6	0.5
1364778	15	3	0.5	2.7	0.4

Table 23

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
819735	15	2.8	0.5	2.4	0.6
1332265	15	2.1	0.7	2.6	0.6

1332269	15	2.5	0.6	2.7	0.5
1332268	15	2.9	0.5	2.3	0.6
1318756	15	2.2	0.7	2.4	0.6
1333508	15	2	0.6	2.2	0.6
1332251	15	2.9	0.5	1.9	0.7
1332249	15	2.3	0.7	2.3	0.7

Example 4: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, single dose (70 µg)

Activity of modified oligonucleotides was tested in human SMN2 transgenic mice essentially as described above in Example 2. The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of 70 µg modified oligonucleotide. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels.

Table 24

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
1212969	70	2.5	0.4	2.4	0.3
1212970	70	2.7	0.3	2.6	0.3

Example 5: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, multiple dose

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice essentially as described above in Example 2. Comparator Compound No. 396443 was also tested in this assay. The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of modified oligonucleotide at multiple doses as indicated in the tables below. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from coronal brain and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. ED₅₀ for exon inclusion (exon 7⁺) was calculated in GraphPad Prism 7 using nonlinear regression, 4-parameter dose response curve [$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (10^{\log \text{EC}_{50} / X})^{\text{HillSlope}})$].

Table 25

Effect of modified oligonucleotides on human SMN2 RNA splicing in homozygous transgenic mice

Compound No.	Dose (µg)	CORONAL BRAIN		ED ₅₀ (µg)	SPINAL CORD		ED ₅₀ (µg)
		exon 7 ⁺	exon 7 ⁻		exon 7 ⁺	exon 7 ⁻	
PBS	-	1.0	1.0		1.0	1.0	
396443	3	1.4	0.9	32.5	1.3	0.9	22.1

	10	1.8	0.8		2.0	0.7	
	30	2.6	0.5		2.6	0.4	
	100	3.5	0.4		3.2	0.3	
	300	4.2	0.1		3.6	0.2	
1263789	3	1.5	0.9	38.3	1.5	0.8	13.3
	10	2.0	0.7		2.2	0.6	
	30	2.3	0.6		3.0	0.4	
	100	3.4	0.3		3.4	0.3	
	300	3.9	0.1		3.7	0.2	
1287717	3	1.3	0.8	38.7	1.3	0.9	20.5
	10	1.8	0.7		1.9	0.7	
	30	2.4	0.7		2.7	0.5	
	100	3.5	0.4		3.3	0.3	
	300	4.1	0.1		3.8	0.2	
1358996	3	1.6	0.9	16.6	1.7	0.8	7.4
	10	2.5	0.6		2.6	0.5	
	30	3.0	0.4		3.5	0.2	
	100	4.0	0.2		3.6	0.2	
	300	4.0	0.1		3.9	0.1	
1287745	3	1.5	0.8	22.8	1.7	0.7	8.8
	10	2.1	0.6		2.4	0.5	
	30	3.0	0.3		3.3	0.3	
	100	3.6	0.1		3.5	0.2	
	300	4.2	0.1		3.8	0.1	

Example 6: Tolerability of modified oligonucleotides complementary to SMN2 in wild-type mice, 3 hour study

Modified oligonucleotides described above were tested in wild-type female C57/Bl6 mice to assess tolerability. Wild-type female C57/Bl6 mice each received a single ICV dose of 700 µg of modified oligonucleotide listed in the tables below. Comparator Compound No. 396443 was also tested in this assay with a dose of 350 µg. Comparator Compound Nos. 387954, 396442, 443305, and 819735 were also tested in this assay with a dose of 700 µg. Each treatment group consisted of 4 mice. A group of 4 mice received PBS as a negative control for each experiment (identified in separate tables below). At 3 hours post-injection, mice were evaluated according to seven 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 (the functional observational battery score or FOB). After all 7 criteria were evaluated, the scores were summed and averaged within each treatment group. The results are presented in the tables below. Each of Tables 26-49 represents a different experiment.

Table 26**Tolerability scores in mice at 350 µg dose**

Compound Number	3 hr FOB
PBS	0
396443	4.0

Table 27**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
443305	4.75

Table 28**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0
396442	2.5
524403	3.25
1210339	1.25
1210340	2.25
1210341	3.75
1210342	0
1210343	0
1212817	0
1212818	0
1212819	0
1212820	0
1212821	0
1212822	0
1212823	0
1212824	0
1212825	1
1212826	0
1212827	0
1212828	0
1212829	0
1212830	0
1212831	0

Table 29**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
396442	2.50
1210340	3.50
1212850	0.50
1212851	0.75
1212852	0.00
1212853	0.00
1212854	0.25
1212855	0.25
1212856	0.00
1212857	0.00
1212858	0.00
1212859	0.00
1212860	0.75
1212861	1.00
1212863	2.00
1212864	0.00
1212866	0.75
1212867	0.00
1212868	0.00

Table 30**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
396442	3.25
1212961	0.00
1212963	1.00
1212964	2.00
1212965	1.25
1212966	1.25
1212968	0.00
1212971	1.00
1212972	3.25
1212973	0.50
1212974	2.00
1212975	0.50
1212976	1.75

Table 31
Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1212977	0.75
1212978	0.00
1212979	1.75
1212980	1.50
1212981	0.00
1212982	0.50
1212983	0.75
1212984	2.75
1212985	0.00
1212986	1.00
1212987	1.75
1212988	4.50
1212989	1.75
1212990	4.50
1212991	1.25
1212992	3.75

5

Table 32
Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1212993	7.00
1212994	6.50
1212995	4.25
1212996	3.25
1212997	4.00
1212998	2.00
1212999	1.00
1213000	1.25
1213001	3.00
1213002	2.00
1213003	4.00
1213004	3.00
1213005	3.75
1213006	4.00
1213007	4.00

1213008	3.50
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Table 33**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1212832	0.00
1212833	0.00
1212834	0.00
1212835	0.00
1212836	0.00
1212837	0.00
1212838	0.00
1212839	0.00
1212840	0.00
1212841	0.00
1212842	0.00
1212843	0.00
1212844	0.25
1212845	1.00
1212846	0.00
1212847	0.00
1212848	0.00
1212849	0.00

5

Table 34**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
396442	1.75
1210339	1.00
1212865	1.00
1212962	0.00
1212967	0.50
1212969	0.50
1212970	1.25

Table 35**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
819735	2.00
1212869	2.00
1212870	4.75
1212871	1.00
1212873	0.00
1212874	0.00
1212875	0.00
1212879	3.00
1212880	0.00
1212881	4.00
1212885	1.00
1212887	2.25
1212931	2.00
1212936	2.00
1212941	1.25

Table 36**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1263778	0.00
1263781	0.00
1263783	0.00
1263785	1.00
1263787	0.00
1263789	0.00
1263791	0.00
1263793	0.00
1263795	0.00
1263797	0.00
1263799	0.00
1263800	0.00
1263802	0.00
1263804	0.00
1263806	0.00
1263808	1.00
1263810	0.00

1263812	0.00
1263814	1.00
1263816	0.50
1263818	0.00
1263820	0.00
1263822	0.25
1263824	0.00

Table 37**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1263826	0.00

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Table 38**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
387954	4.00
1287048	0.00
1287049	0.00
1287050	2.00
1287051	3.25
1287052	3.50
1287053	2.75
1287054	2.00
1287055	3.25
1287056	4.00
1287057	3.00
1287058	4.00
1287059	4.00
1287060	4.00
1287061	4.00
1287062	3.50

Table 39**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1287106	3.50

1287107	4.00
1287108	3.75
1287109	3.25
1287110	3.00
1287111	4.75
1287112	4.00
1287113	3.50
1287114	3.25
1287115	3.50
1287116	4.00
1287117	4.25
1287118	3.00
1287119	3.50
1287120	3.75
1287121	2.75

Table 40**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1287063	0.00
1287064	0.00
1287065	1.00
1287066	3.75
1287067	1.00
1287068	2.50
1287069	2.25
1287071	1.00
1287072	3.00
1287073	3.75
1287074	1.75
1287075	3.50
1287076	2.00

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Table 41**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1287070	2.00
1287701	2.50

1287702	3.75
1287703	3.75
1287705	4.00
1287706	4.00
1287707	4.00
1287709	4.75
1287710	4.00
1287711	4.75
1287712	4.00
1287713	4.00
1287714	3.50
1287715	4.00
1287716	4.00
1287717	3.25

Table 42
Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1287728	1.00
1287729	1.25
1287730	2.00
1287731	2.50
1287732	3.00
1287733	3.25
1287734	3.00
1287735	0.50
1287736	2.50
1287737	4.00
1287738	3.00
1287739	2.50
1287740	2.75
1287741	3.75
1287742	3.00
1287743	2.75
1287744	2.25
1287745	1.00

Table 43**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1287122	0.00
1287123	0.00
1287124	3.50
1287125	3.00
1287126	3.00
1287127	0.00
1287128	0.00
1287129	4.00
1287130	2.75
1287131	2.50
1287132	2.75
1287133	3.25
1287704	3.50
1287708	3.50

Table 44**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1318748	2.00
1318765	4.00
1318767	4.25
1318770	3.75
1318771	4.50
1318772	4.25
1318773	4.25
1318774	3.50
1318775	3.75
1318776	3.75
1318777	4.00
1318778	4.00
1318779	4.00
1318780	4.00
1318781	4.00
1318782	1.00
1318783	4.00

1318784	2.00
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Table 45**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1318757	4.00
1318758	4.25
1318759	3.75
1318760	3.75
1318761	4.00
1318762	4.00
1318763	4.00
1318764	3.75
1318766	3.75
1318768	4.00
1318769	4.00

5

Table 46**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1318749	4.25
1318750	2.25
1318751	4.00
1318752	3.75
1318753	2.25
1318754	3.00
1318755	3.75
1318756	0.00

Table 47**Tolerability scores in mice at 700 µg dose**

Compound Number	3 hr FOB
PBS	0.00
1332247	1.75
1332248	0.25
1332249	0.00
1332250	3.75

1332251	0.00
1332252	3.00
1332263	2.00
1332265	1.50
1332266	1.00
1332267	3.75
1332268	2.75
1332269	1.25
1332270	2.25
1332271	2.50
1333508	0.00

Table 48

Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1332255	1.00
1332256	2.00
1332257	1.25
1332258	1.25
1332259	2.25
1332260	2.25
1332261	2.50
1332262	2.00

5

Table 49

Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1358996	0.00
1364777	2.00
1364778	3.00
1364779	3.50
1364780	3.50
1364781	5.25
1364782	2.50
1364783	3.50
1364784	3.50

Example 7: Tolerability of modified oligonucleotides complementary to human SMN2 in rats, long-term assessment

In separate studies run under the same conditions, selected modified oligonucleotides described above were tested in Sprague Dawley rats to assess long-term tolerability. Comparator Compound Nos. 396442 and 819735 were also tested in this assay. Sprague Dawley rats each received a single intrathecal (IT) delivered dose of 3 mg of oligonucleotide or PBS. Beginning 1 week post-treatment, each animal was weighed and evaluated weekly by a trained observer for adverse events. Adverse events were defined as neurological dysfunction not typical in PBS-treated control animals, including, but not limited to: abnormal limb splay, abnormal gait, tremors, abnormal respiration, paralysis, and spasticity. The onset of the adverse event is defined as the week post-dosing when the dysfunction was first recorded. If no adverse event was achieved, there is no onset (-). The onset of adverse events typically correlates with a failure to thrive as defined by a lack of body weight gain/maintenance similar to PBS-treated animals. Similar tolerability assessments were described in Oestergaard et al., *Nucleic Acids Res.*, 2013 Nov, 41(21), 9634–9650 and Southwell et al., *Mol Ther.*, 2014 Dec, 22(12), 2093–2106.

At the end of the study, the rats were sacrificed and tissues were collected. Histopathology was performed on sections of cerebellum using calbindin stain. Purkinje cell loss was observed in calbindin stained cerebellum sections as indicated in the table below. Cerebellum and spinal cord were also evaluated using an antibody specific for modified oligonucleotides. Animals demonstrating no oligonucleotide uptake were excluded from histopathology analysis. Histology was not completed for animals that were sacrificed early due to adverse events. Additionally, cortical GFAP, a marker of astrogliosis (Abdelhak, et al., *Scientific Reports*, 2018, 8, 14798), was measured using RT-PCR, and average elevations >2-fold are noted below.

Table 50

Long-term tolerability in rats at 3 mg dose

Compound Number	Adverse event onset, weeks post-treatment, individual animals	Purkinje cell loss (# animals with loss/# animals tested)	Cortex GFAP mRNA >2-fold PBS Control
PBS	No	Not observed	N/A
396442	6, 6, 2	2/3	Yes
819735	4,6,6,-	1/4	Yes
1263789	-, -, -	0/3	No
1287717	-, -, -, -, -, -, -	0/8	No
1287745	-, -, -, -, -, -	0/7	No
1358996	-, -, -, -	0/4	No
1263783	-, -, -, -	0/4	No
1263785	-, -, -	0/3	No
1263787	-, -, -, -	0/4	No
1263800	-, -	0/2	No

1263802	-,-,-	0/3	No
1263806	-,-,-	0/3	No
1263808	-,-,-	0/3	No
1263810	-,-,-	0/3	No

Example 8: Tolerability and pharmacokinetics of modified oligonucleotides in non-human primates, single or repeat dosing

5 Cynomolgus monkeys are treated with modified oligonucleotides to determine the local and systemic tolerability and pharmacokinetics of the modified oligonucleotides. Each group receives either artificial CSF or modified oligonucleotide as a single intrathecal lumbar bolus dose injection (IT), or, for repeat-dosing groups, an IT bolus dose on day 1 of the study, followed by IT bolus doses at later time points. Tissues are collected 1 week after the final injection.

10 In a single dose study, monkeys are administered a single dose of modified oligonucleotide and tolerability is assessed. Representative doses for single-dose studies in adult cynomolgus monkeys include 1 mg, 3 mg, 7 mg, and 35 mg.

In a repeat-dosing study, monkeys are administered an IT bolus dose on day 1 of the study, followed by weekly (e.g., days 8, 15, and 22 for a four-week study) or monthly (e.g., days 29, 57, and 84 for a 13 week study) IT bolus dosing. Representative doses for repeat-dose studies in adult cynomolgus studies include 1 mg, 3 mg, 7 mg, and 35 mg.

15 Assessment of tolerability is based on clinical observations, body weights, food consumption, physical and neurological examinations including sensorimotor reflexes, cerebral reflexes and spinal reflexes, coagulation, hematology, clinical chemistry (blood and cerebral spinal fluid (CSF)), cell count, and anatomic pathology evaluations. Complete necropsies are performed with a recording of any macroscopic abnormality. Organ weights are taken and microscopic examinations are conducted. Blood is collected for complement analysis. In addition, blood, CSF, and tissues (at
20 necropsy) are collected for toxicokinetic evaluations.

Tolerability of modified oligonucleotides is analyzed in brain and spinal cord tissue by measuring Aif1 and Gfap levels in cynomolgus monkeys treated with the modified oligonucleotide or the control. Brain and spinal cord samples are collected and flash frozen in liquid nitrogen and stored frozen (-60°C to -90°C). At time of sampling, 2 mm biopsy punches are used to collect samples from frozen tissues for RNA analysis. Punches are taken from multiple brain
25 and spinal cord regions.

Example 9: Phase Ia Human Clinical Trial with Compound No. 1263789, 1287717, 1287745, or 1358996

Safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of modified oligonucleotide complementary to human SMN2 is evaluated in a clinical trial setting. Single and/or multiple doses of modified
30 oligonucleotide are evaluated in patients with confirmed SMA, such as Type I SMA, Type II SMA, Type III SMA, or Type IV SMA.

Patient safety is monitored closely during the study. Safety and tolerability evaluations include: physical examination and standard neurological assessment (including fundi), vital signs (HR, BP, orthostatic changes, weight),

ECG, AEs and concomitant medications, Columbia Suicide Severity Rating Scale (C-SSRS), CSF safety labs (cell counts, protein, glucose), plasma laboratory tests (clinical chemistry, hematology), and urinalysis.

Efficacy evaluations are selected that are age and Type appropriate and include, for example, the Hammersmith Motor Function Scale—Expanded (HFMSE), which is a reliable and validated tool used to assess motor function in children with SMA; the Pediatric Quality of Life Inventory (PedsQL™) Measurement 4.0 Generic Core Scale; the Pediatric Quality of Life Inventory 3.0 Neuromuscular Modules; the Compound Muscle Action Potential (CMAP); the Motor Unit Number Estimation (MUNE); the Upper Limb Module (ULM); and the 6-Minute Walk Test (6MWT) (Darras, et al., *Neurology*, 2019, 92: e2492-e2506).

10 Example 10: Design of modified oligonucleotides complementary to a human SMN2 nucleic acid

Modified oligonucleotides complementary to a human SMN2 nucleic acid were designed and synthesized as indicated in the tables below.

Each modified oligonucleotide listed in the tables below is 100% complementary to SEQ ID NO: 1 (GENBANK Accession No. NT_006713.14 truncated from nucleotides 19939708 to 19967777). “Start site” indicates the 5’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. “Stop site” indicates the 3’-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

The modified oligonucleotides in the table below are 18 nucleosides in length. Each nucleoside comprises either a 2’-MOE sugar moiety or a 2’-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each ‘e’ represents a 2’-MOE sugar moiety, and each ‘n’ represents a 2’-NMA sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage, a phosphodiester internucleoside linkage, a methoxypropyl phosphonate internucleoside linkage, or a mesyl phosphoramidate (MsP) internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, is provided in the Internucleoside Linkage Motif column, wherein each ‘s’ represents a phosphorothioate internucleoside linkage, each ‘o’ represents a phosphodiester internucleoside linkage, each ‘x’ represents a methoxypropyl phosphonate internucleoside linkage, and each ‘z’ represents a mesyl phosphoramidate (MsP) internucleoside linkage. Each cytosine is a 5-methyl cytosine. Modified oligonucleotide 449320 has been previously described in WO2015/161170 A2.

Table 51

MOE and NMA modified oligonucleotides with mixed PO/PS, PO/MsP, uniform MsP, or PS/MOP internucleoside linkages

Compound Number	Sequence (5' to 3')	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
449320	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	ss0000000000000ss	27062	27079	23
1287723	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnn	ssssssssssssssxs	27062	27079	23
1287724	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnn	sssssssssssssssx	27062	27079	23
1287727	CACTTTCATAATGCTGGC	nnnnnnnnnnnnnnnn	sssssssssssssssx	27061	27078	21

1405549	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	zzzzzzzzzzzzzzzz	27062	27079	23
1405552	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	sssssssssszzzzzz	27062	27079	23
1405553	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	sssszzzzzzssssss	27062	27079	23
1545359	TCACTTTCATAATGCTGG	nnnnnnnnnnnnnnnn	ss000000000000ss	27062	27079	23
1547773	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	zz000000000000zz	27062	27079	23
1549028	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	zzzz0000000000zz	27062	27079	23
1549029	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	zzzzzz00000000zz	27062	27079	23
1549030	TCACTTTCATAATGCTGG	eeeeeeeeeeeeeeee	zzzzzzzz000000zz	27062	27079	23

The modified oligonucleotides in the table below all consist of the sequence (from 5' to 3'):
TCACTTTCATAATGCTGG (SEQ ID NO: 23). Each modified oligonucleotide listed in the tables below is 100% complementary to SEQ ID NO: 1 (described herein above). "Start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence. "Stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

The modified oligonucleotides in the table below are 18 nucleosides in length. Each nucleoside comprises either a 2'-MOE sugar moiety or a 2'-NMA sugar moiety. The sugar motif for each modified oligonucleotide is provided in the Sugar Motif column, wherein each 'e' represents a 2'-MOE sugar moiety, and each 'n' represents a 2'-NMA sugar moiety. Each internucleoside linkage is either a phosphorothioate internucleoside linkage, a phosphodiester internucleoside linkage, or a mesyl phosphoramidate (MsP) internucleoside linkage. The internucleoside linkage motif for each modified oligonucleotide, is provided in the Internucleoside Linkage Motif column, wherein each 's' represents a phosphorothioate internucleoside linkage, each 'o' represents a phosphodiester internucleoside linkage, and each 'z' represents a mesyl phosphoramidate (MsP) internucleoside linkage. Each cytosine is a 5-methyl cytosine. The modified oligonucleotides in the table below are conjugated to a 6-palmitamidoheptyl phosphate conjugate group attached to the 5'-OH of the oligonucleotide. The structure for the conjugate group is:

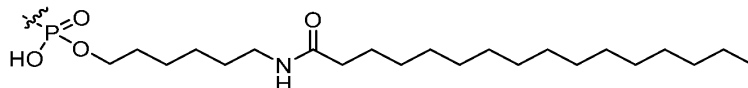


Table 52

6-palmitamidoheptyl conjugated MOE and NMA modified oligonucleotides with mixed PO/PS, PO/MsP, or uniform MsP internucleoside linkages

Compound Number	Sugar Motif (5' to 3')	Internucleoside Linkage Motif (5' to 3')	SEQ ID No: 1 Start Site	SEQ ID No: 1 Stop Site	SEQ ID No.
1545361	eeeeeeeeeeeeeeee	ss000000000000ss	27062	27079	23
1545362	nnnnnnnnnnnnnnnn	ss000000000000ss	27062	27079	23
1547772	eeeeeeeeeeeeeeee	zzzzzzzzzzzzzzzz	27062	27079	23
1547774	eeeeeeeeeeeeeeee	zz000000000000zz	27062	27079	23
1549031	eeeeeeeeeeeeeeee	zzzz0000000000zz	27062	27079	23
1549032	eeeeeeeeeeeeeeee	zzzzzz00000000zz	27062	27079	23
1549033	eeeeeeeeeeeeeeee	zzzzzzzz000000zz	27062	27079	23

The modified oligonucleotides in the table below all consist of the sequence (from 5' to 3'):

TCACTTTTCATAATGCTGG (SEQ ID NO: 23), with a start site of 27062, and a stop site of 27079 on SEQ ID No: 1

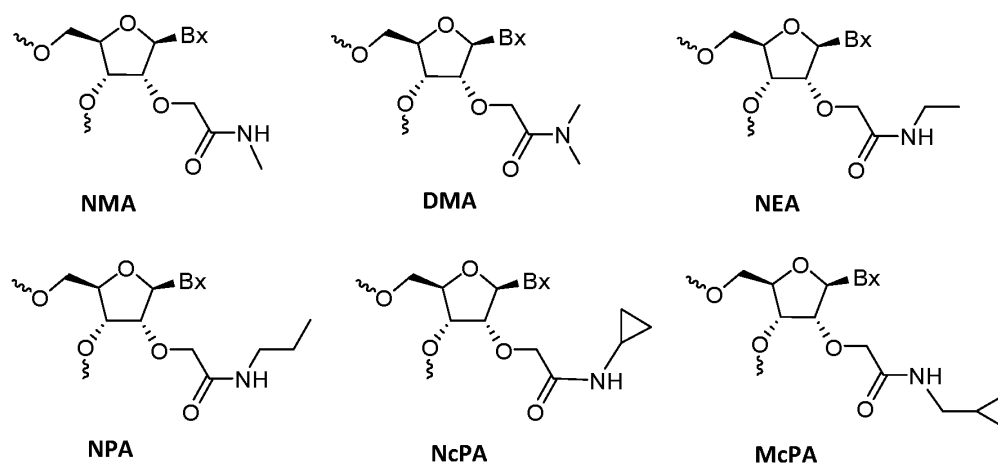
(described herein above), wherein "start site" indicates the 5'-most nucleoside to which the modified oligonucleotide is

5 complementary in the target nucleic acid sequence, and wherein "stop site" indicates the 3'-most nucleoside to which the modified oligonucleotide is complementary in the target nucleic acid sequence.

The modified oligonucleotides in the table below are 18 nucleosides in length. The sugar and internucleoside linkage motifs for each modified oligonucleotide are provided in the Sequence and Chemistry Notation column, wherein each

10 subscript 'n' represents a 2'-NMA sugar moiety, each subscript '[DMA]' represents a 2'-O-(N,N-dimethyl) acetamide moiety, each subscript '[NEA]' represents a 2'-O-(N-ethyl) acetamide moiety, each subscript '[NPA]' represents a 2'-O-(N-propyl) acetamide moiety, each subscript '[NcPA]' represents a 2'-O-(N-cyclopropyl) acetamide moiety, each subscript '[McPA]' represents a 2'-O-(N-cyclopropylmethyl) acetamide moiety, and each subscript 's' represents a phosphorothioate internucleoside linkage. Each cytosine is a 5-methyl cytosine, wherein the superscript 'm' before the

15 cytosine residue (^mC) represents a 5-methyl cytosine. The structures for each of the sugars represented in the table below are:



20

Table 53

NMA and NMA analog modified oligonucleotides with uniform PS internucleoside linkages

Compound Number	Sequence and Chemistry Notation (5' to 3')	SEQ ID No.
1355763	$T_{[DMA]s}{}^mC_{[DMA]s}A_{ns}{}^mC_{[DMA]s}T_{[DMA]s}T_{[DMA]s}T_{[DMA]s}{}^mC_{[DMA]s}A_{ns}T_{[DMA]s}A_{ns}A_{ns}T_{[DMA]s}G_{ns}{}^mC_{[DMA]s}T_{[DMA]s}G_{ns}G_n$	23
1359463	$T_{[NEA]s}{}^mC_{[NEA]s}A_{ns}{}^mC_{[NEA]s}T_{[NEA]s}T_{[NEA]s}T_{[NEA]s}{}^mC_{[NEA]s}A_{ns}T_{[NEA]s}A_{ns}A_{ns}T_{[NEA]s}G_{ns}{}^mC_{[NEA]s}T_{[NEA]s}G_{ns}G_n$	23
1358995	$T_{[NPA]s}{}^mC_{[NPA]s}A_{ns}{}^mC_{[NPA]s}T_{[NPA]s}T_{[NPA]s}T_{[NPA]s}{}^mC_{[NPA]s}A_{ns}T_{[NPA]s}A_{ns}A_{ns}T_{[NPA]s}G_{ns}{}^mC_{[NPA]s}T_{[NPA]s}G_{ns}G_n$	23

1355776	$T_{[NcPA]s}^m C_{[NcPA]s} A_{ns}^m C_{[NcPA]s} T_{[NcPA]s} T_{[NcPA]s} T_{[NcPA]s}^m C_{[NcPA]s} A_{ns} T_{[NcPA]s} A_{ns} A_{ns} T_{[NcPA]s} G_{ns}^m C_{[NcPA]s} T_{[NcPA]s} G_{ns} G_{ns}$	23
1355777	$T_{[McPA]s}^m C_{[McPA]s} A_{ns}^m C_{[McPA]s} T_{[McPA]s} T_{[McPA]s} T_{[McPA]s}^m C_{[McPA]s} A_{ns} T_{[McPA]s} A_{ns} A_{ns} T_{[McPA]s} G_{ns}^m C_{[McPA]s} T_{[McPA]s} G_{ns} G_{ns}$	23

Example 11: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, single dose (35 µg)

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice essentially as described above in Example 2.

Treatment

The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of modified oligonucleotide at doses as indicated in the tables below. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from coronal brain and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. ED₅₀ for exon inclusion (exon 7⁺) was calculated in GraphPad Prism 7 using nonlinear regression, 4-parameter dose response curve [$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (10^{\log EC_{50}} / X)^{\text{HillSlope}})$].

RNA analysis

Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Primer probe set hSMN2vd#4_LTS00216_MGB was used to determine the amount of SMN2 RNA including exon 7 (exon 7⁺). Primer probe set hSMN2_Sumner68_PPS50481 was used to determine the amount of SMN2 RNA excluding exon 7 (exon 7⁻). Total SMN2 RNA levels were measured using primer probe set hSMN2_LTS00935. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels.

Table 54

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
396443	35	3	0.6	3	0.5
1405549	35	1.6	0.9	1.2	0.9
1405552	35	3.2	0.6	2.1	0.7
1405553	35	2.4	0.7	1.7	0.8

Table 55

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	N/A
396443	35	2.7	0.5	3	N/A
1405549	35	1.5	0.8	1.9	N/A
1547772	35	1.8	0.8	1.8	N/A
1547773	35	1.6	0.9	1.9	N/A
1547774	35	1.6	1	1.6	N/A
1549028	35	1.5	1	1.5	N/A
1549029	35	1.4	1	1.7	N/A
1549030	35	1.4	1	1.4	N/A
1549031	35	1.7	0.8	1.6	N/A
1549032	35	1.4†	0.9†	1.7†	N/A
1549033	35	1.4	1	1.3	N/A

† indicates fewer than four samples available

5 **Example 12: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, single dose (15 μg)**

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice essentially as described above in Example 2.

Treatment

10 The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of modified oligonucleotide at doses as indicated in the tables below. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from coronal brain and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. ED₅₀ for exon inclusion (exon 7⁺) was calculated in GraphPad Prism 7 using nonlinear regression, 4-parameter dose response curve [$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (10^{\log \text{EC50} / X})^{\text{HillSlope}})$].

15

RNA analysis

Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Primer probe set hSMN2vd#4_LTS00216_MGB was used to determine the amount of SMN2 RNA including exon 7 (exon 7⁺). Primer probe set hSMN2_Sumner68_PPS50481 was used to determine the amount of SMN2 RNA excluding exon 7 (exon 7⁻). Total SMN2 RNA levels were measured using primer probe set hSMN2_LTS00935. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels.

20

Table 56

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		SPINAL CORD	
		exon 7 ⁺	exon 7 ⁻	exon 7 ⁺	exon 7 ⁻
PBS	-	1	1	1	1
443305	15	3.4	0.4	3.3	0.3
1287723	15	3.9	0.3	3.3	0.3
1287724	15	4.2	0.2	3.7	0.2
1287727	15	4.5	0.2	3.4	0.2

Example 13: Activity of modified oligonucleotides complementary to human SMN2 in transgenic mice, multiple dose

Activity of selected modified oligonucleotides described above was tested in human SMN2 transgenic mice essentially as described above in Example 2.

Treatment

The transgenic mice were divided into groups of 4 mice each. Each mouse received a single ICV bolus of modified oligonucleotide at multiple doses as indicated in the tables below. A group of 4 mice received PBS as a negative control. Two weeks post treatment, mice were sacrificed and RNA was extracted from coronal brain and spinal cord for real-time qPCR analysis of SMN2 RNA. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels. ED₅₀ for exon inclusion (exon 7⁺) was calculated in GraphPad Prism 7 using nonlinear regression, 4-parameter dose response curve [$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (10^{\log \text{EC50} / X})^{\text{HillSlope}})$].

RNA analysis

Two weeks post treatment, mice were sacrificed and RNA was extracted from cortical brain tissue and spinal cord for real-time qPCR analysis of SMN2 RNA. Primer probe set hSMN2vd#4_LTS00216_MGB was used to determine the amount of SMN2 RNA including exon 7 (exon 7⁺). Primer probe set hSMN2_Sumner68_PPS50481 was used to determine the amount of SMN2 RNA excluding exon 7 (exon 7⁻). Total SMN2 RNA levels were measured using primer probe set hSMN2_LTS00935. Results are presented as fold change in RNA levels relative to PBS control, normalized to total SMN2 levels.

Table 57

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		ED50 (μg)	SPINAL CORD		ED50 (μg)
		exon 7 ⁺	exon 7 ⁻		exon 7 ⁺	exon 7 ⁻	
PBS	-	1	1	-	1	1	-
396443	10	2.1	0.7	26	1.9	0.8	22
	30	2.8	0.5		2.6	0.7	
	100	3.2	0.3		2.8	0.4	

449320	10	1.2	1.0	>100	1.2	1.2	>100
	30	1.5	1.0		1.3	1.2	
	100	1.5	0.9		1.3	0.9	
1545361	10	1.4	1.0	>100	1.2	1.1	>100
	30	1.8	1.1		1.6	1.3	
	100	1.3	0.9		1.4	0.9	
443305	10	2.4	0.6	14	2.6	0.5	9
	30	3.5	0.3		3.0	0.4	
	100	3.7	0.1		3.3	0.1	
1545359	10	1.4	1.0	>100	1.4	1.1	>100
	30	2.0	0.9		1.7	1.1	
	100	2.3	0.6		1.7	0.8	
1545362	10	1.4	0.9	95	1.3	1.1	51
	30	1.9	0.8		2.4	1.0	
	100	2.7	0.5		2.6	0.6	

Table 58

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (μg)	CORTEX		ED50 (μg)	SPINAL CORD		ED50 (μg)
		exon 7+	exon 7-		exon 7+	exon 7-	
PBS	-	1	1	-	1	1	-
1263789	3	1.5	0.9	35	1.6	0.8	16
	10	1.9	0.8		2.2	0.6	
	30	2.7	0.6		2.9	0.5	
	100	3.8	0.3		3.5	0.3	
	300	4.3	0.2		3.9	0.2	
1287703	3	1.5	0.7	43	1.5	0.8	28
	10	1.6	0.7		2.0	0.7	
	30	2.7	0.5		2.6	0.5	
	100	3.7	0.3		3.2	0.4	
	300	4.0	0.2		3.5	0.2	
1287717	3	1.4	0.8	31	1.4	0.9	30
	10	1.7	0.7		1.8	0.7	
	30	3.1	0.4		2.4†	0.5†	
	100	3.8	0.3		3.3	0.3	
	300	4.3	0.2		3.8	0.2	
1318768	3	1.3	0.8	49	1.2	0.8	32
	10	1.8	0.7		1.8	0.8	
	30	2.4	0.6		2.4	0.6	
	100	3.5	0.3		3.3	0.4	
	300	4.1	0.2		3.9	0.2	
1287731	3	1.5	0.8	29	1.5	1.0	13
	10	2.0	0.6		2.4	0.6	

	30	2.7	0.4		3.2	0.4	
	100	4.3	0.2		3.8	0.2	
	300	4.2	0.1		3.7	0.1	
1287735	3	1.7	0.7	22	1.5	0.8	15
	10	2.4	0.6		2.2	0.6	
	30	3.0	0.3		3.1	0.3	
	100	4.0	0.2		3.5	0.2	
	300	4.2	0.1		3.9	0.1	
1287745	3	1.5	0.7	35	1.4	0.8	13
	10	2.0	0.6		2.4	0.6	
	30	2.9	0.4		3.2	0.4	
	100	3.7	0.2		3.6	0.2	
	300	3.7	0.2		3.8	0.2	
396443	3	1.7	0.7	47	1.5	0.9	22
	10	1.4	0.7		1.6	0.8	
	30	2.8	0.5		3.1	0.4	
	100	3.3	0.4		3.3	0.5	
	300	4.3	0.2		4.0	0.2	

† indicates fewer than four samples available

Table 59

Effect of modified oligonucleotides on human SMN2 RNA splicing in heterozygous transgenic mice

Compound No.	Dose (µg)	CORTEX		ED50 (µg)	SPINAL CORD		ED50 (µg)
		exon 7+	exon 7-		exon 7+	exon 7-	
PBS	-	1	1	-	1	1	-
396443	3	1.1	0.9	39	1.2	0.9	33
	30	2.0	0.6		2.3	0.5	
	100	2.3	0.4		2.7	0.3	
443305	3	1.4	0.7	13	1.6	0.7	10
	30	2.4	0.3		3.0	0.2	
	100	2.9	0.2		3.1	0.2	
1355763	3	1.1	0.8	54	1.1	0.8	45
	30	1.7	0.6		2.1	0.5	
	100	2.4	0.4		2.6	0.3	
1359463	3	1.3	0.8	18	1.3	0.7	19
	30	2.3	0.3		2.7	0.3	
	100	2.9	0.2		2.7	0.2	
1358995	3	1.2	0.8	45	1.0	0.8	59
	30	2.0	0.4		1.8	0.4	
	100	2.2	0.4		2.6	0.3	
1355776	3	1.1	0.8	25	1.3	0.7	24
	30	2.2	0.4		2.4	0.4	
	100	2.6	0.3		2.9	0.3	
1355777	3	1.0	0.9	107	0.9	0.9	72

	30	1.6	0.7		1.8	0.6	
	100	1.7	0.5		2.2	0.6	

Example 15: Tolerability of modified oligonucleotides complementary to SMN2 in wild-type mice

Modified oligonucleotides described above were tested in wild-type female C57/Bl6 mice to assess the tolerability of the oligonucleotides. Wild-type female C57/Bl6 mice each received a single ICV dose of 700 µg of modified oligonucleotide listed in the table below. Each treatment group consisted of 4 mice. A group of 4 mice received PBS as a negative control for each experiment (identified in separate tables below). At 3 hours post-injection, mice were evaluated according to seven 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 (the functional observational battery score or FOB). 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 tables below.

Table 60

Tolerability scores in mice at 700 µg dose

Compound Number	3 hr FOB
PBS	0.00
1287723	2.00
1287724	1.00
1287727	2.00